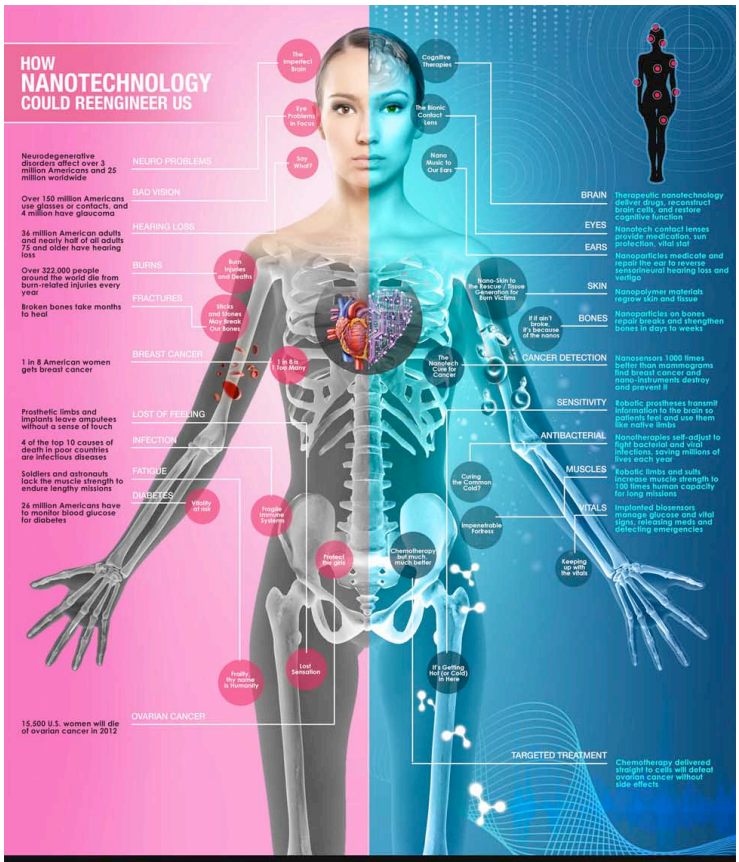

sources

Human

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Enhanced Human

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http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2880812/
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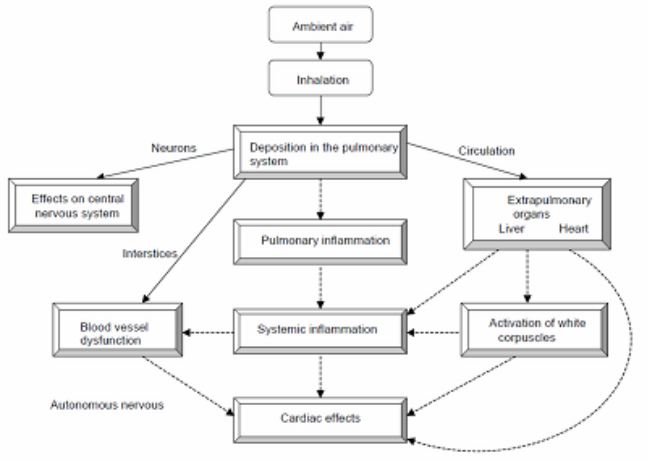
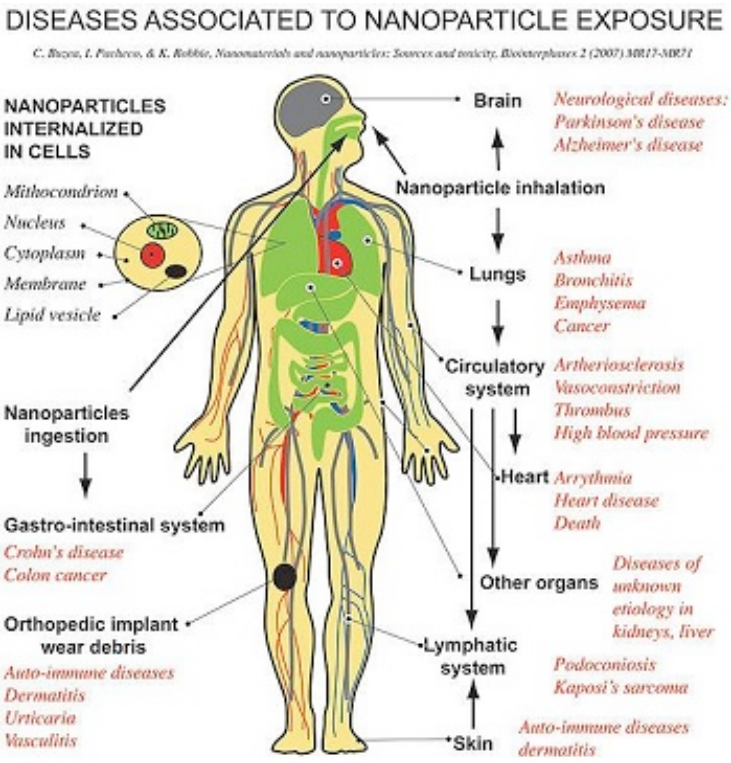
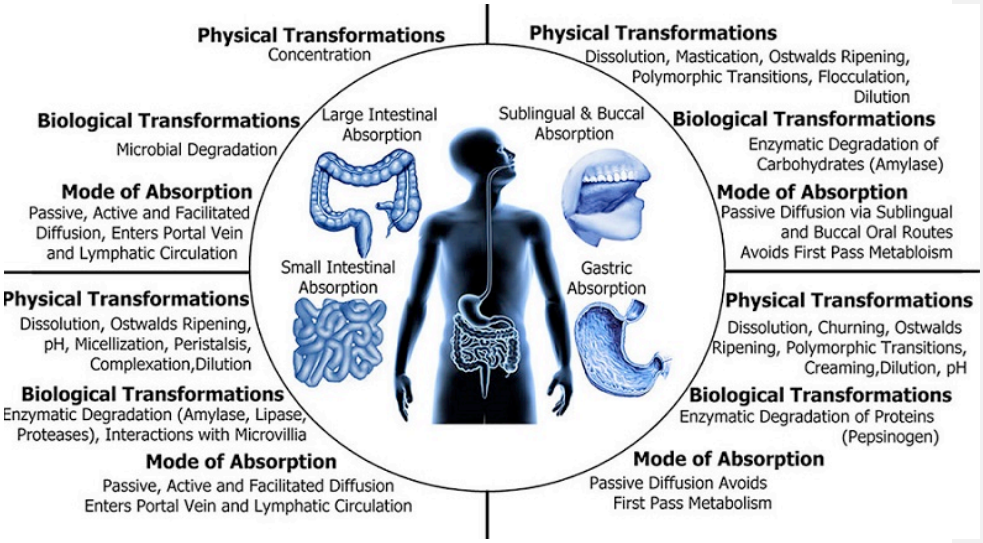
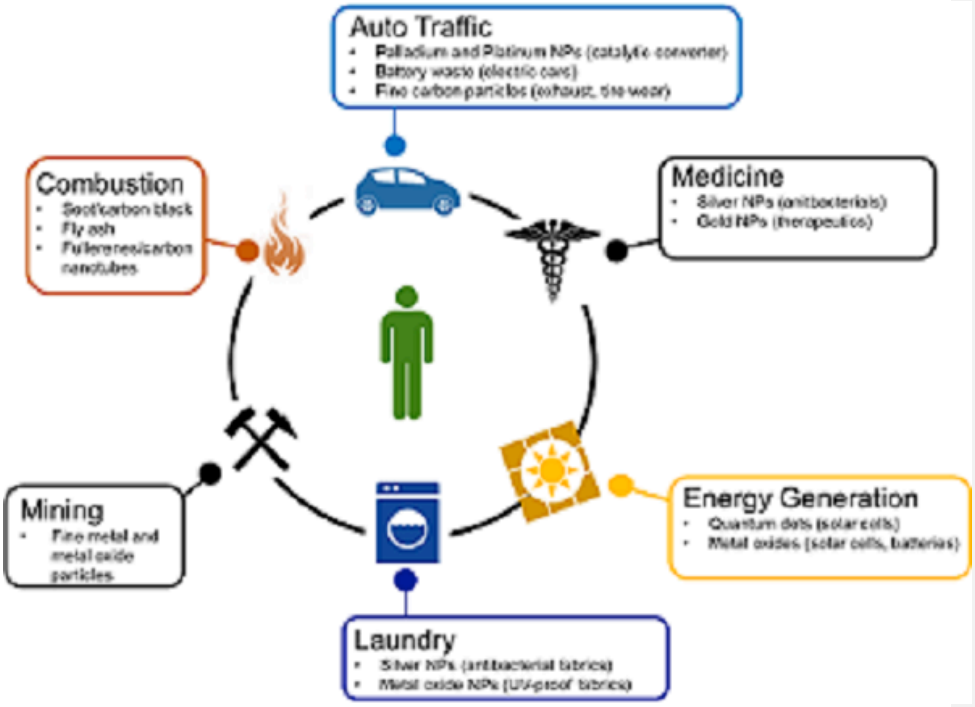

Ambient air
Inhalation
Deposition in the pulmonary system
Neurons
Circulation
Effects on central nervous system
Extrapulmonary organs
Liver
Heart
Interstices
Pulmonary inflammation
Blood vessel dysfunction
Systemic inflammation
Activation of white corpuscles
Autonomous nervous
Cardiac effects

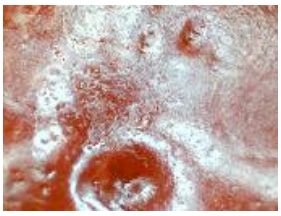

DISEASES ASSOCIATED TO NANOPARTICLE EXPOSURE
C. Buzea, I. Pacheco, & K. Robbie, Nanomaterials and nanoparticles: Sources and toxicity, Biointerphases 2 (2007) MR17-MR71
NANOPARTICLES INTERNALIZED IN CELLS
Mithocondrion
Nucleus
Cytoplasm
Membrane
Lipid vesicle
Nanoparticles ingestion
Gastro-intestinal system
Crohn's disease
Colon cancer
Orthopedic implant wear debris
Auto-immune diseases
Dermatitis
Urticaria
Vasculitis
Nanoparticle inhalation
Brain
Neurological diseases:
Parkinson's disease
Alzheimer's disease
Lungs
Asthma
Bronchitis
Emphysema
Cancer
Circulatory system
Artheriosclerosis
Vasoconstriction
Thrombus
High blood pressure
Heart
Arrythmia
Heart disease
Death
Other organs
Diseases of unknown etiology in kidneys, liver
Lymphatic system
Podoconiosis
Kaposi's sarcoma
Skin
Auto-immune diseases
dermatitis

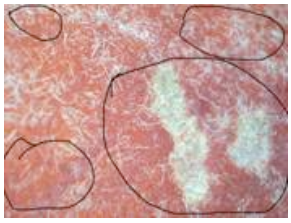

Physical Transformations
Concentration
Biological Transformations
Microbial Degradation
Mode of Absorption
Passive, Active and Facilitated Diffusion, Enters Portal Vein and Lymphatic Circulation
Large Intestinal Absorption
Sublingual & Buccal Absorption
Small Intestinal Absorption
Gastric Absorption
Physical Transformations
Dissolution, Mastication, Ostwalds Ripening, Polymorphic Transitions, Flocculation, Dilution
Biological Transformations
Enzymatic Degradation of Carbohydrates (Amylase)
Mode of Absorption
Passive Diffusion via Sublingual and Buccal Oral Routes Avoids First Pass Metabloism
Physical Transformations
Dissolution, Ostwalds Ripening, pH, Micellization, Peristalsis, Complexation,Dilution
Biological Transformations
Enzymatic Degradation (Amylase, Lipase, Proteases), Interactions with Microvillia
Mode of Absorption
Passive, Active and Facilitated Diffusion Enters Portal Vein and Lymphatic Circulation
Physical Transformations
Dissolution, Churning, Ostwalds Ripening, Polymorphic Transitions, Creaming,Dilution, pH
Biological Transformations
Enzymatic Degradation of Proteins (Pepsinogen)
Mode of Absorption
Passive Diffusion Avoids First Pass Metabolism


Encapsulation

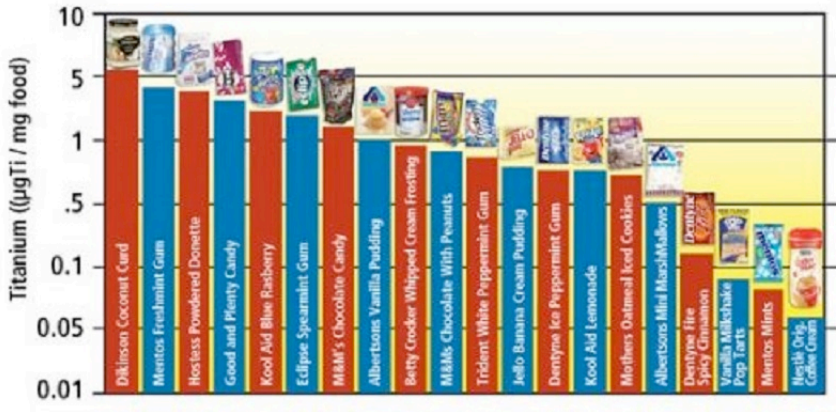
Self Assembling Carbon NanoParticle

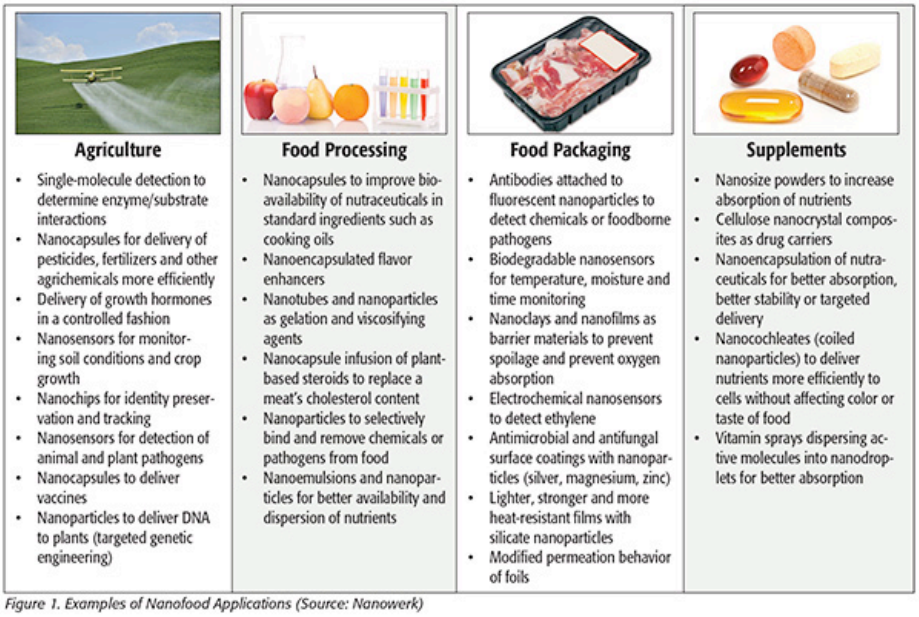

Auto Traffic
Palladium and Platinum NPs (catalytic converter)
Battery waste (electric cars)
Fine carbon particles (exhaust, tire wear)
Combustion
Soot/carbon black
Fly ash
Fullerenes/carbon nanotubes
Medicine
Silver NPs (antibacterials)
Gold NPs (therapeutics)
Mining
Fine metal and metal oxide particles
Energy Generation
Quantum dots (solar cells)
Metal oxides (solar cells, batteries)
Laundry
Silver NPs (antibacterial fabrics)
Metal oxide NPs (UV-proof fabrics)





Foods Containing Nanotitanium





Agriculture

- Single-molecule detection to determine enzyme/substrate interactions
- Nanocapsules for delivery of pesticides, fertilizers and other agrichemicals more efficiently
- Delivery of growth hormones in a controlled fashion
- Nanosensors for monitoring soil conditions and crop growth
- Nanochips for identity preservation and tracking
- Nanosensors for detection of animal and plant pathogens
- Nanocapsules to deliver vaccines
- Nanoparticles to deliver DNA to plants (targeted genetic engineering)

Food Processing

- Nanocapsules to improve bioavailability of nutraceuticals in standard ingredients such as cooking oils
- Nanoencapsulated flavor enhancers
- Nanotubes and nanoparticles as gelation and viscosifying agents
- Nanocapsule infusion of plant-based steroids to replace a meat's cholesterol content
- Nanoparticles to selectively bind and remove chemicals or pathogens from food
- Nanoemulsions and nanoparticles for better availability and dispersion of nutrients

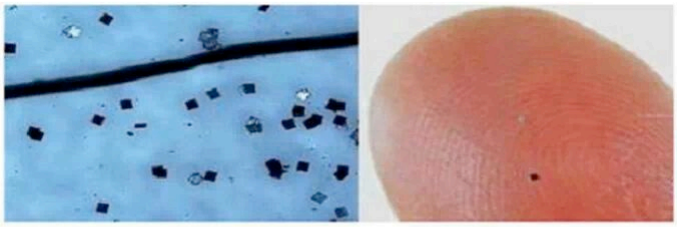
Food Packaging

- Antibodies attached to fluorescent nanoparticles to detect chemicals or foodborne pathogens
- Biodegradable nanosensors for temperature, moisture and time monitoring
- Nanoclays and nanofilms as barrier materials to prevent spoilage and prevent oxygen absorption
- Electrochemical nanosensors to detect ethylene
- Antimicrobial and antifungal surface coatings with nanoparticles (silver, magnesium, zinc)
- Lighter, stronger and more heat-resistant films with silicate nanoparticles
- Modified permeation behavior of foils

Supplements

- Nanosize powders to increase absorption of nutrients
- Cellulose nanocrystal composites as drug carriers
- Nanoencapsulation of nutraceuticals for better absorption, better stability or targeted delivery
- Nanocochleates (coiled nanoparticles) to deliver nutrients more efficiently to cells without affecting color or taste of food
- Vitamin sprays dispersing active molecules into nanodroplets for better absorption

Figure 1. Examples of Nanofood Applications (Source: Nanowerk)



"These are made by Hitachi. They measure only.15X.15 mm each and have GPS capabilities! Sometimes called 'smartdust' as they can be sprayed on us and absorbed or taken in foods,drinks and even injected."

ASSEMBLING OF NANOBOT WITH FULLERENE EXTENDING BIOFILM ENWRAPPING IT SELF WROUND THE NANO BOT

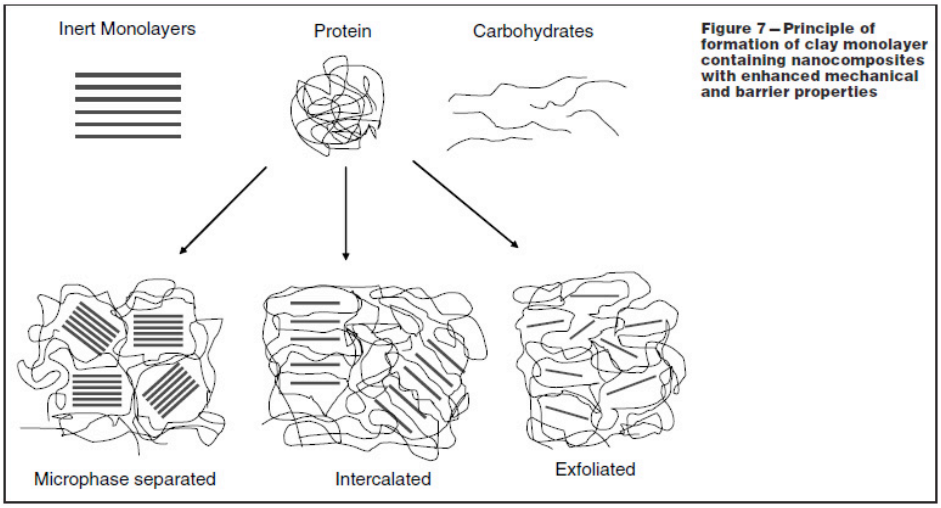


Figure 7 — Principle of formation of clay monolayer containing nanocomposites with enhanced mechanical and barrier properties

BIOMATERIALS MASS PRODUCING NANOMETALS	Engineered nanomaterials- exposures, hazards, and risk prevention	Neurotoxicity of nanoscale materials
Mechanisms of Nanoparticle-Induced Oxidative Stress and Toxicity	The nanosilica hazard- another variable entity	NanoPlastic WSU researchers develop shape-changing 'smart' material
Nano-enabled synthetic biology	Transformations of Nanomaterials in the Environment	Application of dental nanomaterials
NANO CHEMTRAILS	Realizing a nano-enabled synthetic biology	The key to mass-producing nanomaterials
Atmospheric Aerosols	DNA-NANO- cages 'can survive inside living cells'	
Nano Solutions	ANTI NANO PAIL	Health Effects of NANO- Aerosols

Viruses can be made to churn out high-tech nanomaterials

Viruses subvert their hosts to pump out masses of new viruses. In an unusual twist, an MIT researcher reports in the May 3 issue of Science **that she used genetically engineered viruses that are noninfectious to humans to mass produce tiny materials for next-generation optical, electronic and magnetic devices**.--"We've been looking at using genetic tools to grow semiconductor materials," said author Angela M. Belcher, associate professor of materials science and engineering and biological engineering. "**In this case, we took advantage of the viruses' genetic makeup and physical shape to not only grow the material but also to help them assemble themselves into liquid crystal structures that are several centimeters long.**"--Belcher and colleagues at the University of Texas at Austin are interested in using

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Comment [1]: Speculated Quotes From Einstein - These are at this point a presumption that he made these quotes but there is no clear validation that he did but none the less they are true and with this current alien tech that is being pursued on a global level this tech is far more dangerious then anything humanity has ever dealt with and this can not only consume the planet but literally re write everything that has any DNA

I fear the day that technology will surpass our human interaction. The world will have a generation of idiots.

I fear the day when the technology overlaps with our humanity. The world will only have a generation of idiots.

I fear the day when technology overlaps our humanity. It will be then that the world will have permanent ensuing generations of idiots.

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Comment [2]: We are no longer talking tech~ or Biology anymore what we are actually dealing with is Syntethic Life and now it is evolving through other university and research developments

the processes by which nature **makes materials to design new biological-electronic hybrid materials that could be used to assemble electronic materials at the nanoscale**. Her research brings together inorganic chemistry, materials chemistry, biochemistry, molecular biology and electrical engineering. She will join the MIT Department of Material Science and Engineering and the Biological Engineering Division of the School of Engineering in September.--Belcher's approach is to use systems such as viruses that evolved over millions of years to work perfectly at the nanoscale, but to convince the viruses to work on technologically important materials. **Belcher's research team can evolve the viruses to work on the materials of interest over a period of months.--**Building self-assembling and defect-free two- and three-dimensional materials on the nanometer scale is essential for the construction of new devices for optics and electronics. Researchers have been looking at ways to use organic materials to organize molecules of inorganic materials on the nanoscale. Fabricating viral films, Belcher said, may provide new pathways for organizing molecules to help create electronic, optical and magnetic materials.

"We showed that engineered viruses can recognize specific semiconductor surfaces, and these recognition properties can be used to organize molecules in inorganic nanocrystals, forming ordered arrays," she said. "In this system, we can easily modulate the length of the bacteriophage (the type of virus) and the type of inorganic materials through genetic modification and selection. One can easily modulate and align different types of inorganic nanocrystals in 3D layered structures."

This work is supported by the Army Research Office, the National Science Foundation and the Welch Foundation.

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Bio-inspired synthesis of metal nanomaterials and applications

Jiale Huang† [a], Liqin Lin† [b], Daohua Sun [a], Huimei Chen [c], Dapeng Yang *[b] and Qingbiao Li *[abc]
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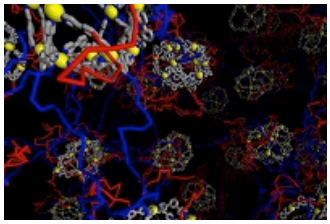
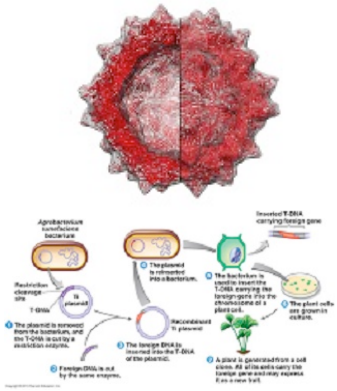
[b]College of Chemistry and Life Science, Quanzhou Normal University, Quanzhou, P. R. China. E-mail: yangdp@qztc.edu.cn
[c]Environmental Science Research Center, College of the Environment & Ecology, Xiamen University, Xiamen 361005, P. R. China

Received 10th February 2015

First published on the web 17th June 2015



This critical review focuses on recent advances in the bio-inspired synthesis of metal nanomaterials (MNMs) using microorganisms, viruses, plants, proteins and DNA molecules as well as their applications in various fields. Prospects in the design of bio-inspired MNMs for novel applications are also discussed.



1. Introduction

1.1 Definition of bio-inspired synthesis

A number of fascinating advances, technologies and possibilities have emerged in recent years in the burgeoning field of nanotechnology.[1–5] As building blocks, **nanomaterials (NMs)** can play a vital role in nanotechnology due to their remarkably different properties as compared to their bulk counterparts.[6] **Metal nanomaterials (MNMs)** including common **nanoparticles (NPs), nanoclusters (NCs**, <2 nm), **nanowires (NWs)** and related nanostructures have received tremendous attention owing to their **unique catalytic, electrical, magnetic and thermal properties**.[2,7] **"Top-down" and "bottom-up" approaches in nanotechnology[8] are also generally applicable to the fabrication of MNMs**. However, in contrast to "top-down" approaches, "bottom-up" protocols enable a comparatively flexible and inexpensive preparation, being consequently more intensively investigated in recent years.[8,9]

MNMs have long existed in natural environments. Some metal ions might be adsorbed and further reduced to elemental metals by

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Comment [3]: Terms of the nano tech Building Blocks-NM –NanoMaterials – NP –NanoParticles-MNM Metal NanoMaterials NW –NanoWires NC-NanoClusters

microorganisms, plants, biomass, etc. Inspired by the biological paradigm

Viruses can be made to churn out high-tech nanomaterials

Viruses subvert their hosts to pump out masses of new viruses. In an unusual twist, an MIT researcher reports in the May 3 issue of Science **that she used genetically engineered viruses that are noninfectious to humans to mass produce tiny materials for next-generation optical, electronic and magnetic devices**.--"We've been looking at using genetic tools to grow semiconductor materials," said author Angela M. Belcher, associate professor of materials science and engineering and biological engineering. "**In this case, we took advantage of the viruses' genetic makeup and physical shape to not only grow the material but also to help them assemble themselves into liquid crystal structures that are several centimeters long**."--Belcher and colleagues at the University of Texas at Austin are interested in using the processes by which nature **makes materials to design new biological-electronic hybrid materials that could be used to assemble electronic materials at the nanoscale**. Her research brings together inorganic chemistry, materials chemistry, biochemistry, molecular biology and electrical engineering. She will join the MIT Department of Material Science and Engineering and the Biological Engineering Division of the School of Engineering in September.--Belcher's approach is to use systems such as viruses that evolved over millions of years to work perfectly at the nanoscale, but to convince the viruses to work on technologically important materials. **Belcher's research team can evolve the viruses to work on the materials of interest over a period of months.--**Building self-assembling and defect-free two- and three-dimensional materials on the nanometer scale is essential for the construction of new devices for optics and electronics. Researchers have been looking at ways to use organic materials to organize molecules of inorganic materials on the nanoscale. Fabricating viral films, Belcher said, may provide new pathways for organizing molecules to help create electronic, optical and magnetic materials.

"We showed that engineered viruses can recognize specific semiconductor surfaces, and these recognition properties can be used to organize molecules in inorganic nanocrystals, forming ordered arrays," she said. "In this system, we can easily modulate the length of the bacteriophage (the type of virus) and the type of inorganic materials through genetic modification and selection. One can easily modulate and align different types of inorganic nanocrystals in 3D layered structures."

This work is supported by the Army Research Office, the National Science Foundation and the Welch Foundation.

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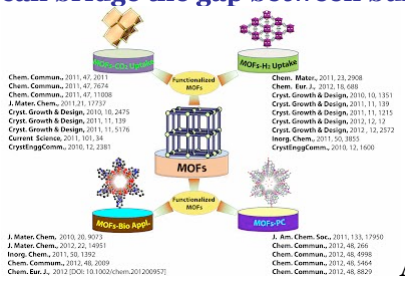
Bio-inspired synthesis of metal nanomaterials and applications

for the formation of MNMs, bio-inspired syntheses have emerged as innovative and alternatively attractive synthetic protocols for MNMs. Seminal reports on bio-inspired synthesis date back to the late 90s when **Ag and Au NPs were prepared from Pseudomonas stutzeri AG259[10] and alfalfa plant biomass,**[11] respectively. **Along with microorganisms and plants, viruses,**[12] **proteins**[13] **and DNA**[14] **have also become potentially useful candidates for bio-inspired synthesis of MNMs**  . Herein, based on these biological candidates, bio-inspired synthesis encompasses a combined application of biological concepts, mechanisms and functions for the design and development of innovative **bio-derived (nano)materials** with a number of applications.[15–18] **Such combined application could be applicable to the synthesis or assembly of a wide range of inorganic NMs.**[19–25]

1.2 Scope of the present review

Based on the scale of bio-inspired candidates, **bio-inspired syntheses can be divided into two different types, namely synthesis using (1) biomatrices with sizes ranging from nanometers and microns to macroscale, e.g., microorganisms, live plants and viruses** ((a) in Fig. 1) and (2) **water soluble DNA, proteins or those biomolecules secreted or extracted from microorganisms and plants** ((b) in Fig. 1). **Biomatrices can bridge the gap between bulk materials and MNMs**.



As-synthesized MNMs may not be immobilized onto some support prior to application. Such matrices can consequently play the same role as supports ((a) in Fig. 1). As far as the synthesis with those soluble biomolecules is concerned, the resulting MNMs may be immobilized onto some support for further applications ((b) in Fig. 1). Herein, broadly speaking, supports include not only common catalytic supports but also substrates able to support bio-inspired MNMs. **Fig. 1** Schematic representation of bio-inspired synthesis of MNMs **using microorganisms, viruses, plants, proteins and DNA molecules**. **(a) Reduction of metal ions with**

biomatrices; (b) reduction of metal ions with water-soluble biomolecules

The complex interaction between bio-inspired candidates, metal ions and MNMs has been the subject of intensive research efforts from chemical, biotechnological or chemical engineering communities in the past decade. The design of simple and useful bio-supported MNMs as well as interfacing bio-MNMs with diverse supports has attracted some attention for different applications in recent years. The present contribution has been aimed at providing an overview of recent progress related to the **bio-inspired synthesis of MNMs (mainly noble metals) from microorganisms, plants, microbial or plant biomass, viruses, proteins and DNA**. The chemistry of the bio-metal-ions-MNM interfaces as well as applied interfaces of bio-MNMs or bio-MNMs-support is critically reviewed in this work. Furthermore, prospects in the field of bio-inspired synthesis of MNMs have also been discussed. Recent overviews have touched upon these topics[26,27] but not limited to MNMs. Readers are also kindly referred to related reviews exclusively focused on the synthesis of NMs using plant extracts.[28–32]

2. General mechanism of bio-inspired synthesis of MNMs

The basic mechanism of bio-inspired synthesis of MNMs differs from that of bio-inspired candidates. Microorganisms are ubiquitously present on earth**. Live microorganisms host a significant array of metabolic reactions required for functions including nutrient processing, growth, and energy release**.[33] The metabolic process of live microorganisms might be involved in **the bioreduction of metal ions to reduce the toxicity of metal ions**. Through the transport system, microbes can intracellularly accumulate metals ions. **Metal ions can be reduced by various reducing species present inside microbes (i.e. on cell walls, etc.)[34] as well as extracellularly reduced by different metabolites.**[35,36] In contrast to live microorganisms, dead entities are not dependent on metabolic processes. **Metal ions are bound by microbial cells which then provide preferential nucleation sites for MNM growth on their surface**. **Various functional groups including thiol, hydroxyl, carboxyl, imidazole, amino, guanidine and imino groups have been demonstrated to have high affinity to bind metal ions.**[37] **Upon reduction of metal ions (with microbial cells themselves or auxiliary reductants), MNMs are formed through nucleation and surface growth. As-synthesized MNMs can be entrapped or confined by the surface, often exhibiting an excellent stability**. ---- **Similar to microorganisms, viruses usually provide binding sites for metal ions and nucleation sites for MNM formation** in the presence (and even absence) of additional reducing agents. Though there are many coating protein molecules in wild-type (WT) viruses, some active groups embedded inside the viral matrices are not accessible to metal ions.[12] Certain essential procedures should be therefore adopted to condition WT viruses prior to their use for the synthesis of MNMs (i.e. surface modification**) to**

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Comment [4]: thiol, hydroxyl, carboxyl, imidazole, amino, guanidine and imino groups have been demonstrated to have high affinity to bind metal ions.—these can also act as a chelating agent to remove from the body metal build up

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Comment [5]: In other words as the material become bound the MNM then forms as the virus is attached and starts to assemble or to cause the metals to adhere and join and go into a construct of what ever it is programmed to display or function

promote the metallization of viruses by increasing their low affinity for metal precursors.[12,39] The capsids of viruses can be modified by various strategies including charge and genetic modifications to provide uniform and precisely spaced binding sites for metal ions.[38] In some cases, auxiliary reductants are also required for the virus-templated synthesis of MNMs.

Inspired by the bioremediation of heavy metals by plants,[40] live plants have emerged as an alternative candidate in MNM biosynthesis.[41] However, the general mechanism for the bio-inspired synthesis of MNMs by live plants has not been fully understood.[41,42] Water-soluble biomolecules from microbial or plant biomass generally play dual roles as reducing and protecting agents in bio-inspired syntheses.[43–45] Functional groups (e.g. hydroxyl groups) play reductive roles in the formation of MNMs while the strong interaction between biomolecules and MNMs leads to an excellent stablity of as-synthesized MNMs.[44,45]

For the protein-templated synthesis of MNMs, there are a significant number of chemical functional groups (such as –COOH, –SH, $–NH_2$ and –OH) on proteins providing different sites to bind a variety of metal ions.[46] Upon addition of metal ions into an aqueous protein solution, the protein molecules are able to rapidly bind and entrap metal ions.[13] The interactions between metal ions and functional groups can lead to conformational changes of proteins in aqueous solution. As a result, the hydrophobic residues of proteins can be exposed to outside aqueous phases which, due to changes in solution conditions (i.e. temperature, pH as well as the introduction of reducing agents), favour the transformation of entrapped metal into MNMs. Furthermore, a self-assembly process often occurs under the influence of hydrophobic interactions and multiple nanoscale forces.[47] The structure and morphology of resulting MNMs is consequently dependent on protein/metal ions ratio, temperature, amount of reducing agents, etc.[48] In addition, denatured protein molecules are also responsible for directing crystal growth and subsequent capping of the resulting MNMs.[49]--DNA has unique advantages in the formation of ordered nanostructures and nano-assemblies. The building-up process can be divided into two strategies, namely (1) in situ reduction and growth and (2) electrostatic interaction or molecular hybridization. The first strategy generally entails a two-step metal precursor binding to the DNA template followed by in situ reduction into MNMs with DNA directing NP growth. NCs, spherical NPs and one-dimensional (1D) MNMs can be prepared using this strategy. Comparatively, DNA is used to direct the assembly of pre-synthesized NPs into desired nanostructures through electrostatic interaction or molecular hybridization in the second approach. The assembly can be tuned by varying the number of DNA strands on each metal NP, the base sequence as well as the length and

Owner 4/17/2017 10:45 AM
Comment [6]: Does this sound more like a weaponizing construct interacting a metal deliverys system to spread a contagion

Owner 4/17/2017 10:45 AM
Comment [7]: Bioremediation is a waste management technique that involves the use of organisms to remove or neutralize pollutants from a contaminated site

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Comment [8]:
Alanine ala a CH3-CH(NH2)-COOH

Arginine arg r HN=C(NH2)-NH-(CH2)3-CH(NH2)-COOH

Asparagine asn n H2N-CO-CH2-CH(NH2)-COOH

Aspartic acid asp d HOOC-CH2-CH(NH2)-COOH

Cysteine cys c HS-CH2-CH(NH2)-COOH

Glutamine gln q H2N-CO-(CH2)2-CH(NH2)-COOH

Glutamic acid glu e HOOC-(CH2)2-CH(NH2)-COOH

Glycine gly g NH2-CH2-COOH

Histidine his h NH-CH=N-CH=C-CH2-CH(NH2)-COOH
... |_____|

Isoleucine ile i CH3-CH2-CH(CH3)-CH(NH2)-COOH

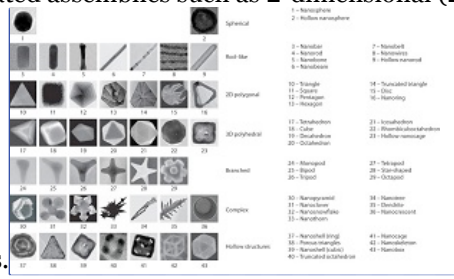
Leucine leu l (CH3)2-CH-CH2-CH(NH2)-COOH

Lysine lys k H2N-(CH2)4-CH(NH2)-COOH
... [1]

Owner 4/17/2017 10:45 AM
Comment [9]: meaning no water or little water

Owner 4/17/2017 10:45 AM
Comment [10]: • Denaturation, in biology, process modifying the molecular structure of a protein. Denaturation involves the breaking of many of the weak linkages, or bonds (*e.g.,* hydrogen bonds), within a protein molecule that are responsible for the highly ordered structure of the protein in its natural (native) state
... [2]

structure of DNA. This strategy can be implemented in the synthesis of various assemblies including dimers, trimers and polymeric structures. Modern DNA self-assembly techniques including DNA-tile or DNA-origami approach can facilitate the design of more complicated assemblies such as 2-dimensional (2D) or 3-dimensional (3D) nanostuctures.



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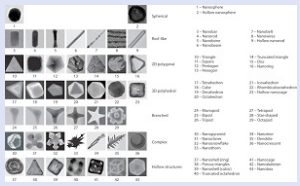
Comment [11]:



Plasmonic nanoparticles can be categorized based on geometrical parameters. Rows one to five contain spherical shapes[12], rod-like shapes[13, 14, 15, 16, 17, 18, 19], 2D polygons[20, 21, 22, 23, 24, 25], 3D polyhedrons[26, 27, 28, 29, 30] and branched shapes[31, 32, 33]. From left to right in each row, particles become geometrically higher-ordered in terms of aspect ratios, number of sides and facets, or number of branches. The last particle in each row has a hollow structure. Row six contains nanoparticles of various complexities[32, 34, 35, 36, 37, 38]. Row seven contains various other hollow polygonal and polyhedral nanoparticles[7, 30, 39, 40, 41, 42, 43]. Some images have been cropped, rotated, recoloured and/or had their backgrounds filled in; see the original papers for scale bars and other information. Figure reproduced with permission from: 2–9, 13–16, 19, 23–27, 29, 31, 35–37, 39, 40, refs 12, 13, 14, 15, 16, 17, 18, 19, 23, 23, 24, 25, 28, 30, 31, 31, 31, 31, 33, 35, 38, 34, 39, 30, 41 respectively, © 2006, 2007, 2008, 2009, 2006, 2008, 2008, 2006, 2005, 2005, 2005, 2004, 2008, 2002, 2003, 2003, 2003, 2003, 2009, 2010, 2008, 2004, 2008, 2002, 2006 respectively ACS; 10, 43, refs 20, 43 respectively © 2001, 2002 respectively AAAS; 11, 22, 34, refs 21, 29, 37 respectively, © 2005, 2010, 2007 respectively RSC; 12, 17, 21, 28, 30, 33, 34, 42, refs 22, 26, 26, 3 ... [3]

3. Chemistry of bio-inspired synthesis for MNMs

3.1 Microorganism-mediated synthesis

Microorganisms can be essentially classified as prokaryotic and eukaryotic. Bacteria and fungi represent prokaryotic and eukaryotic microorganisms as main bio-inspired candidates for the synthesis of MNMs. Live microorganisms not only exhibit complicated biological activity but also possess a **complicated hierarchical structure**. An increasing number of insights into metal resistance in the bioreduction process mediated by microorganisms have been recently achieved but have not been well addressed in currently available reviews. **Metal NPs can be found in the periplasmic space, on the cell wall and outside the cells**. It is believed that various enzymes take an active part in the **bioreduction process of transporting electrons from certain electron donors to metal electron acceptors for different microorganisms,.**[50,51] The roles of enzymes as well as MNM formation have been extensively studied in recent years. -- Dead entities are comparably not dependent on metabolic processes with respect to living microorganisms. As previously mentioned, **the surface structure in non-enzymatic alternatives provided an excellent biotemplating medium to grow MNMs**. **Cell-free extracts from microorganisms have also been utilized to prepare MNMs. Without microbial cells, the extracts are more flexible for shape/size control, downstream processing of MNMs and design of bio-microreactors.** The synthesis of MNMs through enzymatic and non-enzymatic reduction corresponding to living and dead microorganisms, respectively, as well as cell-free extracts from microorganisms will be critically reviewed in the next sections.

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Comment [12]: **Here we are utilizing bacteria and fungi ti intiate the building or assembling of nanostructures or nanocomponents or nanofoundations to construct and operate as the program dictates and the bacteria or DNA material can be utilized by any host**

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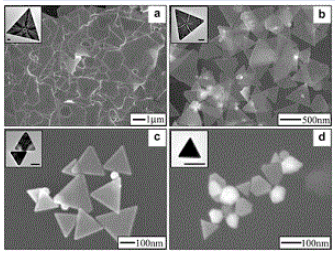
Comment [13]: **This is basically saying the materials whether dead or alive are usable or recyclable~ in other words it will use anything or integrate with anything**

3.1.1 Enzymatic reduction.

3.1.1.1 Metal resistance. As noble metal ions are toxic to microorganisms, metal resistance in the microbial reduction of these metal ions should be considered. **Both Ag(I) ions and Ag NPs are well known toxic elements to live**

microorganisms. However, Klaus et al.[10] employed Pseudomonas stutzeri AG259 in the preparation of Ag NPs. Ag-resistance can involve the formation and accumulation of Ag precipitates outside the cytoplasmic membrane, possibly accompanied by metal efflux and metal binding.[10] Mukherjee et al.[52] later reported the use of eukaryotic microorganisms Verticillium fungus (Verticillium sp.) in the intracellular synthesis of metal NPs. They showed that the toxic effects of Ag(I) could be minimized by reducing Ag(I) ions to elemental Ag NPs within the cells, which were alive and could further reproduce after reduction.[52] Ag NPs were also prepared via $AgNO_3$ reduction with Lactobacillus strains.[34] While the exact Ag-resistant mechanism was not elucidated in this work, further studies related to the extracellular formation mechanism of Ag NPs by Ag-resistant Morganella sp. demonstrated by three homologous genes silE, silP and silS were closely associated with Ag-resistance.[35] Several Ag-specific proteins were found to be secreted outside the cell during the growth and were suggested as being responsible for the reduction of Ag(I) ions and formation of Ag NPs in the extracellular microenvironment.[35] Interestingly, Ramanathan et al.[53] further proved that a kinetically controllable growth of Ag-resistant Morganella psychrotolerans could allow the fine-tuning of anisotropic Ag NPs. The silE protein-based Ag-binding machinery of bacteria was activated due to the exposure to Ag(I) ions, leading to the cellular uptake of Ag(I) ions.[53] Very recently, Lin et al.[54] also investigated the biosynthesis of Ag NPs upon reduction of Ag(I) ions by the periplasmic nitrate reductase c-type cytochrome subunit NapC in a Ag-resistant Escherichia coli (E. coli) under anaerobic conditions. Results showed that c-type cytochromes such as NapC located in the periplasm could reduce Ag(I) ions to Ag NPs (Fig. 2).[54]



A proposed model of biosynthesis of nano-Ag by periplasmic c-type cytochrome NapC in the Ag-resistant E. coli strain 116AR. Reprinted with permission from ref. 54. Copyright © 2014, Royal Society of Chemistry.

Compared to Ag(I) ions, Au(III) ions are less toxic to microorganisms. For some microorganisms, Au(III) ions can be reduced to obtain Au NPs. Das et al.[55] demonstrated a comprehensive study on the characterization of bioreduction process of Au(III) ions in the fungus Rhizopus oryzae (R. oryzae) to form Au NPs. Growth of R. oryzae in the presence of sublethal Au(III) concentrations (130 μM) induced

Owner 4/17/2017 10:45 AM
Comment [14]: NANOSILVER being used in the construction through the usage of a Pseudomonas stutzeri a bacterium found in plants and soils and this binds with the silver and uses it to grow the MNM

Owner 4/17/2017 10:45 AM
Comment [15]: So with this you have now a mutation of bacterium that will not get impacted but will essentially morph with the silver and grow whatever the program decides

Owner 4/17/2017 10:45 AM
Comment [16]: Basically this can now incorporate the silver NP into it reducing or neutralizing the impact of the silver and now integrate ~ this now is showing clearly how NanoSilver can be Negated and made into an ally and becomes integrated so any addition with the nanosilver will have no effect and in fact maybe used by this construct to further morph or build

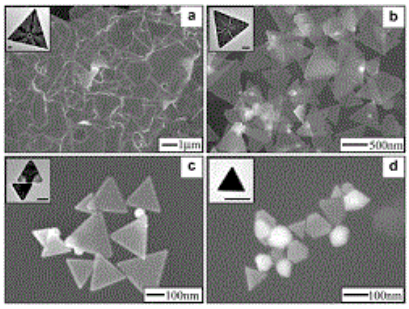
stress response proteins which were correlated with the biosynthesis of Au NPs.[55] At Au(III) concentrations of >250 ☐M, **the cellular ultrastructure was damaged due to Au toxicity and the biosynthesis of Au NPs was drastically suppressed**. Au-specific genetic responses were further identified as two cytoplasmic proteins of ☐45 and ☐42 kDa likely responsible for the biosynthesis of Au NPs at Au(III) concentrations of <130 ☐M. An ☐80 kDa protein was comparatively proposed to serve as a capping agent for Au NPs and stabilized Au NP bioconjugates **through electrostatic repulsion**.[55]--- Very recently, Rösken et al.[56] examined the time-dependent growth of in vivo Au NPs in cyanobacteria Anabaena sp., when exposed to Au(III) ions at 0.8 mM. **All microorganisms seemed to be dead after 8 days due to the incorporation of Au NPs**. However, in some cases, **Au(III) ions could not be completely reduced by microorganisms.** Instead, the complete reduction of Au(III) ions required the input of electron donors as demonstrated in the rapid preparation of Au NPs by Shewanella algae (S. algae) ATCC 51181 using H_2 as electron donor (Au NPs formed in the periplasmic space).[57,58] The microbial reduction of **Pd(II)** and Pt(IV) ions was reported to be conducted in the presence of an electron donor[59–62] as in the recent reduction of Pd(II) ions by Desulfovibrio desulfuriacans (D. desulfuriacans) NCIMB 8307 in the presence of formate or hydrogen as the electron donor.[59] Pd **NPs were unable to be generated by bacteria without the addition of an electron donor**. Similarly, Pt NPs could be prepared using D. desulfuricans **NCIMB 8307 and H_2 as electron donors.**[60] **Using sodium lactate as an electron donor, Pt NPs could also be prepared from S. algae ATCC 51181**[61] **and E. coli MC4100**[62] **(using H_2 as the electron donor).** Recently, Yates et al.[63] demonstrated that Pd NPs primarily formed outside the Geobacter sulfurreducens cells could reduce the toxicity of metal ions and allow the recovery of Pd NPs without cell destruction. However, the resistance of the related microorganisms against Pd(II) and Pt(IV) ions was little discussed; therefore it is unclear whether Ag or Au resistance is applicable to that of Pd(II) and Pt(IV) ions. As far as the resistance against other metals is concerned, a few reports are available for the microbial reduction. **A recent demonstration pointed to the synthesis of stable Cu NPs by Ag-resistant Morganella morganii (M. morganii) RP42.**[64] The authors claimed that Cu(II) ions were uptaken by M. morganii using the same/similar proteins involved in Ag(I) ion uptake from previous studies.[53] **The resulting Cu NPs were then released into the media using the bacterial efflux system.**[64]--- 3.1.1.2 Identification of enzymes and formation of MNMs. Besides the metal resistance summarized above, **enzymes play vital roles in the microbial reduction of metal ions**. Although some pioneering studies were conducted,[10,34,52,65] the identification of enzymes involved and understanding of their roles in microbial reduction remains a significant challenge. Table 1 summarizes some examples of enzymes involved in the microbial reduction of metal ions.

Table 1 Examples of enzymes involved in the microbial reduction of metal ions

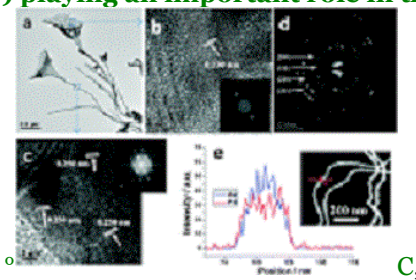
Owner 4/17/2017 10:45 AM
Comment [17]: Here again a gold particle in a fungus can assist in the fungal growthand forms a gold NP in a presence of a gold particle in a micon size so this is another example of some of these particulates can actually increase the volume of the component and in some cases increase lethality or toxicity—this would appear to be a gold salt (possibly a citrate of gold)

Owner 4/17/2017 10:45 AM
Comment [18]: Palladium(II)

Microorganisms_MNMs		Size		Enzymes__a Not mentioned in the corresponding reference.
__Bacteria_				
D. desulfuriacans ATCC 29577[67]_Pd NPs		—[a]		Hydrogenase and cytochrome C3__M. psychrotolerans [53] _Ag nanoplates_Edge length of 100–150 nm_Ag reductase__S. maltophilia [68] _Ag NPs_□93 nm_Chromium reductase__Shewanella oneidensis [69] _Ag NPs_24.4 ± 0.8 nm_c-Type cytochromes__E. coli [54] _Ag NPs_5–70 nm, average size: 26.9 nm_Nitrate reductase_____Fungi_	
F. oxysporum [70] _Ag NPs	20–40 nm	NADH-dependent reductases	_R. oryzae [55]	Au NPs	□15 nm_Cytoplasmic proteins__L. edodes [71] _Au NPs_5–50 nm_Laccase, tyrosinase, and Mn-peroxidase__F. oxysporum [72] _Ag NPs_10–20 nm_Nitrate reductase_
Thermomonospora sp.[73]	Au NPs	2–6 nm, average size: 3 nm	**Sulfite reductase**		
F. oxysporum IRAN 31C[74]	Ag NPs	Average size: 50 nm	**Nitrate reductase**		



Intracellular synthesis of MNMs was generally achieved using bacteria. Nair et al.[34] prepared Au NPs through reduction of $HAuCl_4$ with Lactobacillus strains, in one of the earliest reports on microbial synthesis. **Au(III) ions were believed to be reduced by sugars and enzymes on cell walls.**[34] However, no experimental evidence was provided. Ahmad et al.[66] subsequently demonstrated the use of single-spore bacteria (Thermomonospora sp.) for the reduction of Au(III) ions to Au NPs at 50 °C. **The results pointed to proteins (molecular weight 80–10000) playing an important role in the reduction of Au(III) ions at 50 °C**, also favorable for the survival of the thermophilic bacteria.[66] The reduction of Pd(II) ions to produce Pd NPs with D. desulfuriacans ATCC 29577 was conducted in the presence of sodium pyruvate, formate or H_2 as an electron donor.[67] Hydrogenase and cytochrome C3 were claimed to be potentially involved in the reduction of Pd(II) ions.[67]

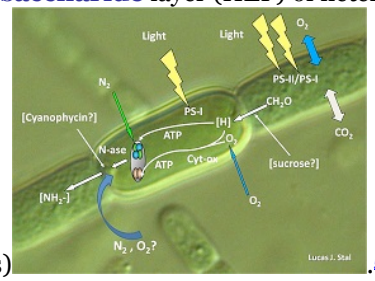


Intracellular synthesis of metal NPs using fungi was first demonstrated by Mukherjee et al.[52,65] **A comprehensive study was conducted on the intracellular biomineralization mechanism of Au by zygomycete fungi R. oryzae.**[55] The authors showed that **the majority of Au(III) was transported into the cytoplasmic region where reduction to Au NPs took place facilitated by cytoplasmic proteins (i.e. metal reductases). The accumulation of spherical Au NPs inside the mycelial cells of L. edodes was also recently reported.**[71] The results of enzyme assays showed that the intracellular phenol-oxidizing enzymes (laccases, tyrosinases, and Mn-peroxidases) were involved in Au(III) reduction **to give electrostatically stabilized colloidal solutions.**[71] Extracellular synthesis of metal NPs using

Owner 4/17/2017 10:45 AM
Comment [19]: Gold ions reduced by the sugar and enzymes on clee walls

fungi were also first reported by Mukherjee et al.[70] for the particular example of Au NPs which could be extracellularly synthesized by Fusarium oxysporum (F. oxysporum). **Results also showed that F. oxysporum was able to release a large number of coenzyme (NADH)-based proteins to reduce Au(III)**.[70] Reductases were a class of characteristic enzymes of F. oxysporum, while intracellular or extracellular reduction of Au(III) could not be achieved by other strains such as F. moniliforme. **Protein-bound Au NPs through the amino groups from cysteinyl acid residues and lysine residues provided Au NPs with long-term stability**. **These studies proved that the use of fungi has the added advantages of simplicity in processing and handling of biomass for extracellular synthesis**.[70] Based on the same reduction mechanism, extracellular biosynthesis of Ag,[75] Au–Ag[76] and other NPs with F. oxysporum was further investigated at the National Chemical Laboratory of India. **In recent years, the synthesis of MNMs using cell-free extracts from microorganisms also confirmed the reductive ability of some enzymes, which will be separately discussed**.

Studies of MNM formation and size control clearly demonstrated that cell walls of microorganisms usually provide binding sites for metal ions and preferential nucleation sites for the synthesis of MNMs in the presence of enzymes. In the intracellular synthesis of metal NPs, **electrostatic interactions between Au(III) or Ag(I) ions and the charged groups (such as lysine residues) of enzymes within the cell wall of fungus Verticillium sp. can lead to entrapping of metal ions on the cell surface, where enzymes facilitated the reduction of metal ions to NPs**.[52,65] Similarly, Au(III) ions initially bound by the cell surface of R. oryzae could be reduced to intermediate Au(I)–protein complexes and eventually to Au NPs.[55] **Bacterial cells were able to provide enzymes as reducing agents (with cells as nucleation sites to favor crystal growth)** in the reduction of Pd(II) ions by D. desulfuricans NCIMB 8307 using formate or hydrogen as electron donors.[59] Very recently, a time-dependent study on Au NP growth using X-ray powder diffraction (XRD) and transmission electron microscopy (TEM) pointed out that the formation of Au NPs started at the **heterocyst polysaccharide** layer (HEP) of heterocysts



(HCs).[56] **At longer times, the vegetative cells (VCs) were the most important area of synthesis**.[56] Even after one day,

Owner 4/17/2017 10:45 AM
Comment [20]: NADH = Nicotamide orrr Niacinamide or Niacin suggesting that B3 maybe able to reduce the gold salts (citrate for of this)

Owner 4/17/2017 10:45 AM
Comment [21]: This is what so horrific about this technology –it is unregulated and with these different species of fungi or vacterium or other plant life they can not only construct the nanotech but can then convert the metals to nano size –complete hijacking of humanity

Owner 4/17/2017 10:45 AM
Comment [22]:
The following sequences take place in formation of heterocysts from a vegetative cell:
•The cell enlarges.
•Granular inclusions decrease.
•Photosynthetic lammele reorients.
•The wall finally becomes triple-layered. These three layers develop outside the cell's outer layer.
○The middle layer is homogeneous.
○The inner layer is laminated.
•The senescent heterocyst undergoes vacuolation and finally breaks off from the filament causing fragmentation. These fragments are called hormogonia and undergo asexual reproduction

the number of Au NPs inside the HCs as well as inside HEP was inferior as compared to that in VCs.[56]

Microorganisms used for the extracellular synthesis of metal NPs need to be extensively screened.[77] **Metal NPs were in most cases simultaneously produced intracellularly and extracellularly, with the associated challenges in controlling their particle size**. Furthermore, microorganisms possess a hierarchical cell structure, which is essentially detrimental for the production of metal NPs with a narrow particle size distribution. Adjusting the synthetic conditions can potentially facilitate particle size control in MNM synthesis. **As an example, the formation rate of intracellular Au NPs using two types of fungi (V. luteoalbum and Isolate 6-3)** could be influenced by changes in pH value, reaction temperature, Au(III) concentration and reduction reaction time, affecting the size of Au NPs.[78,79] A genetic approach to size control was recently proposed by Ng et al.,[69] comparing the **particle size of the extracellular NPs from WT Shewanella oneidensis and its mutant**. Results showed that the mutant lacking outer membrane c-type cytochromes produced significantly smaller NPs with respect to WT.[69]

3.1.2 Non-enzymatic reduction. **The non-enzymatic reduction of metal ions to NPs based on dead cells** has also received some attention in recent years. This approach is independent on the metabolic process of microorganisms[80–83] and consequently different from enzymatic reduction. **The protocol essentially entails a simple adsorption and reduction of metal ions on cell surfaces, which result in completely extracellular metal NPs**. **Reported examples include the formation of Au NPs using Shewanella oneidensis through a fast biosorption but slow reduction process in the presence of an electron donor.[84] The reduction was claimed to be non-enzymatic as microorganism cells were killed at high Au precursor concentrations.**[84] A similar study could yield Au NPs using dried yeast Pichia pastoris (P. pastoris) in the absence of electron donors.[37] **The cell surface also exhibited high affinity for Au(III) species in aqueous solution. Au(III) ions were rapidly absorbed and slowly reduced to Au(0) by $-NH_2$, $-OH$ and other functional groups on the surface**. **As a result, as-synthesized Au NPs were tightly bound to the cell surface**. In a separate study, the adsorption and reduction of Pd(II) ions by P. pastoris cells was also investigated.[85] **Analogously to Au(III) ions, biosorption of Pd(II) ions was also rapid.** However, Pd(II) reduction was comparatively very slow and incomplete, in good agreement with previous reports for the reduction of **Pt(IV) ions** with Bacillus megatherium D01 biomass.[86] The adsorption ability of the cells for Pd(II) ions was greatly enhanced

Owner 4/17/2017 10:45 AM
Comment [23]: Doesn't that look like Lyme!!!?? Maybe some Misdiagnostics happening with about 99/100 people

Owner 4/17/2017 10:45 AM
Comment [24]: This is essentially saying that once the NP kills off the pathogen that the left over material then increases through usage of what is left and further spreads throughout or increases

Owner 4/17/2017 10:45 AM
Comment [25]: Pt(IV) ions= Palladium 4

after pretreatment with aqueous HCl, aqueous NaOH and methylation of amino groups. Nevertheless, even slower reduction rates were observed by pretreated P. pastoris cells as compared to untreated cells for the reduction of the Pd(II) ions.[85]

Such observed slow reduction rates have also been one of the most important issues in the synthesis of Ag NPs. In this regard, various methodologies have been proposed to accelerate the reduction of Ag(I) ions (i.e. adjusting the pH of the reaction solution).[87] Wang et al.[88] synthesized stable Ag NPs with narrow size distribution using dried **Aeromonas sp. SH10 cells in the presence of hydroxyl ions. $[Ag(NH_3)_2]^+$ ions first reacted with OH^- to form Ag_2O species, and subsequently non-enzymatically reduced by the cells to Ag NPs on the surface of the Ag_2O particles**. Sun et al.[89] demonstrated that Ag NPs prepared by the bioreduction method exhibited a superior thermal and chemical stability as compared to those synthesized via sodium citrate reduction. Results indicated that Ag NPs remained stable even after heat treatment at 100 °C for 6 h. **The stability of the Ag NPs was significantly influenced by hydrogen ions and the electrolyte with multivalent cations, while it was little affected by the presence of hydroxide anion**s.[89]

Owner 4/17/2017 10:45 AM
Comment [26]: Gold NP gets affected by hydrogen -

The biosorption and bioreduction of metal ions has been extensively studied by Lin et al.[82,86,90,91] at Xiamen University, China**. S. cerevisiae was demonstrated to have a remarkable affinity for Au(III) ions due to the presence of oxygen-containing functional groups (hydroxyl and carboxylate ion groups) on the cell wall.[90] Reduction of Au(III) ions to zero-valent Au was mainly affected by the free aldehyde group of the reducing sugars**.[90] Using infrared spectrometry studies, polypeptides were proposed to be potentially activated by the intervention of Au(III) ions via the molecular reconformation and profoundly affected the course of Au(0) nucleation and crystal growth.[91] **In the case of Pt(IV) ions bound by proteins on cell walls, polypeptide chains might change from β-folded to α-helical forms, with α-helical being potentially more advantageous than β-folded to prevent Pt NPs from aggregation.[86] The secondary constructions of proteins (e.g. α-helical, β-folded, etc.) and pores of the net-like structural polysaccharides on peptidoglycan layers of cell walls may play important roles in stabilizing Pt NPs and promoting the formation of uniform particles.**[86]

As a matter of fact, microbial biomass has been used to remove heavy metal ions from wastewater for a long time. The biosorption process is not simply the adsorption of metal ions onto the microbial biomass and may be accompanied by the reduction of metal ions.[50] For example, an interesting demonstration was reported by Salvadori et al.,[92] **who showed that the dead biomass of Rhodotorula mucilaginosa may be considered to be a fast and low-cost bioprocess for the formation of Cu NPs in which the biomass acted as a nano-adsorbent of Cu(II) ions in wastewater**.

3.1.3 Reduction with cell-free extracts from microorganisms. Besides microorganisms, **cell-free extracts from microorganisms have been used in the synthesis of MNMs**. **The use of cell-free extracts confers the advantage of flexible shape control of MNMs**. For example, He et al.[93] used the cell-free extract of Rhodobacter capsulatus for the preparation of Au NWs. **At higher Au(III) concentration conditions, quasi-spherical Au NPs easily cascaded to form curly Au NWs with wide diameter and length distributions.**[93] Wadhwani et al.[94] showed that polyhedral Au NPs could be produced by mixing cell suspensions of Acinetobacter sp. SW 30 with $HAuCl_4$ solutions. Lee et al.[95] **reported the use of recombinant E. coli cell extracts, a hydrogel polymer and a microdroplet based microfluidic device to fabricate a mass amount of artificial cellular bioreactors with uniform sizes and shapes as reactors to synthesize diverse metal NPs** (Fig. 3). The hydrogels were able to protect the encapsulated cell extracts from the surrounding environment and maintained the functionality of cellular components in cellular bioreactor applications.

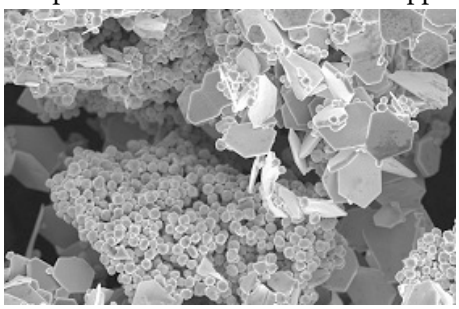


Fig. 3 Schematic representation of the microdroplet-generation model using a microfluidic device. (a) Microdroplets are produced with the mixture of cell extracts and NIPAM monomers in a microfluidic device. (b) Polymerized NIPAM monomers serve as an artificial membrane. (c) Different types of precursor solutions are dispersed in the artificial cellular bioreactors. (d) The precursors are transferred into the cells,

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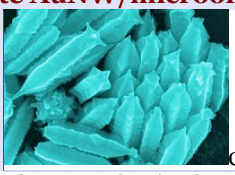
Comment [27]: This why some of the afflicted feel the crawley creepy of because of to much exposure and may see different shapes and images which would be all in actuality nothing more then the shaping of the tech with the specific bacterium or fungi or mold

and NPs are subsequently formed in the artificial cellular bioreactors. Reprinted with permission from ref. 95. Copyright © 2014, American Chemical Society.

_The identification of **active biomolecules and MNMs formation** understanding under reduction with cell-free extracts is comparatively simpler to those of **processes in the presence of microbial cells**. Velmurugan et al.[96] studied the use of **nitrate-reducing Bacillus subtilis EWP-46 cell-free extracts for the preparation of Ag NPs**. **Nitrate reductase enzymes with a molecular weight of 43 kDa might be responsible for the formation of Ag NPs**. Talekar et al.[72] demonstrated that NADH-dependent nitrate reductases from F. oxysporum cell extracts were directly immobilized as cross-linked enzyme aggregates (CLEAs) and investigated for the synthesis of Ag NPs from silver nitrate.

3.1.4 **Microorganism-mediated surfactant-directed synthesis**. Microbial reduction emerged as a novel and viable alternative to chemical and physical methods for synthesis of metal NPs in recent years. **Generally, metal ions was adsorbed and reduced by the microbial surface, resulting in very small NPs that gradually grew over the microorganisms**. However, the shape of MNMs cannot be effectively controlled under microbial reduction. Inspired by the seed-mediated, surfactant-directed synthesis of MNMs,[97] Wang et al.[98,99] **developed a microorganism-mediated, surfactant-directed (MSD) synthesis to fabricate AuNW/microorganism and Au-nanohorn/microorganism**  composites using P. pastoris cells. Such nanohorns are difficult to synthesize by pure chemical reduction. Yang et al.[100] expanded the **MSD approach to synthesize hierarchically branched AuNWs by using E. coli cells**. Au nanohorns could be also synthesized using the MSD approach with E. coli cells and cetyltrimethylammonium chloride (CTAC).[101]

The interesting phenomenon of microbially induced aggregation of Au nanostructures around E. coli cells in the presence of cetyltrimethylammonium bromide (CTAB) and ascorbic acid (AA) was also observed**. A microorganism-mediated CTAB-directed approach could be used to rapidly recover Au from aqueous solutions**.[102] The work provided a new concept in which Au recovery could be strengthened through engineering Au nanostructures, thereby

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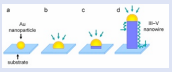
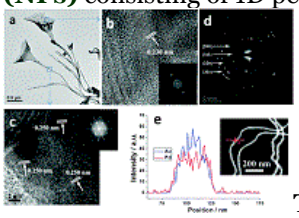
Comment [29]: AUNW= gold nanowire And they are making these with different micro organisms 

opening a new avenue to enhance the efficiency of recovery of precious metals.[102] Huang et al.[103] further investigated the effect of surfactants on recovery rate and morphology of recovered Au. Results showed that the carbon chain length had a minimum effect on the recovery rate and morphology in the presence of Br^{-}. In contrast, the recovery rate of Au was much lower in the presence of Cl^{-} due to the formation of horn-like nanostructures. Interestingly, the recovered Au together with cell residues could be used as a surface-enhanced Raman scattering (SERS) substrate.[103]

As far as the role of microorganisms in the MSD approach is concerned, the microbial surface was demonstrated to provide biosorption of Au ions and preferential nucleation sites for Au nuclei.[98–100,102] CTA^{+} was selectively adsorbed onto specific crystal facets to enable preferential growth of other facets.[98,100] **The resulting anisotropic particles tended to connect together to form AuNWs due to the shape-directing effect of Br^{-}.**[98,100,104] In the case of CTAC, further anisotropic growth of the branch-like nanostructures led to film-like nanostructures part of the growing AuNHs.[99,101]

Binary metal ions can be simultaneously adsorbed by microorganisms, which may provide nuclei for the growth of bimetallic nanostructures.[105] Very recently, Chen et al.[106] described a MSD proof of concept approach to synthesize novel **AuPd bimetallic nanoflowers (NFs)** consisting of 1D pedicels and 3D horns in the presence of CTAC (Fig. 4).



The authors justified the advantage of the MSD approach to obtain bimetallic nanostructures in one pot protocol at room temperature. Furthermore, the problem of metal leaching that occurs in the galvanic replacement reaction for synthesizing bimetallic nanostructures can be circumvented. **In addition, the bimetallic nanostructures could form application-oriented nano-composites with microorganisms which, for example, could be directly used as active catalysts** in the selective hydrogenation of 1,3-butadiene. Results also showed that all obtained materials were alloys with Pd-enriched surfaces. The diameters of horns increased while those of pedicels decreased with the increase of Pd precursor feeding concentration.[106] **The presence of the Pd precursor was vital for the formation of the nanowire part of the NF structure**. Such NFs have not been reported, partially due to synthetic difficulties under pure chemical reduction methods. Therefore, the work opened up a new avenue for the shape control of novel bimetallic NMs.

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Comment [30]: NANO FLOWER a protein encased in copper phosphate "petals." As well as looking pretty, these petals perform two important functions. First, they stabilize the protein to prevent it from breaking down. Secondly, if the protein has catalytic properties—that is, if it speeds up other chemical reactions—encasing it in a nanoflower makes it a more effective catalyst. Nanoflower catalysts therefore work better and last longer than the bare proteins

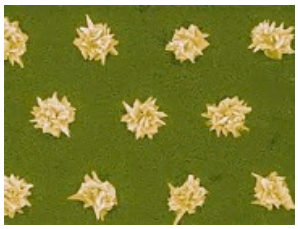

Fig. 4 (a) TEM image of AuPd NFs, (b) HRTEM image of blossom, (c) HRTEM image of pedicel, (d) SAED pattern of AuPd NFs, and (e) EDX line profiles of an individual AuPd nanowire **(pedicel)** corresponding to the framed part (dash) in (a). The insets indicate the corresponding fast Fourier transform (FFT) pattern. Reprinted with permission from ref. 106. Copyright © 2014, Royal Society of Chemistry.

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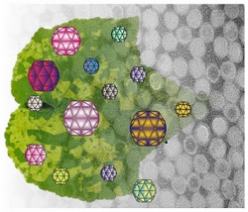
Comment [31]: A pedicel is a stem that attaches a single flower to the inflorescence.[1] In the absence of a pedicel, the flowers are described as sessile. Pedicel is also applied to the stem of the infructescence.

_3.2 Virus-templated synthesis

A variety of viruses have emerged as promising candidates in the bio-inspired synthesis of MNMs in the past decade. Some viruses have the advantages of unique dimensions and structures, well-spaced functionalities, high chemical stability and high yields, which stimulated research interest in the synthesis of virus-templated MNMs. Structurally, most viruses consist of two parts:[107] **(i) the genetic material from either deoxyribonucleic acid (DNA) or ribonucleic acid (RNA); (ii) the capsid comprising of a**

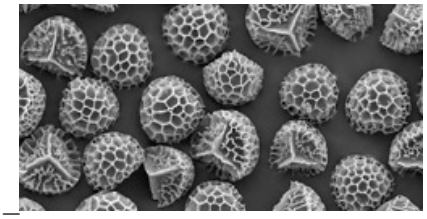


number of identically coated protein molecules. **Generally, the average size of viruses is about one-hundredth that of bacteria.[107] Importantly, the capsids of viruses can be conveniently modified by various strategies to provide uniform and precisely spaced binding sites for metal ions**. At present, viruses for the synthesis of MNMs included tubular **(e.g. Tobacco Mosaic Virus (TMV)),[108] filamentous (e.g. M13),[109] icosahedral (e.g. Cowpea Mosaic Virus (CPMV),[110] fd bacteriophages[111]) and elongated icosahedral viruses (e.g. T4 bacteriophages[112])** as detailed in Table 2.

Table 2 Characteristic dimension of some viruses for bio-inspired synthesis

Virus_Characteristic dimension	**Subunits**		
_Tobacco mosaic virus	300 nm-long hollow cylinder with an outer	2130 identical coat protein

		(TMV)	diameter of 18 nm and a 4 nm-wide inner cavity[12]	molecules following the right-handed helix of an associated RNA strand[12]_
M13 virus	☐6.5 nm diameter, 880 nm length[113]	☐2700 major coat proteins (p8) helically wrapped around its single-stranded DNA, with minor coat proteins (p3, p6, p7, and p9) at each end[113]		
fd bacteriophages	The same to M13 virus[111]	The same to M13 virus[111]		
Cowpea mosaic virus (CPMV)	Icosahedron, ☐28 nm diameter.	60 asymmetric units (with a small, 24 kDa, and a large, 41 kDa, subunit)[114]		
T4			Elongated icosahedron, ☐60 nm diameter[112,115]	A regular pattern of four kinds of coat proteins: gp23*, gp24*, highly antigenic outer capsid, and small outer capsid.[112]



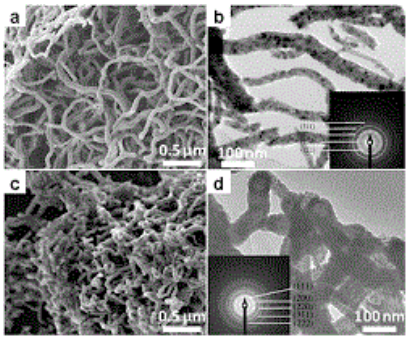
Wild type (WT) genetically modified and chemically modified viruses have also been employed to synthesize MNMs. As shown in Table 3, TMV, M13 virus, fd virus, CPMV and T4 virus are the main strains used for templated synthesis of MNMs. Generally, as-synthesized MNMs are metal clusters or spherical NPs ranging from 1 nm to more than a dozen nanometers in size. **Pure metal NWs could be obtained only in some exceptional cases though metal**–virus NWs could be prepared using tubular or filamentous viruses. Mild reductants including dimethylamine borane complexes (DMAB),[119] sodium cyanoborohydride ($NaBH_3CN$),[39] hydrazine hydrate (N_2H_5OH),[12] hydroxylamine (NH_2OH),[118] hydroxylamine hydrochloride ($NH_2OH{\cdot}HCl$),[125] sodium hypophosphite hydrate (NaH_2PO_2), and strong reductants such as $NaBH_4$[109] as well as UV irradiation[12] have been employed to reduce metal precursors during virus-templated synthesis of MNMs.

Table 3 Synthesis of MNMs with viruses

Virus_Activator or modifier	**Reductant**	**pH**	**MNMs**	**Size/diameter**	**Metalized viral diameter**	**_a Not mentioned in the corresponding reference. b Diameter of ☐20 nm.**
		UV irradiation	2.3	Ag NPs	<5 nm (channel) 10–15 nm (outer)	
WT TMV[116]_Pd(II)	Pd(II)	6–8	Ni NWs	☐3 nm	—[b]	–
WT TMV[117]	Pd(II)	Pd(II)	6–8	CoFe NWs	☐3 nm	—[b]
6His mutant of TMV[118]	Genetic modification	NH_2OH	5	Au NWs (annealed)	☐40 nm	—[b]
TMV1cys[39]	Cysteine	DMAB, $NaBH_3CN$	—[a]	Pd NPs	5–15 nm (DMA	—[b]

_E95Q/D109N mutant of TMV[12]	Amides_N_2H_5O nm_—[b]__	
Pt(II)	Pt(II)__Co NWs	

					B) 4–6 nm		
TMV1cys[119]	Cysteine	DMAB	—[a]	Pd NPs	11.4 nm	—[b]	
TMV1cys[120]	Cysteine	DMAB	—[a]	AuPd NPs	<5 nm	—[b]	
TMV1cys[38]	Cysteine	No	—[a]	Pd NPs	1–2 nm	—[b]	
TMV1cys[121]	Cysteine	No	—[a]	Ag NPs	2 nm	—[b]	
TMV2cys[122]	Cysteine	DMAB	—[a]	Au NPs	2.9 ± 1.4 nm	—[b]	
				Ag NPs	5.8 ± 2.7 nm		
_			Pd NPs	<5 nm		_TMV2cys[123]	Cysteine
M13 virus[113]_Peptide	Tetraglutamate	—[a]	Au NPs	☐5 nm	—[a]	_M13 virus[109]	No
				AuPt NWs			
			Pd NPs		20 ± 3 nm		Pd(II)
fd virus[111]_No	DMAB			2–3 nm		_WT CPMV[114]	
_			CoPt NPs		34.4 ± 0.3 nm	_WT CPMV[125]	Polyelectrolyte
T4 virus[115]_No	DMAB	—[a]	Pt NPs	3–4 nm	—[a]	_T4 virus[112]	No



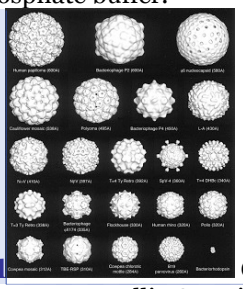
NaBH4_—[a]_Rh
[a]__M13 virus[124]_
NWs_10–50 nm

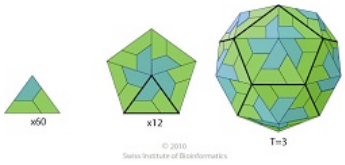
DMAB_—[a]_Co N
0.5 nm_____NaI
NPs__31.0 ± 0.3
nm_____DMAB_
0.4 nm_____NaI
NPs__32.6 ± 0.5
NPs__31.4 ± 0.5

NH2OH·HCl_—[
nm_☐67 nm__
CPMV[126]_Succin
[a]_Co NPs_☐2 n

DMAB_8.5–9_I
Ni NPs_2.0–4.5

3.2.1 Metallization with wild-type viruses. Viruses usually provide binding sites for metal ions and nucleation sites for MNMs formation with or without the need of additional reducing agents. Various strategies have been considered for WT viruses pretreatment to improve their metallization potential for the synthesis of MNMs. A proposed strategy deals with the activation of viral surfaces for enhanced nucleation. Knez et al.[127] first reported the metallization of **TMV plant viruses through a facile Ni deposition inside the TMV channels mediated by Pd(II).[12] In contrast, the high affinity of Ag(I) ions to the most external aminoacid of TMV followed by reduction with formaldehyde led to the exclusive formation of Ag NPs on the exterior surface of TMV.** In this case, Ag(I) played a role as own activator for NP formation. Upon pretreatment of TMV with Pd(II) or Pt(II), Kern's group reported a TMV-templated synthesis of 3 nm Ni and Co NWs via faster electroless deposition in the central channel in the absence of phosphate buffer.[116,128]

Similarly, Aljabali et al.[114] employed WT **icosahedral**  CPMV as a template for the electroless deposition of monodisperse metallic Co, Ni, Fe, Pt, Co–Pt and Ni–Fe NPs at room temperature with reduced Pd as nucleation sites.[114]



Unmodified CPMV empty virus-like particles were also demonstrated to simply encapsulate Co or iron oxide within the capsid interior.[129] Similar metallization was observed in the case of the T4 bacteriophage with the elongated icosahedron.[112,115] Balci et al.[117] also demonstrated an electroless synthesis of 3 nm wide CoFe alloy NWs (Fig. 5) inside 4 nm channels of TMV on the basis of pre-adsorption of Pd(II) ions. They believed that the formation of a Pd catalyst and autocatalytic deposition of the alloy from reduction of metal precursors with BH_3 was responsible for the formation of the CoFe alloy NWs.

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Comment [32]: This again is showing by placing nano materials or metals in a microrgasm that with another compnonent can in fact increase the volume of nano particle production increasing volume and density and with some modifications as well create the type of shape one desires all through programs

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Comment [33]: A polyhedron having 20 faces.
a polyhedron (plural polyhedra or polyhedrons) is a solid in three dimensions with flat polygonal faces, straight edges and sharp corners or vertices. The word polyhedron comes from the Classical Greek πολύεδρον, as *poly-* (stem of πολύς, "many") + *-hedron* (form of ἕδρα, "base" or "seat").
Cubes and pyramids are examples of polyhedr

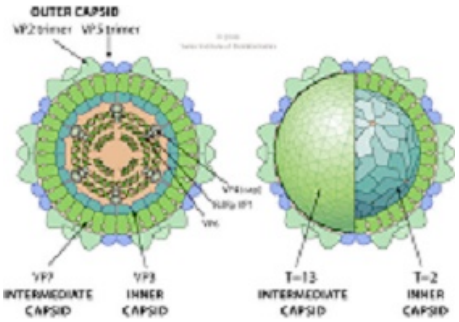
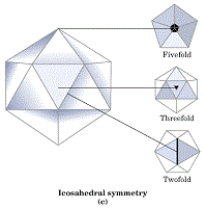


Fig. 5 (a) TEM micrograph of CoFe alloy NWs within the channel of a TMV particle (dark gray). The black line in the center corresponds to an alloy wire of ☐3 nm in diameter and approximately 100 nm in length. In the lower part of the same virion a second small nanowire (☐10 nm long) is located. Another short virion (☐100 nm long) is attached to the lower portion of the metallized virion. (b) High-resolution TEM image of a CoFe nanowire inside TMV. Crystal planes of various orientation and spacing are visible, all compatible with various orientations of CoFe. The image was calibrated by imaging Au particles at the same magnification. (c) TEM image of two short CoFe(Ni) wires located in a single virion cavity. The dark nodule at the bottom part stems from bulk deposition of the alloy. Reprinted with permission from ref. 117. Copyright © 2012, Institute of Physics.

Furthermore, nonspecific electrostatic interactions may give rise to some effective binding sites for the growth of MNMs. For example, Avery et al.[109] showed that metal–M13 NWs can be prepared through the electrostatic interactions between cationic metal complexes and the negatively charged (carboxylate groups) pVIII region of WT M13 bacteriophage, followed by borohydride reduction. In contrast to anionic metal complexes (from K_2PtCl_6 or $HAuCl_4$), the negatively charged pVIII surface could promote the coating of metal NPs.

3.2.2 Promoted metallization by surface modification of viruses. Charge modification and genetic modification have been used to strengthen the binding



of metal precursors onto viruses. As far as charge modification is essential for the enhanced metallization, Dujardin et al.[12] replaced aminoacid residues glutamate 95 (Glu95) and aspartate 109 (Asp109) in TMV mutant E95Q/D109N with the corresponding amides **through site-directed oligonucleotide mutagenesis.** Metal–virus tubular nanocomposites could be

obtained under a **controllable organization of metals (Pt, Au and Ag)** NPs on the external surface or within the 4 nm central channel of TMV **by chemically controlling the surface charge of the virus**.[12] Aljabali et al.[126] **adopted a surface charge modification approach of lysine groups** on the surface of WT CPMV to promote the templated metallization using succinamate. The obtained Co–CPMV NPs are monodisperse with a diameter of 31.2 ± 0.5 nm. The use of polyelectrolyte-modified CPMV was also reported for the templated synthesis of narrowly dispersed Au NPs.[125] Cationic poly(allylamine) hydrochloride (PAH) **was electrostatically bound to the external surface of the virus capsid**. As a result, **the adsorption of anionic Au complexes can be promoted, which facilitated their reduction to form a metallic Au coating under mild conditions**. Templated Au NPs could also be further modified with thiol reagents or assembled into large, hexagonally packed, tessellated-spheres after reaction with PAH-modified CPMV.

Genetic modification was also used to intensify metal precursor binding in viruses. TMV1cys and TMV2cys were obtained by the insertion of one and two cysteine residues into the amino-terminus of the coat protein on the outer surface of TMV, respectively.[122,130] Culver's group in Maryland University demonstrated an improved metal coating density on TMV2cys compared with WT TMV. **The additional sulfhydryl groups on TMV2cys enhanced ion uptake of the virus and provided a significant portion of the potential metal loading onto the TMV surface**.[122,131,132] For example, TMV1cys and TMV2cys **efficiently enhanced the biosorption of metal ions and the corresponding formation of Ag, Au and PdAu alloy NPs**.[122] Yi's group extensively investigated virus-templated growth of Pd NPs with TMV1cys and their catalytic applications. Manocchi et al.[39,133,134] also reported a size controllable growth of Pd NPs based on surfaced assembled TMV biotemplates and TMV solution by grazing incidence small-angle X-ray scattering (GISAXS). Thermal stability studies of Pd NPs on TMV showed that an improved stability of TMV-templated Pd NPs in comparison to Pd NPs formed on the solid substrate surface. The degradation temperature of Pd NPs increased from 214 °C on Au chips to 279 °C for Pd particles formed on TMV. Due to the presence of genetically displayed thiol functionalities, the self-assembly of TMV1cys on Au surfaces was more consistent and uniform as compared to WTTMVs, which results in the formation of high-density Pd NPs on TMV1cys.[39]

Recently, Yang et al.[121] demonstrated TMV1cys to be a sacrificial biomediator in the synthesis of Ag NPs with a tunable average size ranging from 1.9 to 9.5 nm. Under the highly alkaline conditions (pH > 10) of Tollens' reagent, **TMV may thus disassemble into reducing peptides or proteins (amino acids**

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Comment [34]: Genetic modification was also used to intensify metal precursor binding in viruses

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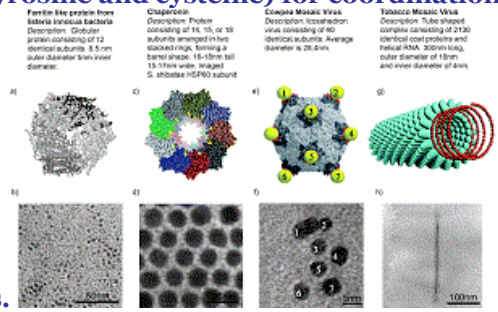
Comment [35]: TMV-Tobacco Mosiac Virus

serine, aspartic acid, tyrosine and cysteine) for coordination and reduction of Ag(I) ions. 

WT viruses can also be modified by peptides to increase binding sites for metal ions. Belcher's group in the Massachusetts Institute of Technology conducted considerable work on virus-templated MNMs with M13 virus. Nam et al.[113] incorporated Au-binding peptides into the filament coat of Co_3O_4/M13 virus composites to prepare hybrid Au–Co_3O_4 wires that improved battery capacity at room temperature. **The combination of virus-templated synthesis at the peptide level and methods for controlling 2D assembly of viruses on polyelectrolyte multilayers can provide a systematic platform to integrate these NMs to form thin, flexible lithium ion batteries.**[113] Aljabali et al.[135] used peptide–CPMV conjugates to produce ca. 32 nm monodisperse NPs coated with Co–Pt and Fe–Pt, which are not easy to synthesize using other methods. Recently, Wn□k et al.[118] engineered the TMV coat protein by incorporating a highly selective His-tag (HHHHHH, 6His) metal-binding peptide that would be readily accessible on the outer surface of assembled protein disks or rods. They demonstrated the use of such **genetically engineered TMV coat protein gene as a bio-template for the fabrication of Au NWs through thermal annealing.--In addition, coating viruses with oxides may enhance the affinity for metal ion adsorption and the stability of viral templates**. For instance, silica coatings on TMV1cys[136] promotes the formation of MNMs on TMV. A pretreatment of TMV with aniline was used to generate a uniform silica attractive surface for the growth of silica layer of >20 nm in thickness. These silica shells not only enhanced the stability **of TMV, but also promoted the deposition of various metal NPs through conventional silica mineralization chemistries**.

3.2.3 Assembly and size control of virus-templated MNMs. Virus-templated synthesis gives rise to different assembled structures and various MNMs. The assembled structures of MNMs on external surfaces or inside the channels of individual virus have primarily attracted much attention in recent years. **The overall structures depended on geometrical shapes of viruses.** For example, tubular TMV was used to assemble metal–TMV NWs or in the synthesis of metal NWs templated by external surface and interior channels, respectively. Knez and coworkers[128] demonstrated for the first time **that specific spatial selectivity for metal clusters-deposited TMVs could be modulated by a**

careful selection of metal ion, pH and duration of treatment. Metal (Pt, Au and Ag) NPs could be simply deposited on the external surface of TMV to obtain metal–virus tubular nanocomposites.[12] Tunable Au–TMV NWs consisting of TMV and densely packed Au NPs through adjustable addition–reduction cycles were also demonstrated.[137] **Ethanol addition after the first addition–reduction cycle and wrapping of the Au–TMV nanohybrids with poly-L-lysine were adopted to ensure high homogeneity and stability in the nanowire suspension**.[137] Recently, Khan et al.[138] also proved a simple, fast and high-yield binding of citrate-coated Au NPs to deprotonated WT TMV. The deprotonation overcome predominant electrostatic repulsion so that attractive van der Waals forces dominated.[138] In contrast to metal–virus NWs templated at the external surface of TMV, Ni and Co NWs could be synthesized inside TMV's internal channels in the absence of phosphate buffers reported by Knez and coworkers.[128]

Similar to tubular TMV, **filamentous M13 virus can be employed to fabricate metal–virus NWs. Metal**–M13 NWs can be prepared through nonspecific electrostatic interactions between **cationic aqueous metal complexes and the anionic carboxylate groups of the pVIII region of WT M13 bacteriophages**.[109] However, pure metal NWs cannot be simply obtained using M13 viruses lacking tubular channels. To promote the growth of crystalline CoPt and FePt NWs, nucleation of CoPt and FePt particles on genetically modified M13 viruses were **achieved by means of a chemical reduction of metal precursor salts, with assemblies annealed at 350 °C to remove the virus template.**[139] Later, a combination of M13 viral templates and CTAB could lead to stable, high-yield and tunable Au NWs and core–shell Au–Pt NWs using genetically modified M13 templates with specific Au binding peptides.[124] As discussed in Section 3.1.4, CTA^+ and Br^- are both vital for the formation of 1D metal nanostructures. In the absence of CTAB, only metal NPs could be obtained.

Three CPMV mutants through cysteine modification were employed as scaffolds to bind charge-stabilized Au NPs (2 and 5 nm) through Au–sulfur bond formation to produce patterns of tunable inter-particle distances.[110] Recently, Fontana et al.[140] demonstrated **a self-assembly strategy to create 3D icosahedral plasmonic Au–CPMV NPs with precisely spatial and orientational order mediated by a genetically engineered CPMV, which led to superstructures with new electromagnetic properties. Au–CPMV NPs exhibited sensitive magnetic response to the positioning of each Au particle.**

The size and loading density control of virus-templated MNMs should also be considered. Manocchi et al.[133] showed that the amount of Pd NPs formed on TMV1cys templates could be tuned by simply adjusting Pd precursor concentrations. Higher Pd precursor concentration led to Pd NPs with higher density on TMV. They also carried out a comprehensive study on the effect of Pd precursor concentration, type of

reductant and concentration and metallization time on the size of Pd NPs. **Results showed that sodium hypophosphite concentration was a key parameter affecting NP sizes**. Other common reductants such as $NaBH_4$ resulted in polydisperse Pd NPs with no size-reductant concentration relationship and inconsistent particle formation.[39] Pd NPs growth quickly occurred under the investigated conditions and the particles reached their final size after 1 min of incubation.[39] In the case of CPMV, Aljabali et al.[114] demonstrated that variations in the type of reducing agent did not have any significant effects on the size of Pd^0–CPMV particles. However, in the case of metallization with cobalt, the thickness of the metallic coating was dependent on the incubation time in a electroless deposition solution.[114]

Yang et al.[141] reported a tunable assembly of TMV on Au chips on which electroless deposition of Pd NPs could be achieved. By varying the concentration of TMV solutions for surface assembly or the Au surface area on patterned silicon chips, the surface density and spatial location of Pd–TMV could be controlled which further correlated well with Pd loadings and catalytic activities. Patterned assembly of metal-coated TMV without particular surface modification of silicon substrates can also be achieved[142] **and even a rapid microfluidic fabrication of hybrid Pd–TMV–PEG microparticles with Pd–TMV directly embedded in polymeric hydrogels for catalytic dichromate reduction**.[143] A similar example of an interesting replica molding approach has been illustrated in Fig. 6 for the encapsulation of spontaneously formed Pd–TMV1cys composites into Pd–TMV–PEG 3D microstructures with controllable Pd NP size, internal Pd–TMV density, internal Pd loading density and external microparticle size.[38] Pd NPs ranged from 1 to 2 nm in size and Pd–TMV–PEG materials could be used as efficient, stable and recyclable catalysts for dichromate reduction. TMV templates provided an alternative and efficient system containing catalytically active and accessible Pd NPs supported on stable microparticles.[143] Based on the previous work on assembly of WT TMV into long nanofibres via aniline polymerization,[144] Zhou et al.[111] demonstrated that PANI–Pd–TMV and PANI–Cu–TMV NWs could be synthesized by coating WT TMV with polyaniline.

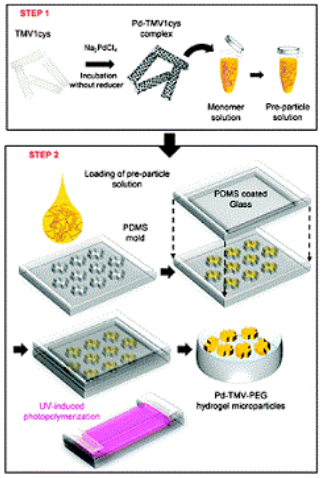


Fig. 6 Synthesis of Pd–TMV encapsulated PEG-based microparticles by replica molding (RM). (Step 1: spontaneous formation of Pd NPs without reducing agent. Step 2: fabrication of PEG-based microparticle via replica molding technique.) Reprinted with permission from ref. 38. Copyright © 2014, American Chemical Society.

M13 phages and Au NPs were recently modified with antigens (tagpeptides) and antibodies, respectively, to specifically interact with each other.[145] **As a result, M13 phages and Au NPs in the hydrogels formed lyotropic liquid crystals and well-ordered network structures through time-dependent cross-linking processes, respectively, leading to highly regular structures and enhanced macroscopic mechanical properties.**[145] Courchesne et al.[146] explored the layer-by-layer (LBL) assembly of a M13 bacteriophage-based template for the organization of Au NPs into nanoporous networks (Fig. 7). The results proved that Au NPs needed to be incorporated into the bacteriophage films during the LBL process in order to remain well-dispersed as well as to preserve the resonance peak location. Otherwise, Au NPs incorporated after LBL-assembly resulted in aggregation with significantly red-shifted and broadened spectra.

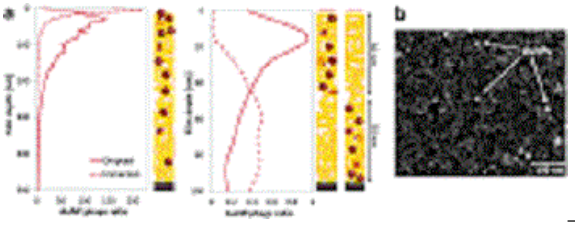


Fig. 7 Tight spatial distribution control of the bacteriophage-mediated incorporation of Au NPs. (a) XPS depth profiling analysis of the Au distribution as a function of the film depth, converted to NP to phage ratio for

different film architectures. The top panel shows the distribution for NPs infiltrated post-assembly, while the bottom panel shows films that were assembled with phage–NP complexes. (b) SEM image of a phage film infiltrated with Au NPs, and coated with titania. Bright circles are imaged Au NPs dispersed within the nanowire mesh. Reprinted with permission from ref. 146. Copyright © 2014 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim.

3.3 Plant-mediated synthesis

Intracellular or extracellular synthesis of MNMs by microorganisms had been extensively studied. However, downstream processing of intracellular MNMs is difficult while screening microorganisms for extracellular synthesis is very laborious.[77] In contrast, plants have attracted significant attention as simple and effective alternative scaffolds for the extracellular synthesis of MNMs. **Live plants, plant biomass and biomolecules extracted from plants or plant biomass have all been employed in the synthesis of MNMs**.

3.3.1 Biomineralization using live plants. The first demonstration of plant-mediated synthesis was reported in 1999 by Gardea-Torresdey et al.,[11] who described the synthesis of Au NPs by bio-precipitation from Au(III) solutions with alfalfa biomass**. Au and Ag NPs were formed using live alfalfa plants**.[41,147] Some examples of similar biomineralization strategies in recent years are listed in Table 4. **Spherical metal NPs (Au, Ag, Pd, Pt and AuAg) with a wide range of size distributions could be synthesized using live plants in most cases**. For example, Bali et al.[148] reported that M. sativa and B. **juncea plants were able to accumulate and translocate Pt from aqueous substrates. Pt NPs** (ca. 3–100 nm in size**) were evenly distributed across all tissue systems including epidermal, cortical and vascular within the roots of both plant species.** The synthesis of AuAg NPs with B. juncea plant was reported by Anderson et al.[149] Very recently, Parker et al.[42] demonstrated the ability of Arabidopsis to produce Pd NPs in a relatively simple way without any requirement for toxic chemicals or energy intensive processes.

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Comment [36]: Plant utilized the palladium all the way through

Table 4 Examples of biomineralization with live plants

Plants	MNMs	Shape	Size (nm)				
Sesbania [150] _Au NPs	Spherical	6–20	_Brassica juncea [151]	Ag NPs	Spherical_2–35__Medicago sativa, Brassica juncea[148]_Pt NPs_Triangular, spherical_3–100_		
Brassica juncea [149]	Au, AuAg NPs	Spherical	<50				
Arabidopsis Pd [42]	NPs	Spherical □32	_Populus deltoides [152]	Au NPs	Spherical	20–40	_Arabidopsis thaliana L.[153] Au NPs Spherical, irregular 5–100

The mechanism of metal uptake and transport into the plant cells has been studied.[152] **Sharma et al.[150] also demonstrated that biomatrix-embedded Au NPs resulted from the reduction of Au(III) ions in the root cells and symplastic transportation of Au NPs to the aerial parts or shoots**. The proposed sequence of Au transformation in Arabidopsis thaliana L. **((1) uptake of the ionic Au followed by (2) subsequent reduction of Au in planta to create NPs) was found to be a plausible route for Au NP formation.**[153] The environment around Arabidopsis thaliana L. may affect the uptake or deposition of metallic ions. Parameters such as the concentration of metal ions, the influence of other metallic ions, etc. have been taken into consideration to evaluate the possibilities of MNM syntheses. Haverkamp et al.[151] demonstrated Ag NPs could be deposited on Brassica juncea if Ag precursor concentrations ($AgNO_3$, $Na_3Ag(S_2O_3)_2$, and $Ag(NH_3)_2NO_3$ solutions) were below a limit of ca. 0.35 wt% Ag on a dry plant basis. **Higher levels of Ag would lead to the concentration of metallic salts within the plant instead of metallic deposition. In vivo effects of Cu and Ag on the synthesis of Au NPs in Brassica juncea plants** were also studied[149] with results showing the formation of discrete Au NPs in shoots in the absence of Cu and Ag. The presence of Cu and Ag reduced the size of Au NPs while also limiting the concentration of Au(0) species in plant tissues. In the controlled experiment, AuAg alloy **NPs were observed in the presence of Ag(I) ions in spite of an equal concentration of Cu ions in the soil had little effect on the synthesized Au and/or Ag structures (and did not alloy with Au and/or Ag)**.

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Comment [37]: A matter of up take and replication and then increase in volume-would appear to be now in the DNA and root base

3.3.2 Synthesis of MNMs using plant extracts. Metal NPs embeded in plant matrices are generally difficult to harvest for further applications. **Plant biomass and extracts can be a comparatively better option to whole plants. Nevertheless, the use of plant biomass poses the difficulty in purification of as-synthesized metal NPs prior to their applications**. Soluble biomolecules from plants or plant biomass have been consequently preferred for extracellular biosynthesis of metal NPs in the past decade.[154,155]

****

Nano-enabled synthetic biology

Mitchel J Doktycz[a,1,2] and Michael L Simpson[b,1,3,4]
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Abstract

Biological systems display a functional diversity, density and efficiency that make them a paradigm for synthetic systems. In natural systems, the cell is the elemental unit and efforts to emulate cells, their components, and organization have relied primarily on the use of bioorganic materials. Impressive advances have been made towards assembling simple genetic systems within cellular scale containers. These biological system assembly efforts are particularly instructive, as we gain command over the directed synthesis and assembly of synthetic nanoscale structures. **Advances in nanoscale fabrication, assembly, and characterization are providing the tools and materials for characterizing and emulating the smallest scale features of biology**. Further, they are revealing unique physical properties that emerge at the nanoscale. Realizing these properties in useful ways will require attention to the assembly of these nanoscale components. Attention to systems biology principles can lead to the practical development of nanoscale technologies with possible realization of synthetic systems with cell-like complexity. In turn, useful tools for interpreting biological complexity and for interfacing to biological processes will result.

Keywords: biological systems, carbon nanofibers, cell mimic, nanotechnology, synthetic biology, synthetic cells

Introduction

Understanding the organizing principles of complex systems presents a significant challenge. Whether in the synthetic or biological domain, there is a growing awareness that design, the reiterative process of creating function through the intentional interconnection of components, is an indispensable tool for unraveling complexity. Whereas modeling, simulation, and experimental analyses have a tendency to focus attention on the details of individual elements, design requires grappling with the trade-offs and compromises needed to enable system function. Along these lines, synthetic biology efforts follow a strategy of constructing deliberately simplified systems to comprehend molecular and cellular regulatory processes from the bottom up (Hasty et al, 2002; Sprinzak and Elowitz, 2005; Andrianantoandro et al, 2006; Guido et al, 2006). Similarly, efforts towards constructing minimal cells either add, subtract or manipulate

components to realize simple systems with desired capabilities (Forster and Church, 2006; Luisi et al, 2006). In both cases, iterative design is a fundamental aspect of the approach and represents a major step towards true bottom-up construction of biological complexity. **However, these approaches still depend on a platform of an existing cellular environment or the use of biomolecules (nucleic acids, proteins, lipids) to jump start cellular function**. Thus, the question remains, what could be learned from a true bottom-up effort to reconstitute cell-like complexity?

Can the deliberate design and assembly of synthetic components lead to systems with cell-like characteristics? The large discrepancy between the functional density (i.e., the number of components or interconnection of components per unit volume) of cells and engineered systems highlights the inherent challenges posed by such a question. **A simple example compares *Escherichia coli* (□2-μm² cross-sectional area) with an equivalent area on a silicon-integrated circuit (Simpson et al, 2001). The *E. coli* cell has an □4.6-million base-pair chromosome (the equivalent of a 9.2 megabit memory) that codes for as many as 4300 different polypeptides under the inducible control of several hundred different promoters, whereas the same space on a silicon chip could provide only a very small fraction of this memory or a few simple logic gates**. Clearly, the operational scale of biological systems is significantly smaller than that of conventionally engineered systems. Beyond just density alone, it is also the drastically different approach to component assembly, interfacing, and organization that differentiates the biological from the synthetic nanoscale system. **In the biological substrate, dynamic systems exploit weak interactions, arranged to provide desired specificity, and take place in a fluid environment.** These features lead from simply high spatial density to high *functional* density and the realization of robust, adaptable systems.

As nanoscience and technology advance, the opportunity to match the scale of biological system components becomes feasible. As a first step, nanotechnology presents the ability to directly interface to the working levels of biology, leading to the emergence of new approaches to therapy and diagnostics. Additionally, the emulation of biological design principles using synthetic components becomes feasible**. Potentially, as systems of such elements approach biological-scale functional density, they can begin to assume cell-like characteristics including: (1) construction from an inhomogeneous mixture of materials with different properties, modes and strengths of interactions, and relative abundances;** (2**) the encoding of information within small populations (e.g., biomolecules or electrons); (3) function emerging from an environment with large stochastic fluctuations (a consequence of (2)); and (4) the efficient transduction of information, energy, and materials that emanates from the molecular scale**. It is an intriguing possibility that, as our ability to control the synthesis and direct the assembly of **synthetic nanoscale elements increases, we may attempt the bottom-**

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Comment [38]: Direct Interface-- the place at which independent and often unrelated systems meet and act on or communicate with each other <the man-machine

up design and construction of nanosystems with cell-like complexity and capabilities. In turn, the design of such systems will lead to an enhanced ability to understand and interface to biological systems.

The intersection of nanoscale science and technology with biology has figured prominently in even the early stages of envisioning nanoscience research directions and goals (Roco, 2003). **In many ways, the biological cell represents an ideal paradigm for nanoscale systems**. Being the fundamental unit of biological systems, their function can be extremely diverse, yet uses only a finite, common set of building blocks. Cells operate under a wide range of environmental conditions with efficiencies unmatched by artificial systems. **They can be highly specialized and carry out tens of thousands of chemical reactions in parallel**. The dimensional characteristics of cells are well conserved and undoubtedly critical for system function (Welch, 1992; Hess and Mikhailov, 1994; Hochachka, 1999; Misteli, 2001; Harold, 2005). **Short distances (nm–μm) enable intra- and intercellular communication using simple diffusion-based mechanisms**. **Also, the small fluid volume of a cell allows for small fluctuations in numbers of specific molecules to result in dramatic changes in cellular state**. **Higher order, nanoscale structuring (Welch, 1992; Hochachka, 1999) and excluded volume effects (Hall and Minton, 2003) are also known to be critical to cellular function**. **In fact, with regard to heredity, the spatial definition of the cell may be as important as the genetic material** (Harold, 2005).

Here, we consider the **potential for a nano-enabled synthetic biology that may be derived from the confluence of systems biology and nanoscale science and technology**. At this confluence, systems biology provides knowledge of the chemical components that comprise the cell and the spatial and temporal interplay between these components**. Initial efforts to mimic cells have followed a path of using soft materials that are similar or identical to cellular materials**. However, the continued progress of nanoscale science and technology provides hope **that many cellular attributes may be transferred to artificial systems through the control of the synthesis and assembly of hard nanoscale materials at the multiple size scales important to cellular function**. In the process, advanced tools for understanding basic questions regarding biological function will be provided. Such developments could benefit both technology and science**. Cell-like complexity in nanoscale systems may lead to significantly higher levels of function**, whereas also forming an experimental system that would allow a much better examination of cellular organizational principles. Here, **we highlight efforts to mimic cell-like systems and the emerging tools of nanoscience that may enable an even more synthetic biology**.

Mimicking cellular systems

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Comment [39]: Again what would you call this—artificial Life or synthetic life and with the interaction of XNA or GNA this would have the capacity to make a template of itself and self replicate—this is again a reckless science with no means to stop something if something goes wrong- the "Super Bugs" are not natural and very well be the result of this "NANOINTEGRATION" of biology and technology on the levels of replication and spread ~ this is what bacterium and viruses and yeast properties convey

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Comment [40]: A means of integrating nano into a cell with shape and density—it has been accomplished-you can see the side effects of this accomplishment with people afflicted with the nanopoisoning—I.E> morgellons

The general concept of mimicking cells dates back several decades with the initiation of efforts to make effective blood substitutes (Chang, 2004). More recently, multiple efforts have evolved and are focused on engineering molecular systems and mimicking functional aspects of cells. **Additionally, synthetic cell efforts are increasingly integrating synthetic materials and nanotechnologies. A common feature of cell mimic pursuits is containment of a small, aqueous volume**. The ability to contain small volumes of liquid **(pico- to nanoliter)** is a critical aspect of biological cells and enabling for the creation of synthetic cell-like systems. Small volume containers obviate the need for mixing and establish local conditions that are favorable for protein function. **Small volumes reduce the number of molecules needed for carrying out a function**. Therefore, they are ideal for studying, or exploiting, reactions that involve single molecules. Further, small volume containers can be used for understanding molecular reaction systems and self organization at the cellular scale (Hess and Mikhailov, 1995; Marijuan, 1995; Chiu et al, 1999; Misteli, 2001; Long et al, 2005; Pielak, 2005). They are also valuable for studies related to understanding questions involving the origin of life (Deamer, 2005). On the applied side, miniaturization of the reaction volume can lead to the creation of massively parallel analytical systems (Wolcke and Ullmann, 2001; Heller, 2002; Khandurina and Guttman, 2002), whereas the *in vitro* aspects of the technology allow the use of physical conditions or the synthesis of products that may be toxic to natural cells. New approaches to high throughput screening, chemical sensing, and drug delivery are being enabled. **The incorporation of synthetic nanomaterials will be key to realizing these diverse applications**. The containing 'membrane' is a distinguishing feature of present approaches to mimic functional aspects of cells (Figure 1). **Natural membrane components, synthetic polymers, emulsion systems, and microfabricated structures are being considered**. An overview of these different systems is described below.

Figure 1

Overview of a genetic-based synthetic cell. The membrane of synthetic cells can be created from a variety of materials, including natural membrane components, synthetic polymers, and micro- or nano-fabricated materials. Alternatively, a water-in-oil emulsion ...

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Comment [41]: BIO-NANOTECH--

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Comment [42]: Trillionth= Pico --- Nano = Billionth

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Comment [43]: With this current tech being applied in pharmaceutucals as well as farming and industry ~exposure to nanopoisoning will be the next wave of

Vesicle-based systems

The most widely studied biomimetic containment systems are based on vesicles prepared from amphiphilic molecules. **These self-assembling structures can be formed from lipids, creating liposomes, or from synthetic molecules such as block copolymers, which are often referred to as polymersomes** (Vriezema et al, 2005). **They are also considered to be ideal biomimetic nanoscale reaction containers (Karlsson et al, 2004).**

Liposomes have long been used to encapsulate enzymes and can be prepared using a variety of techniques (Walde and Ichikawa, 2001). Their potential application as delivery vehicles for therapeutics has garnered much attention**. Liposomes can protect enzymes from degradation, effect slow release of a reagent, or contain chemical reactions. For example, enzymes entrapped in the interior of the liposome can be used for diagnostic applications (Ho et al, 1987), for metabolizing toxic reagents (Petrikovics et al, 1999), or as catalysts** (Yoshimoto et al, 2003). Further, multi-component systems have been designed that allow for targeting and stimuli-dependent release of encapsulated reagents (Guo and Szoka, 2003).

Gene-based reactions systems are also being developed. Reactions involving nucleic acids and polymerases have been described (Walde and Ichikawa, 2001; Monnard, 2003). **Further, simple genetic constructs, involving a promoter and gene sequence, and cell-free extract (Spirin et al, 1988; Shimizu et al, 2001) can be pooled in liposomes to produce the corresponding protein**. The expression of green fluorescent protein) enables easy assessment of the reaction (Yu et al, 2001; Oberholzer and Luisi, 2002; Nomura et al, 2003; Ishikawa et al, 2004; Sunami et al, 2006). More complex reactions have also been demonstrated. For example, Ishikawa et al (2004) **demonstrated a two-stage genetic network, where the protein product of the first stage is necessary for driving protein synthesis of the second stage. Other multi-stage reaction systems have been described leading to the possibility of constructing cell-free genetic circuits** (Noireaux et al, 2003; Noireaux and Libchaber, 2004).--The use of natural lipids facilitates biocompatibility, including the use of membrane proteins to facilitate material exchange with the enclosed volume. However, the long-term stability of these structures can be problematic. Nanotechnologies can effectively address this shortcoming. **Related efforts have investigated the use of synthetic polymers to create polymersomes**. Block copolymers are finding multiple applications in nanotechnology. **These polymers are composed of at least two parts of differing solubility and can self assemble into a variety of structures** (Forster and Antonietti, 1998; Klok and Lecommandoux, 2001; Park et al, 2003). They can also be formed into vesicles. **Vesicles with a broad range of chemistries and physical properties that are based on the choice of polymer type, block ratio, and molecular weight, can be constructed** (Discher and Eisenberg, 2002). As with liposomes, applications in chemical sensing, reagent delivery and reaction containment are pursued. For example, **enzyme activity can be preserved when encapsulated within polymersomes and such systems can be used for sensing and can be made stimulus responsive** (Napoli et al, 2004a, 2004b). **Facilitating the use of polymersomes for incorporation into biological systems is the discovery that natural membrane spanning proteins can incorporate into block copolymer shells. Nardin et al (2001) demonstrated that the *E. coli* porin protein OmpF can form a stable protein/polymer hybrid membrane and act as a size selective filter.** In this case, protein incorporation occurs even though the polymer membrane is two- to threefold

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Comment [44]: Liposome enzyme--

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Comment [45]: This is what nano does it uses the proteins and nanoparticles to construct a circuit-for the self assembling

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Comment [46]: This is a construct that can be used internally by these polymers in a host to self assemble

FREEDOM 4/17/2017 10:45 AM
Comment [47]: A broad range---of chemistries and Physical properties-another thought –XNA-Proteom Nucleic Acids~ nanobots~ self assembling bots~ maybe other mixtures of genes

thicker than a conventional lipid bilayer. Subsequent work has demonstrated the ability to use other block copolymer systems and incorporate other proteins (Ho et al, 2005; Ranquin et al, 2005). For example, Ho et al (2005) have incorporated the energy-transducing membrane proteins bacteriorhodopsin and cytochrome *c* oxidase into block copolymer vesicles. This system was shown to generate transmembrane pH gradients and highlights the potential use of hybrid nanosystems for harnessing the energy conversion processes of natural systems.

Emulsion-defined systems

Small volume reaction containers can also be created by **water in oil (w/o)** emulsions (Tawfik and Griffiths, 1998; Griffiths and Tawfik, 2000, 2003; Ghadessy et al, 2001; Pietrini and Luisi, 2004). Enclosed, femtoliter scale volumes can be defined through simple shaking, stirring or extrusion of a mixture containing an aqueous solution, oil and appropriate surfactants to stabilize the emulsion. **The typical size of the water droplets is on the order of a few microns, comparable to that of a microbial cell. Even smaller droplets can be prepared using ultrasonication** (Musyanovych et al, 2005). The simplicity and ability to create $\square 10^{10}$ containers/ml volume has enabled a variety of applications. For example, complete genomic libraries can be created, amplified and characterized using w/o techniques (Margulies et al, 2005; Shendure et al, 2005). **Alternatively, genetic variation within individual alleles can be quantified** (Dressman et al, 2003). **In these applications, the DNA is diluted such that, on average, a single template is contained within an aqueous droplet. These individual DNA strands can then be amplified by the polymerase chain reaction in separate aqueous volumes within a single tube.**

A notable feature of the w/o emulsion technique is the ability to link genotype with phenotype in a small volume reactor. An *in vitro* compartmentalization system has been described that is useful for high-throughput screening and selection (Aharoni et al, 2005b). In this approach, a gene sequence, along with the appropriate reagents for transcription and translation, is contained within the aqueous compartment. As a large number of compartments can be created and tested simultaneously**, entire libraries of genetic variants can be assessed. This enables 'directed evolution' of protein function by selection for the appropriate activity**. For example, DNA polymerases (Ghadessy et al, 2001) and methyl transferases (Tawfik and Griffiths, 1998; Lee et al, 2002) have been selected for by **simply breaking the emulsion and identifying the remaining gene sequences**. Other approaches exploit a physical connection between the gene and its product (Doi and Yanagawa, 1999; Sepp et al, 2002; Griffiths and Tawfik, 2003) or novel approaches (Aharoni et al, 2005a; Mastrobattista et al, 2005) to allow for subsequent sorting.

Enhancing the ability to transport reagents into and out of w/o emulsion-based reaction vessels is still under investigation. In general, reaction extent inside the

vessel is limited by the availability of precursor reagents, as transport within the oil phase is unlikely**. Some reagent exchange is believed to take place upon contact between individual compartments** (Ghadessy et al, 2001). **Fusion of compartments is also a potential mechanism for exchanging or feeding reagents to reaction vessels** (Bernath et al, 2004; Pietrini and Luisi, 2004). A related approach exploits microfluidics technology for creating aqueous droplets of defined size and composition (Dittrich et al, 2005**). Specific reagents can be mixed and compartmentalized by the merging of microfluidic flow streams**. This approach allows facile manipulation of the droplet and can be combined with sensitive detection techniques.

Nanomaterials: from individual elements to cell-like complexity

As described above, most efforts to mimic cells have relied on the self-assembly properties of organic materials. However, many applications have benefited from **small scale and high parallelism afforded by advanced microfabrication techniques, and these techniques have become better integrated with biological materials to enable greater functionality**. One use of these fabrication techniques is for creating robust reaction containers of defined volume and contents. Various etching, drilling, embossing or molding techniques can be used to create containers of a range of sizes (zl–□l). **The integration of such structures with fluids and biological materials is enabling multiple applications**. For example, small volume reaction containers are being considered for high-throughput screening, (Grosvenor et al, 2000; Angenendt et al, 2005), single molecule enzymology (Rondelez et al, 2005; Rissin and Walt, 2006), and analyses of single cells (Cooper, 1999; Johannessen et al, 2002). These reaction containers are also being developed for the cell-free synthesis of proteins (Nojima et al, 2000; Tabuchi et al, 2002; Yamamoto et al, 2002; Angenendt et al, 2004; Kinpara et al, 2004; Mei et al, 2005). In addition to creating a new approach to the parallel production of various proteins, these structures are permitting novel functional assays (Angenendt et al, 2005; Mei et al, 2005). **The use of microfabricated structures allows for the controllable exchange and mixing of reagents** (Nojima et al, 2000; Wang et al, 2005) and the integration of sensitive techniques for sampling and analysis of reaction products.

However, the use of microfabrication technology to achieve or interface to cell-like complexity is ultimately limited by the shortcomings of top-down synthesis processes that require layer-by-layer definition of structure through a very well-controlled series of deposition, lithography, and etching steps. Instead, synthetic systems must exploit characteristics similar to natural components, and nanoscale materials are especially suited to this challenge **as they reside on the same size scale as the components of biological processes, whereas exhibiting electrical, magnetic, optical, thermal, and chemical properties conducive to the construction of complex networks of functional parts**. By definition, nanoscale materials have a limited extent

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Comment [48]: There are 2 ways to grow this tech one is from top down dropping something on a containment and allowing it to construct going downward and the other means is to grow it bottom up where the construction is more readily controlled

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Comment [49]: Almost sounds Biological – or a synthesized biology

(nominally defined as less than 100 nm) in at least one of the three spatial dimensions. **Zero-dimensional (0-D) materials include semiconductor quantum dots (QD), colloidal metal particles, and atomic or molecular clusters that are confined in all three spatial dimensions; 1-D structures (which we refer to collectively as nanowires) are confined in two spatial dimensions; and 2-D structures (thin films such as silicon nitride or lipid bilayer membranes) are confined in only one spatial dimension (Figure 2).**

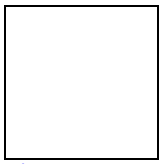

Figure 2
Collage of synthetic nanoscale materials. (**A**) 0-d nanoscale material shown in a Z-STEM image **of 655 Qdots™** (CdSe/CdS core/shell**, Quantum Dot** Corporation; image courtesy of Professor S Rosenthal, Vanderbilt University (adapted with permission from McBride **...**

Much of the early effort in nanoscience has focused on the synthesis and characterization of individual or homogeneous arrays of nanoscale elements, and numerous techniques have been developed for the synthesis of a variety of nanoscale materials: QDs composed of periodic groups II–VI (e.g., CdSe) or III–V (e.g., InP) **materials are synthesized by injecting liquid precursors into hot (300°C) coordinating organic solvent** (Murray et al, 1993; Peng et al, 1998); **semiconductor 1-d nanowires can be grown in a vapor-liquid-solid process** (Wagner and Ellis, 1964) **in which a liquid metal cluster or catalyst acts as the energetically favored site for absorption of gas-phase reactants; and carbon nanowires, which include carbon nanotubes** (Ajayan and Ebbesen, 1997) **and carbon nanofibers** (CNFs) (Rodriguez, 1993; Melechko et al, 2005), are synthesized in numerous processes including laser-vaporization (Kroto et al, 1985), arc discharge (Kratschmer et al, 1990; Iijima, 1991), catalytic chemical vapor deposition, and catalytic plasma-enhanced chemical vapor deposition (C-PECVD).

Although these materials synthesis efforts have been foundational for nanoscale science, taken alone they do not provide the means to construct or interface to cell-like complexity. It is the collective behaviors of interacting nanoscale components, where scale and complexity lead to '**entirely new properties**' (Anderson, 1972). The question then is not only of the synthesis of nanomaterials, but also that of how these materials should be organized into ensembles that **exhibit new levels of functionality**. Addressing this question shifts the focus from synthesis of individual elements to the controlled synthesis and directed assembly of systems of nanoscale components that are capable of assuming cell-like organization**. By controlled synthesis, we mean a process of mass nanostructure growth, where the pertinent attributes (location, size,**

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Comment [50]: Pay Attention to this especially as previously mentioned things are being made that are not even listed on the periodic table and as a result the interface with the biology has a completely different result and effect

orientation, composition, electrical, mechanical, and thermal properties, etc.) of the individual elements can be selected *a priori* by the choice of the growth conditions or the preparation of the growth substrate. Much like biological materials (e.g., silica biomineralization (Morse, 1999; Hildebrand, 2003; Hildebrand et al, 2006)), **or embryogenesis** (Carroll et al, 2004)), directed assembly in this context may more appropriately **be thought of as hierarchical assembly**, as each stage in the process forms the template for the **next layer of added complexity**. We illustrate these concepts in a synthetic system using the example of CNFs below. In this example, we emphasize the use of self-organization, hierarchical assembly, and the emergence of functional order from stochastic processes.

Controlled synthesis and hierarchical assembly

Carbon nanofibers are grown in a PE-CVD process from metal catalyst materials supported on substrates of various types (Melechko et al, 2005). The complex **plasma environment can be manipulated to produce changes in nanofiber aspect ratio, diameter, orientation, shape, and chemical composition** (Merkulov et al, 2001, 2002a, 2002b, 2002c; Melechko et al, 2002). Likewise, CNF morphology, crystalline structure, and composition can be varied through manipulation of the growth substrate (Klein et al, 2005; Fowlkes et al, 2006b). For example, the patterning of the catalyst material allows the selection of either randomly spaced forests or deterministically placed isolated fibers (Figure 3); the selection of the plasma source gases control nanofiber composition; and nanofiber shape can be controlled by selection of the catalyst material **crystallographic orientation** (Fowlkes et al, 2006b). **The forest morphology is particularly interesting, as it is the result of a self-organization process initiated by the plasma-induced fragmentation and reordering of the initial microscale catalyst pattern followed by nanofiber growth from each of the nanoparticles**. Although the placement of individual nanofibers exhibits a high degree of stochasticity, the distributions of interfiber spacing and fiber size are strongly influenced by the choice of catalyst material, thickness, and crystallographic orientation.

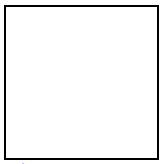

Figure 3
Micrographs of carbon nanofibers. (**A**) Vertically aligned carbon nanofibers can be prepared from a continuous catalyst stripe yielding an array of CNFs that are randomly arranged. (**B**) Individual CNFs can be precisely positioned using electron beam lithography **...**

A concurrent self-organization is the emergence of nanoscale pores that form between the randomly spaced nanofibers. **These structures can act as**

passive membrane mimics in microfluidic devices (Zhang et al, 2002; Fletcher et al, 2004). Whereas the pores are relatively large (e.g., 150–250 nm) in as grown forests, hierarchical processing that adds to the diameters of the nanofibers (e.g., conformal **SiO_2 coating or electroplating of conductive polymers)** leads to the formation of planar or 3-D pore networks (Fowlkes et al, 2005). Within these forest structures, diffusive transport is a strong function of the excluded volume (i.e., space taken up by the nanofibers) and the placement of nanofibers. The stochastic nature of the interactions between diffusing molecules and nanofibers leads to regions of anomalous diffusion (i.e., time-variant diffusion coefficient), whereas a large excluded volume leads to significantly reduced molecular mobility (Fowlkes et al, 2006a). The net effect is that the diffusive transport properties of these membrane mimics may be controlled through self-organization of **stochastic** nanofiber forests and hierarchical processing to define the structure of a nanoporous network.

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These membrane structures can be patterned in arbitrary shapes and used for creating cell mimic structures (Fletcher et al, 2004; Fowlkes et al, 2005). As shown in Figure 4, **advanced, multiscale (nano to macro) fabrication techniques allow for the integration of nanomaterials, fluids, and biological reagents to create structures that mimic functional characteristics of a cell**. In this example, **the enclosed volume can be tuned to closely match those of a natural cell.** Such a container would be useful for experimentally characterizing reaction systems and material organization in a contained fluid environment. Further, this structure allows for controlled transport between the contained volume and the local environment through design of the membrane properties.

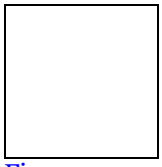

Figure 4
Fabrication of a cell mimic array. The device is created using (**A**) contact photolithography and ICP-RIE etching of silicon to define a fluidic channel approximately 10 □m in depth. (**B, C**) Metal lift-off of Ni catalyst is followed by PECVD growth **...**

These structures can advance from being simple, passive structures that control transport based on physical size to more sophisticated, active nanostructures. For example, additional control of the membrane properties is possible through the application of chemical coatings (Fowlkes et al, 2005; Fletcher et al, 2006; McKnight et al, 2006). **Such coatings can bestow chemical specificity in addition to size-selective transport. Polymeric coatings on the CNFs can be exploited to create active interfaces**. Polypyrrole can be selectively patterned onto CNF-based electrodes (Chen et al, 2001; Nguyen-Vu et al, 2006; Fletcher et al, 2007). **Such coatings can be reversibly actuated to expand**

or contract with the application of an electrical signal (Smela, 2003). Other polymers are responsive to chemical or physical stimuli (Gil and Hudson, 2004; Yoshida et al, 2006).

Realizing a nano-enabled synthetic biology

Nano-enabled synthetic biology is in its early stages. For biological systems, functionality at any scale begins at the cellular level. **The efforts described above illustrate the different approaches involved in attempting the bottom-up construction of simple cellular-like structures**. Nanotechnologies are becoming increasingly involved. Nanoscale science has delivered the ability to synthesize a variety of nanoscale components that provide the means to **dramatically increase the density of elements**. Controlled synthesis and hierarchical assembly allow these **components to mimic passive, and even active, cell-like behaviors. As described, carbon-based nanostructures and block co-polymers match many of the design requirements of synthetic membranes.** Future efforts will likely see the integration of other nanomaterials for potentially transducing energy, conveying signals, or **controlling the arrangement of biological and synthetic structures**.

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Comment [52]: An example of this is a carbon based element made with this nano tech which is 200 times stronger then steel or diamond – graphene and is one of the most conductive materials ever used for any type of conductivity~ it also has the capacity to store energy and is extremely versatile and pliable and can be utilize in a multifunctional capacity

Understanding and improving the interface between natural and synthetic structures represents a key next step for **nano-enabled synthetic biology**. Related challenges in controlling synthesis across the multiple length scales relevant to biology and in the development of tools that are useful in characterizing interactions at these scales also need continued attention. Effective emulation, interpretation, or control of biomolecular events will depend on this interface. As stated at the beginning of this article, it is both scale and complexity (interconnectivity of the elements) that lead to higher levels of functionality, and further progress hinges on increases in the latter. Perhaps paradoxically, increasing complexity requires a renewed, albeit redirected, focus on synthesis. Nanoscale materials have been pursued with an eye on unique properties that emerge usually due to electron confinement, the increased ratio of surface area to volume, or the physical properties that result from precise molecular arrangements. Increased attention towards control of surface properties to allow site-specific functionalization of nanomaterials will be required. The binding affinity between natural and synthetic structures will need to be carefully prescribed. **Nanoelements will need to be multifunctional, possibly requiring a mix of soft/hard material functionality and hybrid synthesis techniques** that. Synthetic nanostructures will need to become active participants in the feedback mechanisms of biological networks for effective interfacing.

Ultimately, the development of nanotechnology-based tools will enable hybrid systems that will substantially enhance the synthetic biology toolbox. Practical biomedical devices will also result. Of course, interfacing to biological systems is not a requirement for systems composed of synthetic nanoscale components. As

nanostructured materials take on the characteristics of biological materials, synthetic systems of high functional density and cell-like complexity may also be realized. Learning how to assemble these components into functional networks will require a close coupling with systems biology efforts. Such bio-inspired nanomaterial systems would not be restricted to operation in aqueous environments or a narrow range of physical conditions. Considering the diversity observed in biology and the commonality of their system architecture, even simple synthetic systems have the potential for addressing multiple applications.

Acknowledgments

We gratefully acknowledge the staff of the Molecular-Scale Engineering and Nanoscale Technologies and the Biological and Nanoscale Systems research groups for contributions and critiques of the manuscript. Our efforts are supported by NIH Grant EB000657, the Center for Nanophase Materials Sciences, which is sponsored at Oak Ridge National Laboratory by the Division of Scientific User Facilities, US Department of Energy and the DOE Office of Science. This work was performed at the Oak Ridge National Laboratory, managed by UT-Battelle, LLC, for the US DOE under Contract no. DE-AC05-00OR22725. This manuscript has been authorized by a contractor of the US Government under contract DE-AC05-00OR22725. Accordingly, the US Government retains a nonexclusive, royalty-free license to publish or reproduce the published form of this contribution, or allow others to do so, for US Government purposes.

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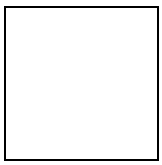

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Engineered nanomaterials- exposures, hazards, and risk prevention

Robert A Yokel1*, Robert C MacPhail2

Abstract Nanotechnology presents the possibility of revolutionizing many aspects of our lives. People in many settings (academic, small and large industrial, and the general public in industrialized nations) are either developing or using engineered nanomaterials (ENMs) or ENM-containing products. However, our understanding of the occupational, health and safety aspects of ENMs is still in its formative stage. A survey of the literature indicates the available information is incomplete, many of the early findings have not been independently verified, and some may have been over-interpreted. This review describes ENMs briefly, their application, the ENM workforce, the major routes of human exposure, some examples of uptake and adverse effects, what little has been reported on occupational exposure assessment, and approaches to minimize exposure and health hazards. These latter approaches include engineering controls such as fume hoods and personal protective equipment. Results showing the effectiveness - or lack thereof - of some of these controls are also included. This review is presented in the context of the Risk Assessment/Risk Management framework, as a paradigm to systematically work through issues regarding human health hazards of ENMs. Examples are discussed of current knowledge of nanoscale materials for each component of the Risk Assessment/Risk Management framework. Given the notable lack of information, current recommendations to minimize exposure and hazards are largely based on common sense, knowledge by analogy to ultrafine material toxicity, and general health and safety recommendations. This review may serve as an overview for health and safety personnel, management, and ENM workers to establish and maintain a safe work environment. Small start-up companies and research institutions with limited personnel or expertise in nanotechnology health and safety issues may find this review particularly useful. 1. Introduction
A. The objectives of this review

Although there has been considerable work to advance nanotechnology and its applications, understanding the occupational, health and safety aspects of engineered nanomaterials (ENMs) is still in its formative stage. The goals of this review are to describe some general features of ENMs, how a worker might be exposed to ENMs, some potential health effects, and approaches to minimize exposure and toxicity. The target audience includes industrial hygienists, investigators working with these materials, institutes and universities conducting research, and start-up companies that may not have the

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necessary occupational health and safety expertise, knowledge, and/or staff. A comprehensive review described the field of nanotoxicology six years ago, including some mechanisms of toxicity, portals of ENM entry, their translocation, and the state of their risk assessment at the time [1]. More recent reviews have focused on the major challenges, key questions, and research needs to assess ENM toxicity and risk [2-7]. This review addresses issues not extensively covered in prior reviews, including recent exposure-assessment studies, and engineering and personal protective equipment (PPE) options and their efficacy to minimize ENM exposure. This review also includes accepted but not yet published reports, recently completed studies not yet published, and ongoing work. Our goal was to provide up-to-date information on ENM exposures, their health hazards, and ways to minimize risk.

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B. Engineered nanomaterials

Nano is a prefix derived from the Greek word for dwarf. The parts of the U. S. National Nanotechnology Initiative (NNI) definition that are relevant for this review define nanoscale materials as having **at least one dimension in the range of 1 to 100 nanometers (nm)**, with properties that are often unique due to their dimensions, and that are intentionally manufactured [8]. There are many definitions of nanoscale materials, which generally encompass the same bounds on ENM size [9,10]. **This is in contrast to naturally occurring and unintentionally-produced materials on the same scale, which are referred to as ultrafine particles**. **The term ultrafine has been used by the aerosol research and occupational and environmental health communities to describe airborne particles smaller than 100 nm in diameter** [11]. Ultrafine particles are not intentionally produced. **They are the products of combustion and vaporization processes such as welding, smelting, fuel combustion, fires, and volcanoe**s [1,12,13]. In this review, intentionally-manufactured nanoscale materials will be referred to as ENMs. **They are usually produced by bottom-up processes, such as physical and chemical vapor deposition, liquid phase synthesis, and self-assembly** [5,14]. The health and environmental effects of ENMs are not well understood, leading some to caution development of this technology [15-19]. **Some understanding of ENM effects can be derived, however, by analogy from ultrafine particles, which have been shown to produce inflammation, exacerbation of asthma, genotoxicity, and carcinogenesis following**

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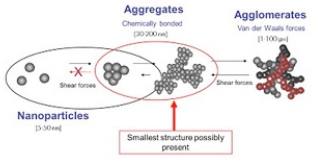
inhalation. The following sections describe ENMs, and some of their uses and uncertainties, providing the context of this review.

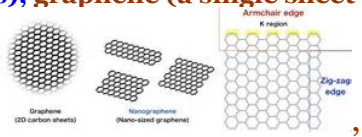
C. Common ENM size, composition, and quality to 2 and 2 to 50 nm wide, respectively, and can be > 1 m long. The C60 diameter is ~1 nm. Metal and metal oxide **ENMs most commonly studied are cadmium in various complexes, gallium arsenide, gold, nickel, platinum, silver, aluminum oxide (alumina), cerium dioxide (ceria), silicon dioxide (silica), titanium dioxide (TiO2, titania), and zinc oxide**. The size of ENMs is in the same range **as major cellular machines and their components**, **such as enzymes, making it likely that they will easily interact with biochemical functions** [22]. Some ENMs contain contaminants, such as residual metal catalysts used in the synthesis of CNTs. ENM toxicity has been attributed to these residual metals, as discussed in II, B, 1. **ENM exposure effects in the lung**. The physico-chemical properties of ENMs, when tested prior to their use, are often different from those stated by the supplier [23,24]. **A major cause of changes in the physico-chemical properties of ENMs over time and in various media is**



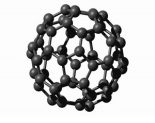
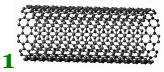
agglomeration , discussed in II, A, 2. The physico-chemical properties of ENMs that impact their uptake. When ENMs are not sufficiently characterized to identify their composition or properties it makes the prediction of toxicity, when added to the insufficient understanding of their biological effects, even more difficult [25].

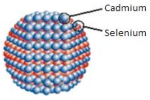
D. **Some uses of ENMs and the projected market and workforce**

Figure 1 relates ENM size to other chemical and biological materials. There are a staggering number of ENM compositions and shapes. Over 5000 patents have been issued for carbon nanotubes (CNTs) and > 50,000 varieties of CNTs have been produced [20]. The sheer number of ENMs contributes to the lack of our adequate understanding of ENM health and safety. **They are primarily composed of carbon or metal/metal oxide, as illustrated by the representative manufactured nanomaterials selected for testing** by the Organisation for Economic Co-operation and Development (OECD) [21]. Carbon-based ENMs **include single-walled and multiwalled carbon nanotubes (SWCNTs and MWCNTs), graphene (a single sheet of carbon atoms in**



a hexagonal structure) **, spherical fullerenes (closed cage structures composed of 20 to 80 carbon atoms consisting entirely of three-coordinate carbon atoms, e.g., C60**

[Buckyballs, buckminsterfullerene]),  **and dendrimers, which are symmetrical and branched. SWCNTs and MWCNTs are ~1** 

There is considerable interest in developing ENMs because their properties differ in fundamental and valuable ways from those of individual atoms, molecules, and bulk matter. Nanoscale products and materials are increasingly being used in optoelectronic, electronic (e. g., computer hard drives), magnetic, medical imaging, drug delivery, cosmetic and sunscreen, catalytic, stain resistant fabric, dental bonding, corrosion-resistance, and coating applications [26]. Major future applications are expected to be in motor vehicles, electronics, personal care products and cosmetics, and household and home improvement. These applications capitalize on their electromagnetic, catalytic, pharmacokinetic, and physico-chemical properties, including strength, stiffness, weight reduction, stability, anti-fogging, and scratch resistance. **Current products contain various ENMs including nanotubes, metal oxides, and quantum dots** 

(semiconductors developed as bright, photostable fluorescent dyes and imaging agents). Nanowerk identified ~2500 commercial nanomaterials, including ~27% metal oxides, 24% CNTs, 18% elements, 7% quantum dots, and 5% fullerenes [http://www.nanowerk.com/ phpscripts/n_dbsearch.php]. There are > 1000 consumer products available that contain ENMs. **They are primarily composed of silver, carbon, zinc, silica, titania and gold.** The main application is in health and fitness Yokel and MacPhail Journal of Occupational Medicine and Toxicology 2011, 6:7 http://www.occup-med.com/content/6/1/7

Figure 1 The sizes and shapes of some ENMs compared to more familiar materials. Shown for comparison are materials that are below, within, and above the nanoscale range, to put ENM size in perspective. Three to four new nanotechnology containing consumer products are introduced weekly into the market, according to The Project on Emerging Nanotechnologies [http://www.nanotechproject.org/ inventories/consumer/]. The anticipated benefits of ENM applications resulted in expenditure of $18 billion worldwide on nanotechnology research and development in 2008. In 2004 Lux Research predicted that nanotechnology applications will become commonplace in manufactured goods starting in 2010 and become incorporated into 15% of global manufacturing output in 2014 [https://portal.luxresearchinc.com/research/document_excerpt/2650]. The ENM workforce is estimated to grow ~15% annually [29]. An epidemiological feasibility study of CNT workers initiated in 2008 revealed most manufacturers

were small companies that had no environmental/occupational health and safety person and little knowledge about this topic [30]. By 2015, the global market for nanotechnology-related products is predicted to employ 2 million workers (at least 800,000 in the U.S.) to support nanotechnology manufacturing, and $1 trillion in sales of nanotechnology-related products [31].
E. Uncertainties regarding the adverse effects of ENMs

There have been concerns about the safety and public acceptance of this burgeoning technology, particularly in the past 5 years, due to the lack of much information about potential adverse effects [32]. This resulted in an increase from 2.9 to 6.6% of the NNI budget for environmental health and safety from 2005 to 2011. Prior to 2005 it does not seem funds were specifically allocated for this purpose nor was the U.S. National Institute for Occupational Safety and Health (NIOSH) a contributor to NNI funding [33,34]. The United Nations Educational, Scientific and Cultural Organization (UNESCO) compared the concerns of the public over new products with their perception of genetically modified foods/ organisms to nanotechnology. **They noted that the lack of knowledge can result in restrictions, outright bans, and international conflicts over production, sale, and transport of such materials** [35]. Public acceptance can influence the success of an emergent technology, as public opinion is considerably influenced by information prior to the adoption of the technology. However, individuals form opinions often when they do not possess much information, based on factors other than factual information, including values, trust in science, and arguments that typically lack factual content [36]. This creates a challenge to earn public acceptance of nanotechnology. There is a notable lack of documented cases and research of human toxicity from ENM exposure. It is widely recognized that little is known about ENM safety.

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Comment [54]: There is still a huge amount of information not surfaced on the actually lethality this tech has on the genetic code and the altering effects it has on bio life or how it can integrate into a dna code or alter the genetics of anything it integrates with

An uncertainty analysis revealed knowledge gaps pervade nearly all aspects of ENM environmental health and safety [4]. Owing to their small size and large surface area, ENMs may have chemical, physical, and biological properties distinctly different from, and produce effects distinct from or of a different magnitude than, fine particles of similar chemical composition. This is discussed in II, A, 2**. The physico-chemical properties of ENMs that impact their uptake. ENM properties often differ from individual atoms, molecules, and from bulk matter**. **These differences include a high rate of pulmonary deposition, the ability to travel from the lung to systemic sites, and a high inflammatory potential** [1]. Further contributing to our lack of understanding of the potential health effects of ENMs is that most production is still small scale. As such, potential adverse effects from the anticipated increase in large scale production and marketing of ENM-containing products and use are generally unknown. Furthermore, the number of novel ENMs being created continues to grow at a high rate, illustrated by the accelerating rate of nanotechnologyrelated patent applications [37,38]. II. A Framework for Evaluating the Risk of ENMs We elected to review the existing literature on ENM effects in the context of the Risk Assessment/Risk Management framework as originally described in the U.S. National Research

Council report "Risk Assessment in the Federal Government: Managing the Process", often called the Red Book, that mainly dealt with chemical threats to health [39]. The framework is depicted in Figure 2. A similar approach was advanced by the European Chemicals Bureau for biocidal products (http:// eur-lex.europa.eu/pri/en/oj/dat/2003/l_307/ l_30720031124en00010096.pdf). Although the NRC framework is portrayed as a sequential approach, in practice it is dynamic with considerable interaction between risk assessors, scientists, and often times the affected parties. This general approach has been proposed for evaluating the risks of ENMs [5-7]. A notable alternative is the Nano Risk framework, a joint venture of the Environmental Defense Fund and DuPont [40]. In addition, due to the many different ENMs, and the time and cost to thoroughly assess their potential risks [41], there is currently much interest in developing in vitro models that are predictive of in vivo effects [42], although these are not always successful [42-44], and in developing tiered testing systems [45,46]. Additional efforts are underway to group (band) similar ENMs in order to promote safe handling and use of ENMs, and restrict worker exposure, in the absence of definitive health and safety information [47,48]. Still others are applying computational approaches to predict ENM effects, including toxicity [49,50]. In this review the Risk Assessment/Risk Management framework will be used as a template because it succinctly codifies the diverse practices of risk assessment into a logical framework that collects data to determine Figure 2 The Risk Assessment/Risk Management framework.

(**1) whether an agent causes an adverse effect, (2) how the effect is related to dose, (3) whether exposure is likely, and (4) the probability of adverse effects in the population at current exposure levels**. The framework also embraces research that feeds each of the elements of the risk assessment with the necessary information. For the current review, this framework provides a systematic method to work through the many issues surrounding the potential health effects of ENMs. **The first element, hazard identification, addresses whether there is any evidence that an agent causes an adverse effect**. Hazard identification represents the lowest hurdle in the process, **since the evidence could come from any number of sources, including laboratory or field observations**, and might only be suggestive. **The next element, dose-response assessment**, is more rigorous and asks whether there is a relationship between the dose of the agent and the incidence or magnitude of adverse effect. **This element is based on the fundamental tenet in toxicology and pharmacology of dose response; that is, as the dose increases so does the effect**. This information is often not directly available for humans, so laboratory animal studies are typically used. **Exposure assessment is the next element**. If evidence indicates an agent poses a hazard, and the hazard is dose-related, the **next step is to determine the extent of occupational or daily life exposure.** Information from all elements is then combined into a risk characterization, which estimates the likelihood of an adverse effect occurring in the exposed population or a segment of the population. The Risk Assessment/Risk Management **framework is comprised of 3 essential components; research, risk assessment, and**

risk management. Risk assessment is regarded as a scientific undertaking whereas risk management uses the science to regulate exposure to the agent in ways that take into account social benefits, economic costs, and legal precedents for action. The following sections are arranged to follow the NRC paradigm. Examples are given of adverse effects of ENMs to show why there may be reason for concern. Reports on exposure levels, the likelihood of adverse effects resulting from exposure, and options for minimizing risk are also summarized. This is not, however, an all-inclusive review of the literature; interested readers are referred to the reference section for a number of comprehensive reviews of many of the topics pertaining to ENMs and their effects.

A. **Hazard identification**

Related industries have the potential to be exposed to uniquely engineered materials with **novel sizes, shapes, and chemical properties, at levels far exceeding ambient concentrations**...much research is still needed." [http://www.cdc.gov/niosh/topics/nanotech/about.html]. Information about ENMs might be obtained from well-documented retrospective analyses of unintended exposures. The most extensive exposures to ENMs likely occur in the workplace, particularly research laboratories; start-up companies; pilot production facilities; and operations where ENMs are processed, used, disposed, or recycled [51]. Occupational hygienists can contribute to the knowledge and understanding of ENM safety and health effects by thorough documentation of exposures and effects. In the U.S., NIOSH is responsible for conducting research and making recommendations for the prevention of work-related illnesses and injuries, including ENMs. The U.S. Occupational Safety and Health Administration (OSHA) is responsible for making and enforcing the regulations.

1. **The key routes of ENM exposure**

In the occupational context, hazard identification can be re-stated as "What effects do ENMs have on workers' health?" to which NIOSH has stated: "No conclusive data on engineered nanoparticles exist for answering that question, yet. Workers within nanotechnology-**the four routes that are most likely to result in ENM exposure of the five organ systems which are the major portals of ENM entry**: **skin, gastrointestinal tract, lung, nasal cavity, and eyes** [22]. **It also illustrates the most likely paths of translocation (re-distribution or migration), enabling ENMs to reach organs distal to the site of uptake**. The inhalation route has been of greatest concern and the most studied, because it is the most common route of exposure to airborne particles in the workplace. The skin has also been investigated. Most studies have shown little to no transdermal ENM absorption. **Oral (gastrointestinal) exposure can occur from intentional ingestion, unintentional hand-to-mouth transfer, from inhaled particles > 5 m that are cleared via the mucociliary escalator, and of drainage from the eye socket via the nasal cavity following ocular exposure**. **Direct uptake of nanoscale materials from the nasal cavity into the brain via the olfactory and trigeminal nerves has been shown**. Each of these routes is discussed in more detail below. **Routes that avoid first-pass clearance and**

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metabolism in the gastrointestinal tract and liver include uptake (absorption) from the nasal cavity (either into systemic circulation or directly into the brain), orotransmucosal (e.g., buccal [from the cheek] and sub-lingual), and transdermal. These routes may present a greater risk of ENM-induced adverse effects because more ENM is likely to reach the target organ(s) of toxicity.

2. **The physico-chemical properties of ENMs that impact their uptake**

Hazard identification has revealed that the physico-chemical properties of ENMs can greatly influence their Dermal -Oral GI Tract- Inhalation-Ocular Nasal Cavity- Lymphatic System-Respiratory Tract Circulatory System (Blood) Organs- Brain

The predominant routes of ENM exposure and uptake, and potential routes of ENM translocation. The four gray shaded boxes indicate the primary routes of ENM exposure. The arrows down from these uptake sites show potential translocation pathways. The translocation pathways are described in more detail in Section II, D. Clearance of ENMs, their translocation to distal sites, and persistence. For example, the lung might be the primary route of exposure or might be a distal site after uptake from another route and translocation to the lung. ENMs might enter the brain from the nasal cavity or from blood, across the blood-brain barrier.

Uptake.

ENMs show greater uptake and are more biologically active than larger-sized particles of the same chemistry, due to their greater surface area per mass [52,53]. **Additional ENM characteristics that may influence their toxicity include size, shape, surface functionalization or coating, solubility, surface reactivity (ability to generate reactive oxidant species), association with biological proteins (opsonization), binding to receptors, and, importantly, their strong tendency to agglomerate. An agglomeration is a collection of particles that are loosely bound together by relatively weak forces, including van der Waals forces, electrostatic forces, simple physical entanglement, and surface tension, with a resulting external surface area similar to the sum of the surface area of the individual components** [9,54]. Agglomeration is different from aggregation. **Aggregated particles are a cohesive mass consisting of particulate subunits tightly bound by covalent or metallic bonds due to a surface reconstruction, often through melting or annealing on surface impact, and often having an external surface area significantly smaller than the sum of calculated surface areas of the individual components**

[9,54]. **Agglomerates may be reversible under certain chemical/biological conditions whereas an aggregate will not release primary particles under normal circumstances of use or handling. Airborne ENMs behave very much like gas particles. They**

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Comment [57]: What is cause or activation of Nano Environmental Materials- characteristics that may influence their toxicity include size, shape, surface functionalization or coating, solubility, surface reactivity (ability to generate reactive oxidant species), association with biological proteins (opsonization), binding to receptors, and, importantly, their strong tendency to agglomerate

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agglomerate in air due to self-association (in one study increasing from 8 to 15 nm in 16 min and to ~100 nm in 192 min) and interaction with background aerosols (to ~500 nm agglomerates within min) [55]. Studies of ENMs in occupational settings showed airborne particulates were most commonly 200 to 400 and 2000 to 3000 nm [51,56]. **ENMs also agglomerate in liquids, resulting in micrometer sized particles [**57]. One study showed that concentration and smaller ENM size positively correlated with speed of agglomeration [58]. **Changes in ENM surface area can profoundly uptake and effects**. **The aspect ratio (length:diameter) of ENMs also plays a major role in their toxic potential**. Particles with a length > 5 m and aspect ratio 3:1 are conventionally defined as fibers [59]. Inhaled asbestos containing high aspect-ratio fibers is more toxic than lower aspect-ratio fibers. Foreign materials are often cleared by macrophage phagocytosis**, but when too large to be phagocytosed they are not effectively cleared from the lung**. This results in release of inflammatory mediators, discussed below**. It appears that ~15 to 30 nm is a critical width or diameter for ENMs to have properties different from the solution and bulk chemistry of their components**. **Reactive oxygen species generation in an acellular system to which 4 to 195 nm titania ENMs were added was negligible up to 10 nm, then increased up to ~30 nm, when it reached a plateau** [53]. A review **concluded there is a critical size for ENMs at which new properties typically appear. These new properties are strongly related to the exponential increase in the number of atoms localized at the surface, making metal and metal oxide ENMs with diameters < 20 to 30 nm most different from bulk material** [60]. For example**, 1 and 3 nm gold ENMs, which contain ~30 and 850 atoms, have nearly all and ~50% of their atoms surface exposed, respectively**. **Additionally, the optimal particle radius to accelerate adhesion to a cell-surface lipid bilayer is 15 and 30 nm for cylindrical and spherical particles, respectively [61,62]. Therefore, 10 to 30 nm diameter ENMs that have a spherical or similar shape appear to have the potential for more profound biological effects than either smaller or larger** ENMs. It is prudent to apply the continually improving understanding of the influence of the physico-chemical properties of ENMs on their effects and safety to the development of future ENMs, to enhance their benefit/ risk ratio. Second generation (active) ENMs are being developed, such as targeted control-release systems for drugs. There is utility in the use of CNTs as drug delivery systems. Based on the studies of the role of CNT physico-chemical properties in biological effects it has been concluded that the use of low aspect ratio (length 1 m), high purity (97-99%), low metal catalyst content CNTs **minimizes cytotoxicity** and provides apparent in vivo bio-compatibility [63]. Application of the continued understanding of the influence of physico-chemical properties on biological responses can similarly enhance the benefit/risk ratio of future ENMs, such as: application of the most predictive dose metric; the rate and nature of interacting proteins and effect of opsonization on uptake, translocation and effects; **the influence of size, shape, charge, and surface reactivity on the extent and sites of translocation; and the duration of**

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Comment [60]: This is an indicator how it can cause the damage to system on a cellular level as well this will transfer to the mitochondria and myelin as well

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Comment [61]: Shape and Form will also determine the effect and level of Damage that will occur to the cells and myelin—fullerenes which are cylindrical in shape will cause more profound damage then a circular nano particle and a quantum dot also would cause more damage due to the cluster of particles and how it would adhere to the cells

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Comment [62]: This something to pay close attention to it is not saying it is safe at all but that the risk are minimized meaning there is still a risk of cellular damage ~ or tissue or potential organ or glandular damage~ the other aspect here is exiting this tech if it comes out at all.

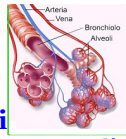
persistence of ENMs in organs and associated effects. Additionally, observations of workers exposed to ENMs can greatly add to this understanding, to increase confidence in the predicted effects of future ENMs.

a. **The role of surface coating in ENM uptake and effects adsorb to the next available surface and other small molecules** [67]. Extensive addition of polyethylene glycol (PEG) to the surface of SWCNTs has been shown to favor uptake into tumors compared to normal organs [68]. **Similarly, addition of PEG to poly(di-lactic acid-comalic acid) coated magnetic ENMs enhanced their uptake by macrophages** [69]. Commercial providers and researchers often add a surface coating to inhibit ENM agglomeration and/or influence their uptake and cellular effects [70]. Cells that line the airways produce mucus. **Pulmonary type II alveolar cells secrete surfactants (a mixture of 90% phospholipids and lung surfactant-specific proteins).** Lung surfactants incorporate ENMs [71,72]. **Mucus, which is secreted by goblet cells in the respiratory tract, eye, nasal cavity, stomach, and intestine, entraps ENMs** [65]. **All of these surface coatings on ENMs would be expected to affect their uptake and effects**.

b. ENM uptake from the initial sites of exposure

ENMs are rapidly coated in biological milieu, **primarily by proteins** [62,64-66]. Due to high energetic adhesive forces close to the surface, ENMs can agglomerate and

To understand ENM-induced effects and their mechanisms of action, cells in culture and other in vitro systems have been utilized. However, these systems cannot model the complexities of the entire organism, **including the limitation of uptake provided by such barriers as the skin and first-pass metabolism, opsonization, metabolism that may inactivate or activate a substrate, translocation to distal sites, activation of homeostatic defenses, or inflammatory processes that release cytokines and other factors that can act at distant sites from their release.** Therefore, this review primarily cites examples of whole-animal studies to address ENM uptake and translocation. i). **Lungs There has been much interest in the health effects of airborne particles, specifically PM10 (thoracic fraction), PM2.5 (respirable fraction**), **PM1, and ultrafine particles (PM0.1), which are 10, 2.5, 1 and 0.1 m (100 nm), respectively. One- to 5-nm air-suspended ENMs that enter the lungs**



are not predicted to reach the alveoli ; instead a high percentage is likely to deposit in the mucus-lined upper airways (tracheo-bronchial region) due to their strong diffusion properties. **On the other hand ~45% of 10-nm, ~50% of 20-nm, and ~25% of 100-nm ENMs deposit in the alveoli** [73]. **Deposition is greater during exercise. Chronic obstructive pulmonary disease increases tracheo-broncheolar and decreases alveolar particle deposition** [74,75]. ii)

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Comment [63]: All factors that determine the lethality of this technology

FREEDOM 4/17/2017 10:45 AM

Comment [64]: So now the question needs to be asked what happens when PEG is utilized in foods and other oral intake does this or will this interact with the current nanoparticles internally bound or trapped into the cells or myelin or mithochondria~ and will this allow more of the nano to penetrate into the system ..

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Comment [65]: So this would mean a removal of the mucous in order to remove the build up of nano particles or Environmental Nano Materials

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Comment [66]: SO what we are talking here is that this is a fundamental perspective at how much it can alter or effect whatever it integrates with or with what ever it accumulates with as well~ so at this juncture and research what we are seeing is only the smallest of view at the danger this has on life and as a result of the self assembling we are also having to contend with and how it can self assemble into a host is yet begun to reset the DNA or Myelin or Mitochondria or Genetic alterations

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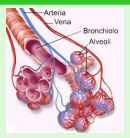
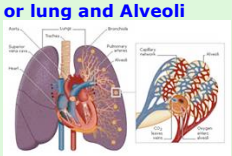


Comment [67]: Alveoli- or lung and Alveoli



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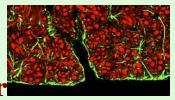
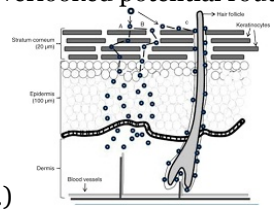
Comment [68]: This is indicating that area of the lung will be hit the most with the highest concentration of the ENM so it may get overwhelmed and at the same time where the leakage may occur causing heart duress as well spread throughout the body

Nasal cavity Uptake from the nasal cavity into the olfactory nerve, followed by retrograde axonal transport to the olfactory bulb and beyond, was shown in studies of the polio virus (30 nm) and colloidal silver-coated gold (50 nm) [76-78]. Uptake of ~35-nm 13C particles along the olfactory pathway to the olfactory bulb, and to a lesser extent into the cerebrum and cerebellum, was shown 1 to 7 days later



[79]. Exposure to ~30 nm agglomerates of Mn by inhalation resulted in up to a 3.5-fold increase of Mn in the olfactory bulb, and lower (but significant) increases in 4 rat brain regions. **The increase of Mn in brain regions other than the olfactory bulb may have resulted from translocation to the brain by route(s) other than via the olfactory nerve, such as through cerebrospinal fluid or across the blood-brain barrier** [80]. The nasal cavity is the only site where the nervous system is exposed directly to the environment. This is an often overlooked potential route of uptake of small amounts of ENMs into the brain. iii.)



Dermal exposure Skin is composed of 3 primary layers, the outermost epidermis (which contains the stratum corneum, stratum granulosum and stratum spinosum), dermis, and hypodermis. The hair follicle is an invagination of the stratum corneum, lined by a horny layer (acroinfundibulum). Dermal uptake routes are intercellular, intracellular, and follicular penetration. Uptake is primarily by diffusion. Materials that diffuse through the lipid-rich intercellular space of the stratum corneum typically have a low molecular weight (< 500 Da) and are lipophilic. **Materials that penetrate the stratum corneum into the stratum granulosum can induce the resident keratinocytes to release pro-inflammatory cytokines. Materials that penetrate to the stratum spinosum, which contains Langerhans cells (dendritic cells of the immune system), can initiate an immunological response**. This is mediated by the Langerhans cells, which can become antigen-presenting cells and can interact with T-cells. Once materials reach the stratum granulosum or stratum spinosum there is little barrier to absorption into the circulatory and lymphatic systems. **Whereas dry powder ENMs pose a greater risk for inhalation exposure than those in liquids, liquid dispersed ENMs present a greater risk for dermal exposure. Consumer materials most relevant to dermal exposure include quantum dots, titania, and zinc oxide in sunscreens, and silver as an anti-microbial agent in clothing and other products.** Prolonged dermal application of microfine titania sunscreen suggested penetration into the epidermis and dermis [81]. However, subsequent studies did not verify

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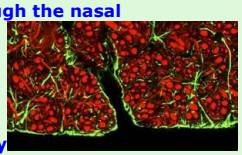
Comment [69]: Brain Penetration through the nasal cavity



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Comment [70]: Alternative routes for entry to the brain

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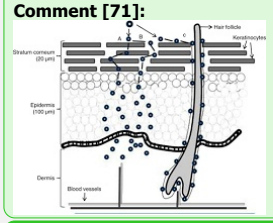
Comment [71]:



FREEDOM 4/17/2017 10:45 AM
Comment [72]: Showing how fat can transport the nano through the skin

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Comment [73]: The exposure is in just the consumable products ~ there is no mention of this in the food supply which can be absorbed from contact either by touch or smell or consumption and nothing is going to tell you that the nano from the sky is also contributing to the skin route and respiratory and nothing is going to tell you the vaccines as well as the pharmaceutical and nutriceuticals are also huge contributors to the uptake of these ENM

penetration of titania from sunscreens into the epidermis or dermis of human, porcine or psoriatic skin [82-87], or find evidence of skin penetration of zinc oxide from sunscreen or positively- or negatively-charged iron-containing ENMs [88,89]. **Nanoparticles with a dye penetrated deeper into hair follicles of massaged porcine skin in vitro and persisted longer in human skin in vivo than the dye in solution** [82,90,91]. Thirty-nm carboxylated quantum dots applied to the skin of mice were localized **in the folds and defects in the stratum corneum and hair follicles**. A small amount penetrated as deep as the dermis. **Ultraviolet radiation increased penetration**, raising concern that these results might generalize to nanoscale sunscreens [92]. **PEG-coated ~37 nm quantum dots accumulated in the lymphatic duct system after intra-dermal injection in mice. Cadmium, determined by ICP-MS, from cadmium-containing quantum dots was seen in liver, spleen, and heart; however, it is uncertain if this was from dissolved cadmium or translocation of the quantum dots** because methods were not used to show the presence of quantum dots. **The above results suggest topically-applied ENMs that penetrate to the dermis might enter the lymphatic system, and the ENMs or dissolved components distribute systemically** [93]. To address these concerns **ENMs intended for dermal application, such as titania, are often surface coated, e.g. with silica, alumina, or manganese**. One goal of the surface treatments is to minimize toxicity by trapping the free radicals of reactive oxygen species (ROS) [94]. **An in vitro study showed that mechanical stretching of human skin increased penetration of 500 and 1000 nm fluorescent dextran particles through the stratum corneum, with some distribution into the epidermis and dermis** [95]. Similarly, mechanical flexing increased penetration of a 3.5 nm phenylalanine-based C60 amino acid ENM through porcine skin in vitro [96]**. The contribution of skin flexing and immune system response was further addressed with three titania formulations applied to minipigs**. **There was some ENM penetration into epidermis and abdominal and neck dermis, but no elevation of titanium in lymph nodes or liver** [97]. Topical exposure of mice to SWCNTs resulted in **oxidative stress in the skin and skin thickening, demonstrating the potential for toxicity not revealed by in vitro studies of ENM skin penetration [**98]. There are no reports of long-term studies with topical ENM exposure. In the absence of organic solvents, the above suggests that topically applied ENMs do not penetrate normal skin. **Not surprisingly, organic solvents (chloroform > cyclohexane > toluene) increased penetration of fullerene into skin that had the stratum corneum removed by tape stripping** [99]. As the fullerenes were not detected in systemic circulation, there was no evidence of systemic absorption. iv.) Oral exposure Little is known about the bioavailability of ENMs from the buccal cavity or the sub-lingual site, or possible adverse effects from oral ingestion. **Particle absorption from the intestine results from diffusion though the mucus layer, initial contact with enterocytes or M (microfold or membranous specialized phagocytic enterocyte) cells, cellular trafficking, and post-translocation events** [100]. **Colloidal bismuth**

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Comment [74]: **So with the UV Sunscreen actually becomes more Lethal not a protectant but a deliverer of nano posioning**

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Comment [75]: **This is incredible as one metal that is highly toxic especially to the male genitalia- titanium dioxide- the other added components are compounding the lethality of the titanium dioxide –the silica and aluminum would bypass the blood brain barrier and the manganese causing a total melt down of the brain and central nervous system –and people are wondering where the dementia and parkinsons and alzheimers is coming from**

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Comment [76]: **Interesting to see that what they did not see in a lab was shown in real life so not everything viewed is going to be present til it manifest in real life**

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Comment [77]: **How it transfers into the Intestinal Region**

subcitrate particles (4.5 nm at neutral pH) rapidly penetrated the mucosa of dyspeptic humans, resulting in bismuth in the blood. Particles appeared to penetrate only in regions of gastric epithelial disruption [101]. Greater uptake of 50 to 60 nm polystyrene

particles was seen through Peyer's patches and enterocytes in the villous region of the GI tract than in non-lymphoid tissue, although the latter has a much larger intestinal surface area [102,103]. **Peyer's patches are one element of gut associated lymphoid tissue, which consist of M cells and epithelial cells with a reduced number of goblet cells, resulting in lower mucin production** [100,103]. It was estimated that ~7% of 50-nm and 4% of 100-nm polystyrene ENMs were absorbed [104]. **Fifty-nm polystyrene ENMs fed to rats for 10 days by gavage showed 34% absorption, of which about 7% was in the liver, spleen, blood, and bone marrow**; no ENMs were seen in heart or lung [104]. **After oral administration of 50-nm fluorescence-labeled polystyrene ENMs, 18% of the dose appeared in the bile within 24 h and 9% was seen in the blood at 24 h; none was observed in urine** [105]. **The mechanism of GI uptake of 4, 10, 28 or 58 nm colloidal (maltodextran) gold ENMs from the drinking water of mice was shown to be penetration through gaps created by enterocytes that had died and were being extruded from the villus**. Gold abundance in peripheral organs inversely correlated with particle size [106]. **In summary, there appears to be significant absorption of some ENMs from the GI tract, with absorption inversely related to ENM size. The absorption site seems to be regions of compromised gastric epithelial integrity and low mucin content**. v.) **Ocular and mucous membrane exposure Ocular exposure might occur from ENMs that are airborne, intentionally placed near the eye (e.g., cosmetics), accidently splashed onto the eye, or by transfer from the hands during rubbing of the eyes, which was shown to occur in 37% of 124 adults every hour** [107]. **This route of exposure could result in ENM uptake through the cornea into the eye or drainage from the eye socket into the nasal cavity through the nasolacrimal duct**. Other than a study that found uptake of a polymer ENM into conjunctival and corneal cells, this route has been largely ignored in research studies of ENM exposure [108].

B. The effects of ENM exposure on target organs and those distal to the site of uptake have led to highly sensitive techniques that can detect chemicals at remarkably low levels (e.g., in parts per billion or parts per trillion). The detectable level may be far lower than any dose shown to produce an adverse effect. Further, a single finding in the literature may garner public attention, and it may be statistically significant, but its scientific importance remains uncertain until it is replicated, preferably in another laboratory. In this regard, a follow-up

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Comment [78]: So basically building a construct internally with this material forming patches

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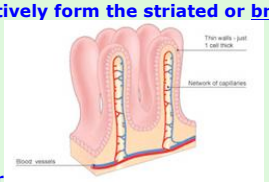
Comment [79]: Indicating that it was trapped internally and nothing was showing

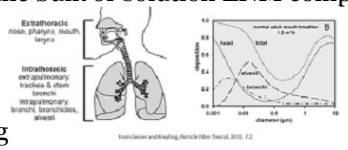
FREEDOM 4/17/2017 10:45 AM

Comment [80]: Intestinal villi (singular: villus) are small, finger-like projections that protrude from the epithelial lining of the intestinal wall. Each villus is approximately 0.5-1.6 mm in length, and has many microvilli projecting from the enterocytes of its epithelium which collectively form the striated or brush



border

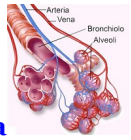
study may be warranted to characterize the relevant parameters of dose, duration, and route of exposure, as outlined in the Risk Assessment/ Risk Management framework. The above discussion reflects many of the issues that have gained prominence in the fields of risk perception and risk communication (see for example [109,110]), neither of which were dealt with by the NRC in their landmark publication. The knowledge of ultrafine-particle health effects has been applied to ENMs. However, the toxicity from ultrafine materials and ENMs is not always the same [111]. Similarly, the effects produced by ENM components do not reliably predict ENM effects. **For example, toxicity was greater from cadmium-containing quantum dots than the free cadmium ion** [112]. Some metal and metal oxide ENMs are quite soluble (e.g., ZnO), **releasing metal ions that have been shown to produce many of the effects seen from ENM exposure** [113,114]. Therefore, one cannot always predict ENM toxicity from the known effects of the bulk or solution ENM components.



1. ENM exposure effects in the lung

Public concerns about ENMs and health may arise with reports of some effect(s) in a laboratory study or their presence in human tissue (or another organism). Any report must be interpreted carefully before concluding ENMs are risky for one's health. **To start with, risk is defined as a joint function of a chemical's ability to produce an adverse effect and the likelihood (or level) of exposure to that chemical**. In a sense, this is simply a restatement of the **principle of dose-response; for all chemicals there must be a sufficient dose for a response to occur**. Additionally, advances in analytical chemistry

Studies of ENM inhalation and intratracheal instillation as well as with lung-derived cells in culture have increased concern about potential adverse health effects of ENMs. An early 2-year inhalation study of Degussa P-25 (a ~3:1 mixture of ~85-nm anatase and 25-nm rutile titania) **resulted in lung tumors in rats** [115]. SWCNTs containing **residual catalytic metals produced greater pulmonary toxicity, including epithelioid granulomas and some interstitial inflammation, than ultrafine carbon black or quartz**.



These effects extended into the **alveolar septa** [116]. A review of eleven studies of **carbon nanotube** introduction to the lungs of mice, rats, and guinea pigs revealed most found **granuloma, inflammation, and fibrosis** [117]. **MWCNTs produced greater acute lung and systemic effects and were twice as likely to activate the immune system as SWCNTs, suggesting the former have greater toxic potential** [118]. Further adding to the concern of ENM-induced adverse health effects are reports that inhaled

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Comment [81]: Multi Walled Carbon Nanotubes

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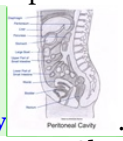
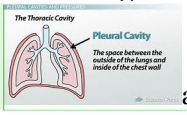
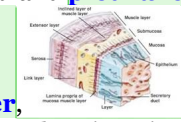
Comment [82]: Single Walled Carbon Nanotubes

CNTs potentiate airway fibrosis in a murine model of asthma [119], and that exposure of a cell line derived from normal human bronchial epithelial (BEAS-2B) cells to **SWCNTs and graphite nanofibers produced genotoxicity and decreased cell viability** [120]. However, a point of contention is that the lung response to intratracheal and inhaled MWCNTs differed among studies. This may have been due to different sizes and distributions of MWCNT agglomerations. These differences create uncertainties regarding the utility of some routes of pulmonary ENM exposure used in laboratory studies to predict potential toxicity in humans [121]. Studies exposing lung-derived cells in culture to ENMs have demonstrated similar effects. **Carbon-based ENMs produced pro-inflammatory, oxidative-stress, and genotoxic effects** [122,123]. Several groups have studied the effects of CNT introduction into the **peritoneal cavity** 

. As there is CNT translocation from the lung to other sites (see II, D. Clearance of ENMs, their translocation to distal sites, and persistence), and the internal surfaces of the peritoneal and **pleural cavities** 

are lined with a **mesothelial cell layer**,  responses in the peritoneal cavity appear to be relevant to the pleural cavity. Single ip injection of high-aspect-ratio MWCNTs (~100 nm diameter and 2000 nm long) **produced inflammation, granulomatous lesions on the surface of the diaphragm, and mesothelioma that were qualitatively and quantitatively similar to those caused by crocidolite asbestos, also a highaspect-ratio fiber** [124]. These effects correlated positively with the MWCNT aspect ratio [125,126]. **Toxicity has also been seen from pulmonary introduction of metal and metal oxide ENMs. Ten and 20 nm anatase titania induced in BEAS-2B cells oxidative DNA damage, lipid peroxidation, increased H2O2 and nitric oxide production, decreased cell growth, and increased micronuclei formation (indicating genetic toxicity)** [52]. Exposure of BEAS-2B cells to **15- to 45-nm ceria or 21nm titania resulted in an increase of ROS, increased expression of inflammation-related genes, induction of oxidative stress-related genes, induction of the apoptotic process, decreased glutathione, and cell death** [127,128]. **Twenty-nm ceria increased ROS generation, lipid peroxidation, and cell membrane leakage, and decreased glutathione a-tocopherol (vitamin E) and cell viability in a human bronchoalveolar carcinoma-derived cell line** (A549) [129]. **Various metal oxides differentially inhibited cell proliferation and viability, increased oxidative stress, and altered membrane permeability of human lung epithelial cells** [130].

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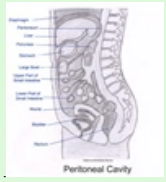
Comment [83]: The peritoneal cavity is a fluid-filled gap between the walls of the abdomen and the organs in the abdomen



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Comment [84]: Definition: Pleural Cavity. The pleural cavity is the space that lies between the pleura, the two thin membranes that line and surround the lungs.

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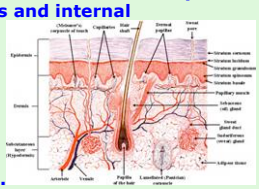
Comment [85]: Mesothelial cells form a monolayer of specialised pavement-like cells that line the body's serous cavities and internal organs.



2. **ENM exposure effects seen in the brain demonstrate the ability of metal oxide ENMs to produce neurotoxicity.**

3. ENM **exposure effects seen in the skin**

Potential toxicity from dermal exposure was demonstrated with silver ENMs, that decreased human epidermal keratinocyte viability [133]. **These results demonstrate the ability of metal oxide ENMs to also produce dermatotoxicity.**

4. **Summary of the effects of ENM exposure** on target organs and those distal to the site of uptake

Common findings of many studies are induction of inflammatory processes and oxidative stress. However, correspondence between responses of cells in culture and in vivo models is often low [24,43]. In light of the pressure to minimize whole animal (e.g., rodent) research, further development of cell-based or in vitro models of the whole organism is expected. Additionally, there has been considerable use of alternative model organisms e.g., C. elegans, which has a genome with considerable homology with vertebrate genomes and is often used in ecotoxicological studies, and zebrafish which are often used in developmental biology and genetic studies [134-136].

C. **Dose-response assessment**

Murine microglial cells were exposed to a commercial 70%:30% anatase:rutile titania (primary crystalline size 30 nm; 800 to 2400 nm agglomerations in test medium). They displayed extracellular release of H 2 O 2 and the superoxide radical and hyper-polarization of mitochondrial membrane potential [131]. **Intravenous ceria administration to rats altered brain oxidative stress indicators and anti-oxidant enzymes** [23,132]. These results Exposure in experimental studies is typically expressed as dose, usually on a mass/subject body weight basis, or as concentration. **Dose or concentration may not be the best metric to predict ENM effects** [42,53,137]. Neutrophil influx following instillation of dusts of various nanosized particles to rats **suggested it may be more relevant to describe the dose in terms of surface area than mass** [138]. **The pro-inflammatory effects of in vitro and in vivo nanoscale titania and carbon black best correlated when dose was normalized to surface area** [122]. **Secretion of inflammatory proteins and induction of toxicity in macrophages correlated best with the surface area of silica ENM** [139]. Analysis of in vitro reactive oxygen species generation in response to different sized titania ENMs could be described by a single S shaped concentration-response curve when the results were normalized to total surface area, further suggesting this may be a better dose metric than concentration [53]. Similarly, using surface area as the metric, good correlations were seen between

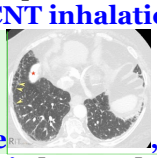
in vivo (PMN number after intratracheal ENM instillation) and in vitro cell free assays [42]. Nonetheless, most studies of ENMs have expressed exposure based on dose or concentration. The relatively small amount of literature has generally shown dose- or concentration-response relationships, as is usually the case for toxicity endpoints. Ceria ENM uptake into human lung fibroblasts was concentration-dependent for several sizes, consistent with diffusion-mediated uptake

[58]. **Positive, dose-dependent correlations were seen in blood, brain, liver, and spleen following iv ceria infusion in rats, measured by elemental analysis as cerium [23], as well as brain titanium after ip titania injection [140], and lung cobalt after inhalation of cobalt-containing MWCN**Ts [141]. Concentration-dependent inhibition of RAW 264.7 (murine) macrophage cell proliferation was seen following in vitro SWCNT exposure, as was lipopolysaccharide-induced COX-2 expression, up to 20 g/ml [142]. Intratracheal instillation of MWCNTs (average length ~6 m) or ground MWCNTs (average length ~0.7 m) produced dose-dependent increases in LDH activity and total protein, but no dose-dependent effect on the number of neutrophils or eosinophils, or TNF-a, in rat lung broncho alveolar lavage fluid [143]. Activated Kupffer cell count increased with iv ceria dose; **the increase in hippocampal 4-hydroxy-2-trans-nonenal and decrease in cerebellar protein carbonyls (indicators of oxidative stress) were dose-dependent up to a maximum that did not increase further at the highest dose** [23]. Some studies demonstrating adverse effects of CNT introduction to the lung have been criticized for using doses or concentrations that far exceeded anticipated human exposure [144]. Most studies assessing potential adverse effects of ENMs have utilized a single exposure. Both of these features make extrapolation of results to prolonged or episodic (periodic) human exposure difficult. However, the study of acute high doses/concentrations to probe potential effects is a standard approach in toxicology and experimental pathology for initially surveying adverse effects (i.e., hazard identification). When adverse effects are seen following some reasonable (e.g., sublethal) dose, subsequent studies must define exposures that do, and do not, result in adverse effects.

D. The clearance of ENMs, their translocation to distal sites, and persistence

As with the above studies that inform about uptake, the clearance and translocation of ENMs from the initial site of exposure to distal sites is best understood from whole-animal studies. The solutes of dissolved particles in the lung can transfer to blood and lymphatic circulation. Some ENMs in the airway wall that slowly dissolve or are insoluble will be cleared within a few days from the lung by cough or the mucociliary escalator. Slowly dissolving and insoluble ENMs that reach the alveoli may be taken up by macrophages. **Macrophage-mediated phagocytosis is the main mechanism for clearing foreign material from the deep lungs (alveoli) and from other organs**. Macrophages are ~20 m in diameter and able to phagocytose materials up to 15 m in length. They engulf the particle in a vacuole (phagosome) containing

enzymes and oxidizing moieties that catabolize it. **Particles resistant to catabolism may remain inside the macrophage**. **After the death of the macrophage the material may be engulfed by another cell.** **Therefore, it may take a long time for insoluble material to be cleared from the body. The elimination half-live of insoluble inert particles from the lung can be years** [145]. This raises the question of the ultimate fate of "poorly digestible" ENMs that are **engulfed by macrophages in the lung, liver (Kupffer cells), brain (microglia), and other organs.** Some ENMs, e.g., those that have a high aspect ratio, are not effectively cleared by macrophages. Alveolar macrophages that cannot digest high-aspect-ratio CNTs (termed "frustrated phagocytosis") **can produce a prolonged release of inflammatory mediators, cytokines, chemokines, and ROS** [146]. **This can result in sustained inflammation and eventually fibrotic changes.** Studies have demonstrated **MWCNT-induced pulmonary inflammation and fibrosis, similar to that produced by chrysotile asbestos and to a greater extent than that produced by ultrafine carbon black or SWCNTs** [117]. Greater toxicity from a high-aspect-ratio metal oxide (titania) ENM has also been shown in cells in culture and in vivo [147]. Studies such as these have raised questions (and concern) about the long-term adverse effects of ENM exposure. Translocation of ENMs from the lung has been shown. **After MWCNT inhalation or aspiration they were observed in subpleural**



tissue, the site of mesotheliomas, where they caused fibrosis [148,149]. Once ENMs enter the circulatory system across the 0.5-m thick membrane separating the alveoli from blood, the sites of reticuloendothelial system function (including the lymph nodes, spleen, Kupffer cells, and microglia) clear most ENMs. **Thirty to 40 nm insoluble 13C particles translocated, primarily to the liver, following inhalation exposure** [150]. **Similarly 15 and 80 nm 192iridium particles translocated from lung to liver, spleen, heart, and brain.** The extent of translocation was < 0.2%, and greater with the smaller ENMs [151]. **ENMs have also been shown to translocate following injection.** **Indirect evidence was shown of fullerene distribution into, and adverse effects in, the fetus 18 h after its injection into the peritoneal cavity of pregnant mice on day 10 of gestation** [152]. Following subcutaneous injection of commercial 25 to 70 nm titania particles into pregnant mice 3, 7, 10, and **14 days post coitum, aggregates of 100 to 200 nm titania were seen in the testes of offspring at 4 days and 6 weeks post-partum and in brain at 6 weeks post-partum**. **Abnormal testicular morphology and evidence of apoptosis in the brain indicated fetal titania exposure had adverse effects on development. The authors attribute these effects to ENM translocation across the placenta [153]. ENM excretion into milk and oral absorption post-partum might contribute to ENM presence in the offspring**, **but we are unaware of any studies assessing ENM translocation into milk.** Non-protein bound substances generally enter milk

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Comment [86]: So what happens is that you have this constant pain and disruption in the body causing huge amounts of imbalances from alkaline/acid to bacterium and fungal and yeast that will seem to not want to go away and this would be one reason why due to the fact that the white blood cells and enzymes are unable to remove these nano then what happens the body will try to shift them out~ in the mean time there is the constant re uptake of these particulates further exasperating the immune system~ so then you will have to find ways to induce this release or else the body will be in prolong suffering

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Comment [87]: Pleural/subpleural
A pleura is a serous membrane which folds back onto itself to form a two-layered membrane structure.
The thin space between the two pleural layers is known as the pleural cavity and normally contains a small amount of pleural fluid. The outer pleura (parietal pleura) is attached to the chest wall. The inner pleura (visceral pleura) covers the lung ... [4]

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Comment [88]: Translocation of Nano from lung to eventually the brain

FREEDOM 4/17/2017 10:45 AM

Comment [89]: This would indicate the same for human or any other life that would be inoculated with any form of nano delivery

FREEDOM 4/17/2017 10:45 AM

Comment [90]: So this is indicating that the nano injections were seen after the offspring were born an in the week to come the adjuvants had eventually moved into the brain a possible connection to autism? Metal poisoning on a nano scale would cause a brain malfunction espec ... [5]

FREEDOM 4/17/2017 10:45 AM

Comment [91]: Interesting –no studies they were aware of does not indicate that the translocation would not occur there since this would pass the blood brain barrier this would cross into any organ or tissue –from what is gathered so far with the study depending on the density volume and material if can pretty welll go ... [6]

by diffusion, and reach an equilibrium between milk and blood based on their pKa and the pH difference between blood and milk, described by the Henderson-Hasselbalch equation. **Given the size of most ENMs, it is unlikely they would diffuse across the mammary epithelium. Within 40 weeks after a single intrascrotal injection of MWCNTs most rats died or were moribund with intraperitoneal disseminated mesothelioma, which were invasive to adjacent tissue, including the pleura.** Fibrous MWCNT particles were seen in the liver and mesenteric lymph nodes, suggesting peritoneal effects might have been due to MWCNT translocation [154]. **The distribution of carbon-, metal- and metal oxide based ENMs after translocation from the lung, skin or intestine is similar to that seen after their iv administration**. They generally appear as agglomerates in the liver and spleen [23,93,132,151,155-158]. **The ENMs are usually in the cytoplasm, with little indication that they enter the nucleus [132,134,158-160]. Due to their small size ENMs may gain access to regions of the body that are normally protected from xenobiotics (sanctuaries), such as the brain**. This feature has suggested their potential application for drug delivery to the brain, which is being extensively pursued [161-164], **but at the same time it raises concern about central nervous system distribution of ENMs when exposure is not intended**. Studies have generally **found << 1% of the administered dose of ceria and iridium ENMs translocate to the brain after inhalation exposure or iv injection** [23,132,151]. **Anionic polymer ENMs entered the brain more readily than neutral or cationic ones**. **Both anionic and cationic ENMs altered bloodbrain barrier integrity** [165]. The persistence of ENMs may be a major factor contributing to their effects. **Many ENMs are designed to be mechanically strong and resist degradation** [22]. Referring to nanoscale fiber-like structures, it has been stated: **"The slower [they] are cleared (high bio-persistence) the higher is the probability of an adverse response"** [166]. The analogy of high-aspect-ratio ENMs to asbestos is one of the contributors to this concern. **The prolonged physical presence of ENMs, that are not metabolized or cleared by macrophages or other defense mechanisms, appears to elicit ongoing cell responses**. **The majority of CNTs are assumed to be biopersistent**. For example, two months after the intratracheal instillation of 0.5, 2 or 5 mg of ~0.7 m and ~6 m MWCNTs, 40 and 80% of the lowest dose remained in the lungs of rats, suggesting adequate persistence to cause adverse effects that are summarized in II, B, 1 ENM exposure effects in the lung [143]. Following oral administration, **50-nm non-ionic polystyrene ENMs were seen in mesenteric lymphatic tissues, liver, and spleen 10 days later** [167]. **Following iv administration, carboxylated-MWCNTs were cleared from circulation and translocated to lung and liver; by day 28 they were cleared from the liver**, **but not from the lung** [168]. No significant decrease of the amount (mass) of cerium was seen in the liver or spleen of rats up to 30 days after iv administration of 5 or 30 nm ceria. **Hepatic granuloma and giant cells containing agglomerates in the cytoplasm of the red pulp and thickened arterioles in white pulp were seen in the spleen**

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Comment [92]: Through IV administration~ meaning once it goes through the system it can translocate~ also this could be possible the immune system trying to remove this as well but again accordingly this nano seems to act out on it's own in what ever it is exposed to

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Comment [93]: Inhalation and injection

FREEDOM 4/17/2017 10:45 AM
Comment [94]: a negatively-charged ion.

FREEDOM 4/17/2017 10:45 AM
Comment [95]: Referring to positively charged ions and their properties

FREEDOM 4/17/2017 10:45 AM
Comment [96]: Another Validation that what does not break down and stays behind will indeed cause a lon term effect –a negative one-- The prolonged physical presence of ENMs, that are not metabolized or cleared by macrophages or other defense mechanisms, appears to elicit ongoing cell responses.

(**unpublished data, R. Yokel) [159,169]. In summary, the persistence of ENMs in tissue raises justifiable concerns about their potential to cause longterm or delayed toxicity**.

E. **The physico-chemical properties of ENMs that impact their hazard** - The role of surface coating in ENM effects

Many surface coatings have been investigated in order to develop ENMs as carriers for drug delivery. Surface modifications can prolong **ENM circulation in blood, enhance uptake at a target site, affect translocation, and alter excretion**. **When ENMs enter a biological milieu they rapidly become surface coated with substances such as fulvic and humic acids and proteins, all of which can alter their effects** [142,170,171]. When 3.5, 20, and 40 nm gold and DeGussa P-25 titania ENMs were incubated with human plasma, proteins **appeared to form a monolayer on the ENMs**. **The abundance of plasma proteins on gold approximated their abundance in plasma, whereas some proteins were highly enriched on titania** [172]. **Metal oxide and carbon-based ENMs rapidly adsorb proteins [66], resulting in changes in their zeta potential (electrical potential at the ENM surface) and toxicity** [142,171]. For circulating ENMs, the surface coating is extremely important, because this is what contacts cells [173]. Although it is understood that ENMs will be surface coated with proteins, lipids or other materials, which may or may not persist on the ENM surface when they enter cells, little is known about the surface associated molecules on ENMs within cells. It is likely, however, that surface coatings profoundly influence ENM effects within cells. Although surface functional groups are known to modify ENM physico-chemical and biological effects, there is little information on the influence of functional groups on health effects. This further complicates the prediction of ENM toxicity in humans from in vitro, and perhaps in vivo, studies.

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Comment [97]: So some of these nanometals will actually feed on specific type of proteins

F. **The effects of ENMs at distal sites**

Reported systemic effects of pulmonary-originating CNTs include acute **mitochondrial DNA damage, atherosclerosis, distressed aortic mitochondrial homeostasis, accelerated atherogenesis, increased serum inflammatory proteins, blood coagulation, hepatotoxicity, eosinophil activation (suggesting an allergic response), release of IL-6 (the main inducer of the acute phase inflammatory response), and an increase of plasminogen activator inhibitor-1 (a pro-coagulant acute phase protein**) [118]. Elevation of the serum analyte ALT was reported up to 3 months after intratracheal MWCNT instillation, suggesting **ENM-induced hepatotoxicity [174]. The translocation of ENMs and their release of cytokines and other factors could potentially affect all organ systems, including the brain**. For example, daily ip injection of titania for 14 days resulted in a dose-**dependent increase of titanium and oxidative stress and a decrease of anti-oxidative enzymes in the brain of rats** [140].

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Comment [98]: It almost appears to be triggering a response so it can aggregate or integrate or "feed" on the materials being triggered – a majority of the responses here are protein triggers

III. **Hazard Assessment from Fire and Explosion of ENMs Some ENMs have very high reactivity for catalytic reactions, thus raising the possibility of fire and/or explosion. As particle size decreases and surface area increases, the ease of ignition and the likelihood of a dust explosion increase**. The latter may create a second hazard due to increased ENM release. **There are no reports that ENMs have been used intentionally, e.g. by terrorists, or unintentionally to cause fires, explosions, or an airborne obscurant effect**. IV. Exposure Assessment Another key element of the Risk Assessment/Risk Management framework is exposure assessment, which includes the most likely routes of ENM exposure. Not much is known about the extent of occupational exposure to ENMs. There are ~20 published studies [51]. "In the absence of solid exposure data, no solid risk evaluation can be conducted" [175]. There is obvious value in conducting exposure assessments in the workplace to identify the routes, extent, and frequency of ENM exposure. In assessing worker exposure, the traditional industrial hygiene sampling method of collecting samples in the breathing zone of the worker (personal sampling) is preferred over area sampling. Only a few of the studies cited [51] conducted breathing zone measurements. On the other hand, area samples (e.g., size-fractionated aerosol samples) and real-time (direct-reading) exposure measurements are useful for evaluating engineering controls, and their efficacy, and work practices. When monitoring potential workplace exposure to ENMs it is critical that background nanoscale particle measurements be conducted before their production, processing, or handling in order to obtain baseline data. Nanosize particles **frequently come from non-ENM sources, such as ultrafines from internal combustion engines and welding** [176,177]. An early study of SWCNT release during its handling in the workplace showed very low airborne concentrations of agglomerated material [178]. The rapid agglomeration of ENMs in air has been repeatedly shown [55,178,179]. **Airborne ENMs associate with other airborne materials when present, or self-associate in their absence. Once formed there was little decrease in the resultant airborne agglomerations for up to 4 h [55].** An on-site monitoring study of carbon nanofibers (CNFs) in a university-based research laboratory showed an increase of **> 500-nm particles in air during weighing and mixing (total carbon levels in inhalable dust samples of 64 and 93 g/m3, respectively**). Handling the bulk partially-dry product on the lab bench generated 221 g/m3, and wet-saw cutting (which sprays water on the object being cut to lessen dusts) of a **CNF composite released > 400-nm particles (1094 g/m 3).** Office background was 15 to 19 g/m3. **Surface samples had up to 30-fold more total carbon than the office floor [180]. Another study showed that wet cutting of a hybrid CNT in an epoxy resin or in a woven alumina fiber cloth using a cutting wheel with water to flush dust particles produced no significant increase of airborne 5- to 1000nm particles in the operator breathing zone, whereas dry machining did** [181]. Production of a nanocomposite containing alumina in a polymer by a twin-screw extrusion process caused release of 5- to 20-nm and **50- to 200-nm alumina in the worker's breathing zone [**182]. Covering the top of the feeding throat and the open mouth of the particle feeder,

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Comment [99]: This is hilarious if there was a situation to investigate unless he device is intact there would be no evidence of the nano involved due to the rapid flow and exchange of heat is 10,000 time faster and that the particle size would also impact the level of heat and conductivity what evidence would there be?

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Comment [100]: This is in a lab – imagine what it is in the outdoors and with the variances of the mixture of environmental and lab created mixture that is not only airborne but in the land and in the oceans and waterways and when you consider this as a technology affecting biology then your now looking at this from a whole new paradym including aquatic avian insect reptilian and amphibian and mammalian and human life and how this is crossing over the boundaries in just about all forms of living including pathologies that may mutate as a result of the exposure~ in general when a virus or bacterium or fungi are initiated to growth they develop a counter to whatever is trying to assimilate them and as a result you get a stronger strain this will eventually carry over into this realm as well and the mutations will not just be in this form it will cross over into everything

thorough cleaning by washing the floor, and water-based removal of residual dust on all equipment significantly decreased airborne particles [182,183]. These results suggest that some engineering controls may be appropriate to safely remove some airborne ENMs, **including maintaining the room at negative pressure relative to the outside,** avoiding the handling of dry ENMs, adequate ventilation, and containment of the ENM material during its use. NIOSH researchers developed a Nanoparticle Emission Assessment Technique (NEAT) for use in the workplace [56]. They used the technique to determine particle number concentrations using two hand-held, direct-reading, particle number concentration-measuring instruments, a condensation and an optical particle counter, to survey 12 sites working with ENMs. This was complemented by collection of particles on filters and transmission electron microscopic visualization. The results demonstrated the utility of NEAT and, in some cases, the source of ENM release and efficacy of engineering controls [179]. Engineering controls are discussed in more detail below.-**There are numerous reports of adverse lung effects, and some reports of human deaths, from nanosized polymer fumes**[26]. Two deaths were reported among seven 18- to 47-year-old female workers exposed to **polyacrylate nanoparticles for 5 to 13 months.** Cotton gauze masks were the only PPE used, and were used only occasionally. The workplace had one door, no windows, and no exhaust ventilation for the prior 5 months [184]. **Workers presented with dyspnea on exertion, pericardial and pleural effusions, and rash with intense itching**. Spirometry showed that all suffered from small airway injury and restrictive ventilatory function; three had severe lung damage. Non-specific pulmonary inflammation, fibrosis, and foreign-body granulomas of the pleura were seen. **Fibrous-coated nanoparticles (~30 nm) were observed in the chest fluid and lodged in the cytoplasm, nuclei, and other cytoplasmic organelles of pulmonary epithelial and mesothelial cells**. Two workers died of respiratory failure. Although presented as the first report of clinical toxicity in humans associated with long-term ENM exposure, many experts have expressed uncertainty that ENMs contributed to these outcomes [22,185,186]. Given the poor environmental conditions of the workplace and lack of effective PPE use, these outcomes could probably have been prevented.

V. **Risk Characterization** The giant insurance firm Lloyd's of London conducted a risk assessment and concluded "Our exposure to nanotechnology must therefore be considered and examined very carefully" [http://www.nanolawreport.com/2007/12/ articles/review-lloyds-new-nano-insurance-report/]. Japan's Ministry of Health, Labour and Welfare funded studies starting in 2005 to establish health risk assessment methodology of manufactured nanomaterials. It was recently concluded that studies of metals and SWCNTs from Japan are not yet sufficient to evaluate ENM risk [187]. However, a new study incorporated a physiologically-based lung model and data of particle sizes of airborne titania ENM during manufacturing t**o estimate anatase and rutile titania ENM burdens and adverse effects in lung cells. The authors concluded that workers exposed to relatively high airborne 10- to 30nm anatase titania are unlikely to have substantial**

risk for lung inflammatory responses, but are at risk for cytotoxicity [188]. Risk characterization and assessment and gap analysis case studies were conducted with fullerenes, CNTs, silver as a example of a metal, and titania as an example of a metal oxide ENM [189]. Numerous additional data gaps were identified for each. VI. Risk Management There are no existing regulations or standards for ENMs within the three jurisdictions that have the largest

nanotechnology funding, the U.S., EU and Japan [190]. In the U.S. OSHA would set standards for occupational exposure to ENMs. Three types of standards are relevant for ENMs under the Occupational Safety and Health Act [191]. 1) Substance-specific standards, for which there are none for ENMs. 2) General respiratory protection standards, under which inhalable ENMs would be considered particulates not otherwise regulated, e.g. "nuisance dust", with a 5 mg/m 3 timeweighted average air exposure limit, determined by breathing-zone air samples. The respiratory protection standard requires employers provide workers with NIOSH-certified respirators or other PPE when engineering controls are not adequate to protect health. 3) The hazard communication standard states producers and importers of chemicals must develop Material Safety Data Sheets [191]. The U.S. EPA, using the legislative authority of the Toxic Substances Control Act has taken steps to limit the use and exposure to ENMs, including CNTs. EPA has required the use of PPE and limitiation on ENM use and environmental exposures [22]. NIOSH prepared a draft Current Intelligence Bulletin: "Occupational Exposure to Carbon Nanotubes and Nanofibers" (http://www.cdc.gov/niosh/docket/review/docket161A/pdfs/carbonNanotubeCIB_PublicReviewOfDraft.pdf). NIOSH recommends an 8-hour time weighted average exposure limit of 7 g carbon nanotubes and nanofibers/m3 air, and that employers minimize exposure to these materials. Suggested implementation includes many of the primary prevention measures discussed in this review and an occupational health surveillance program of exposure and medical monitoring. Given the 7 g/m3 level is below total airborne carbon in non-CNT-production settings (offices) [180], the ubiquitous presence of CNTs which is probably due to hydrocarbon combustion [192], and the necessity to differentiate CNTs from other carbon sources to estimate airborne nanotube and nanofiber concentration, assuring their airborne level of < 7 g/m3 may be difficult. The goal in managing the potential risks from ENMs is to minimize exposure. In the absence of specific information on ENMs, the extensive scientific literature on airborne, respirable aerosols and fibers has been used to develop interim guidance for working safely with ENMs [193] [http://ehs.mit.edu/site/content/nanomaterialstoxicity] [http://www.astm.org/Standards/E2535.htm]. The general approach to minimizing exposure is shown in Figure 4, with the preferred followed by less desireable controls shown by the downward pointing arrow. Occupational health surveillance, which includes hazard and medical surveillance, is the process whereby information from any of these activities is collected and used to support or modify what is done at a higher step in

Figure 4 **Elements of occupational health protection.** The continuum of prevention and the heirarchy of exposure control (left arrow) and occupational health surveillance (right arrow). Adapted from [222] and [194].

The hierachy, as shown by the upward pointing arrow [194]. Those steps in the hierachy that have been investigated for ENMs are further discussed below.

A. Engineering controls

ENM exposure can be reduced through the use of engineering controls, such as process changes, material containment, and enclosures operating at negative pressure compared to the worker's breathing zone; worker isolation; separated rooms; the use of robots; and local exhaust ventilation (LEV). Given the lack of occupational exposure standards to provide guidance, the most prudent approach is to minimize exposure. A survey found that engineering controls in Switzerland were more commonly used in the production of powder than liquid ENMs. For the former, the use of PPE (masks, gloves, safety glasses, or full protective suits) were the norm, although used by only ~16, 19, 19, and 8% of the workers, respectively [195]. This low use of PPE is thought to reflect the early stage of development of the ENM industry. It is anticipated that as this industry matures and knowledge is gained, control will more commonly include superior methods in the hierarchy of exposure control [196]. An international survey of ENM industry managers conducted in 2009-2010 by the

University of California Center for Environmental Implications of Nanotechnology that focused on industry controls of ENM exposure, use of PPE, environmental risks, and perceptions revealed that 46% of the companies had a nano-specific environmental health and safety program, compared to 58% in a 2006 survey [197]. Some companies (a minority) were using inappropriate occupational environmental clean-up methods, such as sweeping and compressed air [198]. These results suggest more widespread adoption of nano-specific environmental health and safety programs and the use of PPE in the absence of superior controls are appropriate.

1. Process containment

Process/source enclosure (i.e., isolating the ENM from the worker) can be aided by glove boxes, chemical fume hoods, biological safety cabinets (BSC), or an externallyvented LEV system. However, one should also consider that these methods can release ENMs into the environment, potentially creating environmental pollution and loss of costly material. ENM handling is often conducted in fume hoods. Field sampling conducted to determine fume hood, work zone, and background concentrations of PM2.5 (< 2.5 m) particles during production of fullerenes and other carbon-containing ENMs showed handling produced aerosolization of 5 to 100 nm particles, which were contained by the fume hood [199]. Monitoring aerosolized particles during chemical vapor deposition (CVD) SWCNT synthesis and aerosol-assisted CVD MWCNT synthesis in a fume hood showed significant release at the source, but not outside of the hood, suggesting fume hood use did not create fugitive airborne emissions and

was necessary to protect workers [144]. These authors also determined the release of dry powder alumina (27 to 56 nm primary particle size, 200 nm agglomerates) and 60 nm silver ENM into the researcher's breathing zone and laboratory environment when poured or transferred in 3 fume hoods; 1) a conventional hood that has a constant air flow with velocity inversely related to sash height, 2) a by-pass hood which attempts to maintain a constant velocity by use of a bypass grill above the hood which becomes uncovered, allowing more air flow through it rather than the hood face as the sash is lowered, and 3) a constant velocity (variable air volume) hood that uses a motor to vary fan speed as the sash is moved. The results showed significant release of ENMs into the researcher's breathing zone and laboratory environment and identified the variables affecting release. These included hood face velocities < 80 ft/min (< 0.4 m/s) (due to room air currents and operator movements) and > 120 ft/min (> 0.6 m/s) (due to turbulence within the hood). The constant velocity hood performed better than the by-pass hood, which in turn performed better than the conventional hood [200]. Tests were also conducted with alumina nanoparticles (primary particle size 27 to 56 nm; present as dry bulk material ~200 nm) to compare particles in the breathing zone during transfer and pouring in constant flow, constant velocity and air-curtain hoods. The newly developed air-curtain hood is evidently not commercially available. The results showed much lower levels with the air curtain hood [201]. Sash height, which affected hood face velocity, affected ENM release. Rapid removal of the worker's arm from a BSC also withdrew ENMs, releasing them outside the cabinet. Worker motion and body size affected ENM release from a traditional, but not the air-curtain, hood. The authors found that ENM handling in traditional fume hoods with a face velocity of 0.4 to 0.5 m/s (~80 to 100 ft/min) and careful motions minimized fugitive ENM emission. In 2008 the Center for High Rate Manufacturing recommended locating equipment at least 6 inches (15 cm) behind the sash, minimizing hood clutter, and avoiding rapid or violent motions while working in the hood [202]. In a study conducted in an industrial setting, use of an exhaust hood during procedures that are more likely to release ENMs (their production, handling, measurement, and reactor cleanout) resulted in no significant increase of ENMs in the workplace [203]. These

studies show that significant reduction of worker exposure to ENMs can be achieved using available fume hoods and consideration of worker activities within these hoods. Labconco Corporation has marketed a modified Class I BSC for handling nanoparticles [http://www.labconco.com/_scripts/editc20.asp?CatID=82]. It has an all stainless steel interior for ease of cleaning, perforated rear baffle to reduce turbulence, and a replaceable HEPA filter. It is available with a built-in ionizer to attract particles to the interior surface of the hood, and an external exhaust for volatiles.

2. Local exhaust ventilation (LEV)

Air-displacement ventilation in an industrial setting was accomplished by introduction of supply air that entered at low velocity at the floor level and was cooler than room air. As the air rose it became warmer and was exhausted close

to the ceiling. This provided efficient clearing of ENMs from the breathing zone [204]. A well-designed exhaust ventilation system with a HEPA filter should effectively capture airborne nanoparticles. A "down flow" booth, "elephant trunk", or fume hood may not provide sufficient protection because they may cause turbulence, spinning the ENM out of the airflow [201]. The effectiveness of engineering controls in ENM production and research facilities has been demonstrated in a few cases. Prior to use of engineering-control measures, total airborne mass concentrations of MWCNTs, measured by area sampling, were 0.21 to 0.43 mg/m3 in a laboratory research facility where the powders were blended to formulate composites. After enclosing and ventilating the blending equipment and re-locating another piece of equipment that produced considerable vibration, the concentration decreased to below the limit of detection [205]. In another study, the effectiveness of LEV was assessed during clean-out of slag and waste, which used brushes and scrapers, of reactors that produced 15 to 50 nm diameter ENMs. A portable LEV unit was used that had been shown to reduce welding fume exposure [206]. The reduction in release of 300to 10,000-nm Ag, Co, and Mn particles during cleanout of reactors used to make nanoscale metal catalytic materials was 75, 94, and 96%, respectively [207]. During the manual sanding of epoxy test samples reinforced with MWCNTs, an order of magnitude more particles, which were predominantly > 300 nm, was observed in a worker's breathing zone when the work was conducted in a custom fume hood rather than on a work table with no LEV. The poor performance of the custom fume hood may have been due to the lack of a front sash and rear baffles, and to low face velocity (0.39 m/sec). Respirable particles were an order of magnitude lower when the work was conducted in a BSC than on a work table [208]. These results illustrate the importance of good exhaust hood design as well as the worker protection provided by a BSC.

B. **Administrative controls**

When engineering controls are not feasible for reducing exposure, administrative controls should be implemented. These are policies and procedures aimed at limiting worker exposure to a hazard [209]. These could include a nanoscale material hygiene plan; preparation, training in, and monitoring use of standard operating procedures; reduction of exposure time; modification of work practices; and good workplace and housekeeping practices. For example, one laboratory was thoroughly cleaned after high air concentrations of nanoscale materials were measured in a facility engaged in the commercial compounding of nanocomposites [183]. A large decrease of airborne 30 to 100 nm particles resulted. Subsequent routine maintenance kept the particles below those originally observed, leading the authors to conclude that this administrative control was beneficial in reducing potential exposure. Biological monitoring and medical examination, a component of secondary prevention (Figure 4), is another administrative control [209]. It is discussed below.

C. Personal protective equipment

deviates around the fiber but large denser-than-air particles do not and impact the fiber due to their inertia, as shown in Figure 5; and 2) interception where the

particle trajectory takes it within a particle radius of the fiber, which captures the particle. Airborne nanoparticles behave much like gas particles. Particles < 100 nm

The last line of defense in the hierarchy of exposure control is the use of PPE, such as respirators, protective clothing, and gloves.

1. **Respirators**

Major types of respiratory protection include dust masks, filtering facepiece respirators, chemical cartridge/ gas mask respirators, and powered air-purifying respirators. Examples can be seen at OSHA's Respiratory Protection Standard site [http://web.utk.edu/~ehss/pdf/rpp. pdf]. NIOSH classifies filter efficiency levels as Type 95, 99, and 100, which are 95, 99, and 99.7% efficient, respectively. The filter's resistance to oil is designated as N, R, and P; N (not resistant to oil), R (resistant to oil), and P (oil proof). Some industrial oils can remove electrostatic charges from filter media, reducing filter efficiency. Efficiency of N filters is determined using 300-nm median aerodynamic, charge neutralized, NaCl particles at a flow rate of 85 l/min; R and P filters are tested with dioctyl phthalate oil. The European Standards (EN 143 and EN 149) rank filtering facepiece (FFP) respirators as FFP1, FFP2, and FFP3, which are 80, 94, and 99% efficient, respectively, indicated by CE (for Conformité Européene) on complying products. They are tested with non-neutralized NaCl at 95 l/min. Particles > 100 nm are collected on filter media by two mechanisms: 1) inertial impaction in which air flow

Figure 5 The mechanisms of ENM association with fiber materials. Each panel shows particles carried by airstreams, indicated by the bands with right pointing arrows. Some particles are retained by the fiber. Those that are not continue on the airstream past the fiber. The upper panel shows a large particle that is unable to follow the airstream around the fiber and collides with the fiber due to inertial impaction. The particle trapped by interception comes close enough to the fiber (within the particle radius) that it is captured by the fiber. **Electrostatic attraction is discussed in the text VI, C,** 1. Respirators. Small particles collide with each other, gas molecules, and other suspended matter in the air stream, resulting in Brownian motion and a random zigzagging path of movement, which may cause the particle to hit the fiber, as shown in the diffusion panel. are collected by diffusion. **Charged particles are trapped by electrostatic attraction, which involves an electrically charged particle and an electrically charged (electret) fiber**. **Electret filters are constructed from charged fibers.** This appears to be a significant mechanism for respirator trapping of ENMs [210]. **Neutral particles that pass through a charged fiber can be polarized by the electric field, thereby inducing charge to the particle**. In dry conditions, ENM penetration decreases with time. With continued use, however, ENM penetration through an electrostatic filter increases; this was suggested to be due to the humidity of exhalation [211]. **Soaking fiber filters in isopropanol removes electrostatic charge.** Studies treating filtering face piece respirators with

isopropanol, and then drying them, **showed increased penetration of particles > 30 nm** [210], **indicating electrostatic charge is a significant mechanism of fiber entrapment of ENMs above this size**. Figure 6 shows results of some studies that assessed the efficacy of different types of dust masks and filtering facepiece respirators to retain ENMs. Most of the studies were conducted with different sizes of NaCl, but a few used silver, graphite or titania. The results show that dust masks purchased at hardware or home improvement stores would not be expected to protect the wearer. The results also show that the NIOSH and CE respirators generally limit penetration of ENMs to concentrations below their ranked efficiency level, which is based on 300 nm particles, except for the N99 filter, which did not retain more than 99% of nanoscale NaCl (Panel D). Most of the results shown were obtained with a flow rate of 85 l/min (modeling heavy work load). **Flow rate affects particle penetration**; an example is shown in Panel C where 30 l/min (modeling low/ moderate intensity work) and 85 l/min flow rates were compared**. Increasing flow rate increased penetration**. This was further shown in a comparison of 30, 85, and 150 l/min flow rates with N95 and N99 filtering facepiece respirators [212], which is not shown in Figure 6. This highest flow rate was intended to model an instantaneous peak inspiratory flow during moderate to strenuous work. A similar result of ENM penetration positively correlating with air flow rate is shown in Panel F, where 5.3 and 9.6 cm/sec face velocity rates were compared. The results shown in Figure 6 also indicate ENM penetration is influenced by particle composition. Panel G shows greater penetration of titania than graphite through FFP3 respirators under the same experimental conditions. Panel H shows greater penetration of 20 to 30 nm NaCl than silver ENMs of the same size through P100 filters. These results suggest further work is warranted to understand the influence of the physico-chemical properties of ENMs, **particularly size, charge, and shape, on their penetration through filtering facepiece respirators**. An issue that significantly impacts filtering facepiece respirator effectiveness is its seal around the face. "The biggest source of leakage is around the respirator seal because of poor fit" (Ronald E. Shaffer quoted in [213]). **It has been estimated that 20% or more leakage occurs in respirators that are not properly fitted** [214]. This underscores the importance of a proper fit for face mask respirators. There is a particle size that maximally penetrates each filter material; the most penetrating particle size (MPPS). The results shown in Figure 6 indicate **that the MPPS is ~40 to 50 nm for ENMs**. This is approximately the same size of spherical ENMs that appear to contribute to their greatest differences in biological systems from solution and bulk forms of the same materials, as discussed in II, A, 2. The physico-chemical properties of ENMs that impact their uptake. This feature raises concern because the size of ENMs that may have the greatest effects in people are those that are best able to penetrate filtering facepiece respirators. Until results are obtained from clinical-laboratory or work-place studies, traditional respirator selection guidelines should be used. These are based on OSHA Assigned Protection Factor values (the workplace level of respiratory protection that a respirator is expected to provide), the British Standards Institution Guide to implementing an effective respiratory protective

device programme (BS 4275), and BS EN 529:2005 (Respiratory protective devices. Recommendations for selection, use, care and maintenance. Guidance document).
2. Protective clothing

No guidelines are available on the selection of clothing or other apparel (e.g., gloves) for the prevention of dermal exposure to ENMs. This is due in part to the minimal data available on the efficacy of existing protective clothing, including gloves. Penetration of 10 to 1000 nm NaCl through woven and fibrous fabrics showed a MPPS between 100 and 500 nm and maximum penetration of 50 to 80% [215]. Comparison of graphite nanoparticle penetration through 650 m thick cotton, 320 m polypropylene, and 115 m non-woven high-density **polyethylene textile (Tyvek®) showed ~30, 12, and 4% penetration of the MPPS (~40 nm), respectively [216]. Tyvek® permitted ~3 orders of magnitude less penetration of ~10 nm titania and platinum than cotton or a 160 m woven polyester** [217]. A study of ten nonwoven fabrics under conditions simulating workplace ENM exposure showed penetration increased with increasing air velocity and particle size (to ~300 to 500 nm). Pore structure of the various fabrics greatly influenced penetration [218]. **Although nonwoven fabrics were much more effective to protect workers from ENM** exposure than woven fabrics, they are much less comfortable to

A
Dust masks
100

B
N95 respirators
100

Penetration (%)

1 0.1 0.01 0.001

Penetration (%)

10

10 1 0.1 0.01 0.001

1

10

100

1000

1

10

100

1000

Particle size (nm)

Particle size (nm)

C
N95 30 and 85 l/min flow rates
100

D
N99
100

Penetration (%)

1 0.1 0.01 0.001

Penetration (%)

10

10 1 0.1 0.01 0.001

1

10

100

1000

1

10

100

1000

Particle size (nm)

Particle size (nm)

E
FFP2
100

F
FFP3
100

Penetration (%)

1 0.1 0.01 0.001

Penetration (%)

10

10 1 0.1 0.01 0.001

1

10

100

1000

1

10

100

1000

Particle size (nm)

Particle size (nm)

G
FFP3
100

H

P100
100

Penetration (%)

1 0.1 0.01 0.001

Penetration (%)

10

10 1 0.1 0.01 0.001

1

10

100

1000

1

10

100

1000

Particle size (nm)

Particle size (nm)

Figure **6 Particle penetration through dust masks and facepiece respirators**. Test material was NaCl, flow rate 85 l/min and values shown are mean, unless noted otherwise. Panel A: Dust masks. Results shown are the mean and most and least efficient of 7 commercially available dust masks, as purchased in home improvement/hardware stores [225]. Panel B. N95 respirators. (Circle) Results from 6 3M Engineered nanoparticles and particulate respirators [http://multimedia.3m.com/mws/mediawebserver?mwsId=66666UuZjcFSLXTtN8T_NXM2EVuQEcuZgVs6EVs6E666666]. (Square) Results from n = 2 [226]. (Triangle) Results from n = 1 at face velocity of 8.6 cm/sec [210]. (Diamond) Results from n = 5 [227]. (Hexagon) Results from n = 1 [212]. Panel C. N95 respirators at two flow rates. Results from n = 2 [226]. Panel D. N99 respirators. Results from n = 2 [212]. Panel E. FFP2 respirators. Results from n = 2 [228]. Panel F. FFP3 respirators. (Circle) Results from n = 1 [228]. (Square) Results from n = 1, with graphite at a face velocity of 9.6 cm/sec,

flow rate not reported [211]. (Triangle) Results from n = 1, with graphite at a face velocity of 5.3 cm/sec, flow rate not reported [211]. Panel G. FFP3 respirators. (Circle) Results from n = 1, with graphite at face velocity of 5.3 cm/sec, flow rate not reported [217]. (Square) Results from n = 1, with titania at face velocity of 5.3 cm/sec, flow rate not reported [217]. Panel H. P100 respirators. (Square) Results from n = 2 with silver particles [229]. (Triangle) Results from n = 2 with NaCl [229]. (Circle) Results from n = 2 with NaCl [228]. wear, suggesting improvements in fabric design or selection are needed to address this disincentive to use more effective PPE. The selection of laboratory coat materials can greatly influence the potential penetration of ENMs, which may end up on or penetrating street clothing, resulting in worker absorption or their even greater dispersion into the environment.

3. **Gloves**

An unpublished study reported in 2005 the interaction of alumina and organoclay ENMs with powder-free (natural rubber) latex, powder-free (synthetic latex) nitrile, and cotton gloves [219]. **Scanning electron microscopy showed that latex and nitrile gloves exhibited micrometer-sized surface pores/intrinsic voids**. Although these surface imperfections were not complete holes, **they may serve as pathways for the penetration of nanoparticles under unfavorable conditions, such as stretching and tearing**. **Stretching the latex and nitrile gloves to 200% of their original size greatly increased the pores/ intrinsic voids**. **The surface pores may be important if they collect nanoparticles and the user does not remove the gloves when going to another location, thereby transporting the ENMs**. Not surprisingly, there were wider gaps between the fibers in cotton gloves. The authors pointed out that ENMs may be treated with special coatings to enhance their dispersion characteristics, which may alter their permeability through glove materials. **This study, however, did not determine the penetration of ENMs through gloves. Nitrile, latex, and neoprene gloves prevented ~10 nm titania and platinum ENM penetration [217].** Double gloving has been suggested [219], which should reduce material penetration when there is glove perforation as well as dermal contamination when removing a contaminated outer glove. However, **double gloving has not been shown to significantly decrease material penetration** [220].

D. **Biological monitoring and medical examination**

includes hazard surveillence, the periodic identification of potentially hazardous practices or exposures in the workplace, assessing the extent to which they can be linked to workers, the effectiveness of controls, and the reliability of exposure measures. A goal is to help define effective elements of the risk management program for exposed workers. Occupational health surveillance also includes medical surveillance, which examines health status to determine whether an employee is able to perform essential job functions [222]. It is required when

there is exposure to a specific workplace hazard (OSHA, 29 CFR 1910.1450). This is different than medical screening or monitoring, a form of medical surveillance designed to detect early signs of work-related illness by administering tests to apparently healthy persons to detect those with early stages of disease or those at risk of disease. NIOSH concluded: "Currently there is insufficient scientific and medical evidence to recommend the specifc medical screening of workers potentially exposed to engineered nanoparticles" [222].
E. Diagnosis, therapy, and rehabilitation

The third level in the continuum of prevention and heirarchy of exposure control, tertiary prevention, includes diagnosis, therapy, and rehabilitation. Owing to the lack of documented episodes of **ENM exposure in humans that have resulted in adverse outcome, there is little experience with treatments of ENM exposure**. One example that illustrates clever application of the knowledge of ENM properties was the use **of UV light to visualize and treat the accidental dermal exposure of a human to quantum dots suspended in solution** [223].
Good work practices

Based on the current knowledge of ENM exposure risks, some good workplace practices have been suggested. They are shown in Appendix 1.
An example of risk analysis and implementation of actions to limit ENM exposure

Secondary prevention in the continuum of the prevention and heirarchy of exposure control (Figure 4) includes biological monitoring and medical examination, the early detection of asymptomatic disease, and prompt intervention when the disease is preventable or more easily treatable [221]. Occupational health surveillance is the process by which information obtained from any activity in the continuum of prevention and heirarchy of exposure control is collected and used to support or alter what is done at a step higher in the heirarchy, as shown in the right upward pointing arrow in Figure 4 and discussed in [194]. Occupational health surveillance is the ongoing systematic collection, analysis, and dissemination of exposure and health data on groups of workers for the purpose of early detection and injury. It

A recent study applied the principles of the Risk Assessment/Risk Management framework to identify and evaluate the potential hazards in a facility manufacturing ENMs [224]. The investigators established a measure of risk for each potential hazard and suggested improvement actions. These were then addressed with administrative controls, environmental monitoring, PPE and good workplace practices.

Some published guidelines for safe handling and use of ENMs

The following are some published guidelines, not regulations, for safe handling and use of ENMs. The Bundesanstalt für Arbeitsschutz und Arbeitsmedizin

(BAUA) provided a "Guidance for handling and use of nanomaterials in the workplace" in 2007 [http://www.baua.de/ en/Topics-from-A-to-Z/Hazardous-Substances/ Nanotechnology/pdf/guidance.pdf;jsessionid=E81EECA3E6B5AD1A0D3D2396C4220AF5.2_cid137?__blob=publicationFile&v=2]. The Environmental Health and Safety office at the University of California provided "Nanotechnology: Guidelines for safe research practices" as their Safety Net #132 guidelines [http://safetyservices. ucdavis.edu/safetynets/Safetynets-Master%20List/Safetynets-Master%20List/safetynet-132-nanotechnologyguidelines-for-safe-research-practices]. Similarly, the Office of Environment, Health & Safety at the University of California prepared "Nanotechnology: Guidelines for Safe Research Practices" as their publication for the Berkeley Campus, Publication No. 73 [http://nano.berkeley. edu/research/73nanotech.pdf]. The Department of Energy, Nanoscale Science Research Centers, updated their "Approach to nanomaterial ES&H" in May 2008, as Revision 3a [http://orise.orau.gov/ihos/nanotechnology/files/NSRCMay12.pdf]. The Institute de recherche Robert-Sauvé en santé et en securité du travail (IRSST) published "Health Effects of Nanoparticles" [http://www. irsst.qc.ca/files/documents/PubIRSST/R-469.pdf]. The Environmental Health and Safety office of Massachusetts Institute of Technology prepared "Nanomaterials Toxicity", which is available at [http://ehs.mit.edu/ site/content/nanomaterials-toxicity]. NIOSH has made available "Approaches to Safe Nanotechnology. Managing the Health and Safety Concerns Associated with Engineered Nanomaterials" [http://www.cdc.gov/niosh/ topics/nanotech/safenano/]. The British Standards Institute prepared "Nanotechnologies - Part 2. Guide to safe handling and disposal of manufactured nanomaterials" in 2007, as their publication PD 6699-2:2007, ICS Number Code 13.100: 71.100.99 [http://www.nanointeract. net/x/file/PD6699-2-safeHandling-Disposal.pdf]. The American Society for Testing and Materials prepared A "Standard Guide for Handling Unbound Engineered Nanoparticles in Occupational Settings", as their publication ASTM E2535-07 [http://www.astm.org/Standards/E2535.htm]. This is a guide for use when no specific information on ENMs or toxicity is available. OSHA prepared "Occupational exposure to hazardous chemicals in laboratories", as their publication 1910.1450 [http://www.osha.gov/pls/oshaweb/owadisp. show_document?p_table=standards&p_id=10106]. This guidance is designed for lab scale (i.e., not industrial) workers. The Center for High-Rate Nanomanufacturing and NIOSH are preparing a guide to safe practices for working with nanomaterials that is anticipated to be released in early 2011. Some websites that have considerable information on nanoscale materials are Nano Safe at [http://www. nanosafe.org], The International Council on Nanotechnology (ICON) [http://icon.rice.edu/], and "The

GoodNanoGuide" [http://goodnanoguide.org/tiki-index. php?page=HomePage].

Conclusions An extensive variety of ENMs has been created. ENMs have already been utilized in many products and much more extensive use is anticipated in the future. There are reports of toxicity following in vitro and in vivo exposure to many ENMs, albeit often after very high doses, and generally lacking dose-response assessment. There is a small amount of exposure assessment information, and a paucity of information required for a risk characterization. Until more research and workplace monitoring information becomes available to refine the current knowledge of ENM risks, good workplace practices and guidelines based on ultrafine materials are guiding the occupational safety and health practices in working with ENMs. Appendix 1. Some good workplace practices - Post signs indicating potential hazards, PPE requirements, and administrative controls at entrances to areas where ENMs are handled. - Use appropriate PPE as a precaution whenever failure of a control, such as an engineering control, could result in ENM exposure, or ensure that engineering controls notify workers (e.g., alarms) when equipment malfunctions. Appropriate clothing and PPE generally includes closed-toed shoes, long pants without cuffs, long-sleeved shirt, laboratory coat, nitrile gloves, eye protection and perhaps a respirator, e.g., a half-mask P-100 or one that provides a higher level of protection, as appropriate to the ENM. - Transfer ENMs between workstations in closed, labeled containers. - Avoid handling ENMs in the open air in a `free particle" state. - Store dispersible ENMs, suspended in liquids or in a dry particle form, in closed (tightly sealed) containers whenever possible. - Clean work areas potentially contaminated with ENMs at the end of each work shift, at a minimum, using either a HEPA-filtered vacuum cleaner or wet wiping methods. Do not dry sweep or use compressed air. - Consider the use of disposable absorbent bench top coverings and laboratory coats. - Place sticky floor mat at exit. - Provide facilities for hand-washing, showering and changing clothes
Prohibit food, beverages and smoking in the work area.
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Acknowledgements The authors gratefully acknowledge the financial support for nanoscale research provided by US EPA STAR Grant RD-833772. The authors thank Matt H. Hazzard and Robin L. Jones, University of Kentucky Teaching & Academic Support Center, for preparing Figures 1, 2, 3, 4, and 5 and Drs. Ronald E. Shaffer, NIOSH, and William K. Boyes, EPA, for their helpful comments on a prior version of this review, and an anonymous journal reviewer whose extensive comments surely improved this manuscript. This manuscript has been reviewed by the National Health and Environmental Effects Research Laboratory and approved for publication. Approval does not signify that the contents reflect the views of the Agency, nor does mention of trade names or commercial products constitute endorsement or recommendation for use. Author details 1 Department of Pharmaceutical Sciences, College of Pharmacy and Graduate Center for Toxicology, University of Kentucky, Lexington, KY, 40536-0082, USA. 2Toxicity Assessment Division, National Health and Environmental Effects Research Laboratory, U.S. Environmental Protection Agency, Research Triangle Park NC, 27711, USA. Authors' contributions RAY initiated this review and created a draft of the manuscript that was discussed with RCM. RCM provided significant guidance on the structure of the review and its content, wrote the Risk

Assessment/Risk Management framework section, integrated that section with the rest of the review, and provided much editorial guidance as RAY and RCM re-wrote and finalized the review. Both authors contributed to the revision of the review, responding to the reviewers' comments. Both authors read and approved the final manuscript. Competing interests The authors declare that they have no competing interests. Received: 8 October 2010 Accepted: 21 March 2011 Published: 21 March 2011 References 1. Oberdörster G, Oberdörster E, Oberdörster J: Nanotoxicology: an emerging discipline evolving from studies of ultrafine particles. Environ Health Perspect 2005, 113:823-839. 2. Stone V, Nowack B, Baun A, van den Brink N, Kammer F, Dusinska M, Handy R, Hankin S, Hassellov M, Joner E, Fernandes TF: Nanomaterials for environmental studies: classification, reference material issues, and strategies for physico-chemical characterisation. Sci Total Environ 2010, 408:1745-1754. 3. Oberdörster G: Safety assessment for nanotechnology and nanomedicine: concepts of nanotoxicology. J Int Med 2009, 267:89-105. 4. Grieger KD, Hansen SF, Baun A: The known unknowns of nanomaterials: describing and characterizing uncertainty within environmental, health and safety risks. Nanotoxicology 2009, 3:222-233. 5. Bergamaschi E: Occupational exposure to nanomaterials: present knowledge and future development. Nanotoxicology 2009, 3:194-201. 6. Hristozov D, Malsch I: Hazards and risks of engineered nanoparticles for the environment and human health. Sustainability 2009, 1:1161-1194. 7. Savolainen K, Alenius H, Norppa H, Pylkkanen L, Tuomi T, Kasper G: Risk assessment of engineered nanomaterials and nanotechnologiesa review. Toxicology 2010, 269:92-104. 8. Nanoscale Science, Engineering and Technology (NSET) Subcommittee of the U.S. National Science and Technology Council (NSTC): Nanotechnology definition, National Science Foundation. [http://www.nsf.gov/crssprgm/ nano/reports/omb_nifty50.jsp]. 9. BSi (British Standards): Terminology for nanomaterials. Publicly available specification, PAS 2007, 136:2007. 10. The Royal Academy of Engineering, The Royal Society: Nanoscience and nanotechnologies: opportunities and uncertainties. 2004 [http://www. nanotec.org.uk/report/Nano%20report%202004%20fin.pdf]. 11. Donaldson K, Stone V, MacNee W: The toxicology of ultrafine particles. Particulate Matter 1999, 115-129. 12. Duffin R, Mills NL, Donaldson K: Nanoparticles-a thoracic toxicology perspective. Yonsei Med J 2007, 48:561-572.

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NANO Overload

Cytotoxicity and Inflammatory Effect of Silver Nanoparticles in Human Cells

Cytotoxicity and Inflammatory Effect of Silver Nanoparticles in Human Cells

Jeong-shin Park, Na Mi Yu, Jinwoo Cheon and In-Hong Choi
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Department of Chemistry;
Nanomedical NCRC, Yonsei University, Seoul, Korea

1. Approaches to practical toxicology tests to assess nanoparticles
2. Cytotoxicity and inflammatory effects of silver nanoparticles

Nanoparticles and toxicity assay

The rapidly developing field of nanotechnology will result in exposure of nanoparticles to humans via several routes (e.g., inhalation, ingestion, skin, etc.). Nanoparticles can translocate from the route of exposure to other vital organs and penetrate cells.

- Toxicity studies to determine the deleterious effects of nanoparticles on living cells are required.
- Due to the nanosize and the nature of agglomeration, simple standard methods to characterize the biological effects of nanoparticles are currently unavailable.
- In this study, practical information regarding the optimal *in*

vitro tests for nanotoxicity were evaluated.

Silver nanoparticles
03/19
Antimicrobial applications

Ink

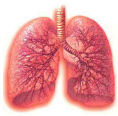
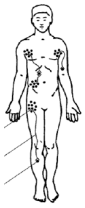
Cosmetics 200nm 200nm 500nm
20 nm (synthetic)
180 nm
(commercial,
Aldrich)

Biological tests Inflammation ➢➢ Annexin staining,caspase activation ➢➢Cytokine production,activation of Signaling molecule ➢➢ROS ➢➢Cytotoxicity➢➢ MTT/CCK-8 ➢➢ Establishment of *in vitro* toxicity assay ➢➢ Identification of mechanisms for toxicity and inflammation	**Synthesis** Production & characterization of physical and chemical properties

In vitro tests for nanoparticles

ISO/TC229 • OECD • U.S NCL **Review *in vitro* methods**	• Production of diverse particles (size, surface) • Assess biological activities **Assess toxicity tests**	Understanding of proper methods for nanoparticles **Establish proper methods**

Exposure routes of nanomaterials

Skin  **Respiratory trac**  **Immune System** 

	Cell line	Origin	Characteristics	

Respiratory	A549	Lung epithelial	Proper for cytotoxicity	
	BEAS-2B	Bronchial epithelial	Proper for cytokine Production	
Immune	U937	Macrophage	Proper for cytotoxicity and cytokine production	
Skin	SK-Mel	Skin epithelial	Proper for cytotoxicity and cytokine production	
	A375	Skin epithelial	Too fast growing	

Standard toxicology tests and silver nanoparticles

***In Vitro* Immunology Properties (Blood contact**	Hemolysis	Release of hemoglobin	Standard	Proper
	Complement activation	Activation of C3 complement	Standard	Inappropriate
		Leukocyte proliferation with mitogen stimulation	Standard	CCK-8
***In Vitro* Immunology (Cell-based assays)**	Leukocyte proliferation	Zymosan assay	Standard	Proper
		Cytokine production	Standard	Proper
	Phagocytosis			Proper
	Cytokine induction			
Toxicity	Oxidative stress	Detection of ROS	Standard	CCK-8
	Cytotoxicity (necrosis)	Cell viability and mitochondrial integrity	Standard	Annexin-V
	Cytotoxicity (apoptosis)			
		Activation of caspase 3	Standard	
Targeting	Cell	N/S	N/S	TEM,

	binding/internalization			confocal microscope or other methods

Characteristics specific to metal nanomaterials

Nanoparticles larger than 100 nm tend to aggregate relatively quickly *in vitro* when compared to nanoparticles smaller than 100 nm. Fresh samples within two weeks after synthesis is recommended for tests.

•

Each standard toxicology method must be verified before use. (ex. interference with a specific wavelength, electrophoresis)

Flow chart for nanotoxicity tests

Small –Nano Particle Size 100nm-Large

Small--Analysis Of Biological --- Nano Particle Size 100nm

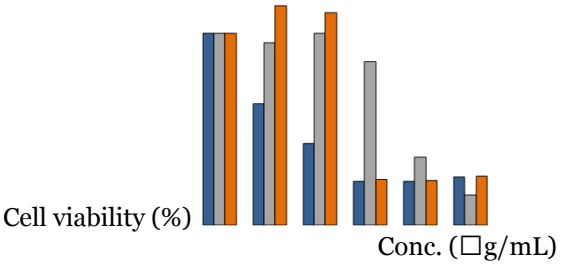
- Particle size
- Cytotoxicity
- Apoptosis
- Cytokine production
- Hemolysis
- Leukocyte proliferation
- ROS production

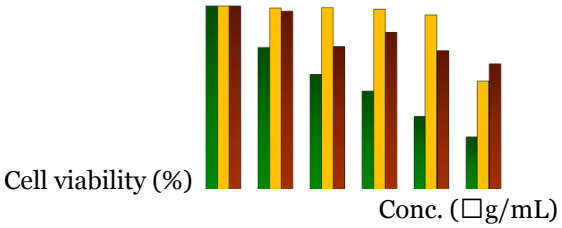
Large— Analysis of chemical/physical properties

- Aggregation
- Particle size

Cytotoxicity of silver nanoparticles

20 nm





SK-Mel28 (skin) A375 (skin) A549 (lung)

Summary

In human cells, epithelial cells from skin or lung, and macrophages, 5 nm and 20 nm silver particles induced stronger cytotoxicity and ROS synthesis than 80 nm particles did.
• 5 nm and 20 nm silver particles induced chemokine production, mainly IL-8, MIF and RANTES, while proinflammatory cytokines, IL-1, IL-6 and TNF-□ were not induced significantly in the same conditions.
• Some MAP kinase signaling pathways were activated during exposure to silver nanoparticles at lower ---concentrations which do not induce cytotoxicity

The toxicity and inflammatory effects of nanoparticles are dependent on their size. In silver nanoparticles smaller than 20 nm induce cytotoxicity significantly *in vitro*.
• Nanoparticles induce inflammatory immune responses at lower concentrations and chemokines are the major cytokines induced at early stages of exposure to silver

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Green synthesis of silver nanoparticles for the control of mosquito vectors of malaria, filariasis, and dengue.

Vector Borne Zoonotic Dis. 2012 Mar;12(3):262-8

Authors: Arjunan NK, Murugan K, Rejeeth C, Madhiyazhagan P, Barnard DR

Abstract
A biological method was used to synthesize stable silver nanoparticles that were tested as mosquito larvicides against Aedes aegypti, Anopheles stephensi, and Culex quinquefasciatus. Annona squamosa leaf broth (5%) reduced aqueous 1 mM $AgNO_3$ to stable silver nanoparticles with **an average size of 450 nm**. The structure and percentage of synthesized nanoparticles was characterized by using ultraviolet spectrophotometry, X-Ray diffraction, Fourier transform

infrared spectroscopy, and scanning electron microscopy methods. The median lethal concentrations (LC) of silver nanoparticles that killed fourth instars of Ae. aegypti, Cx. quinquefasciatus, and An. stephensi were 0.30, 0.41, and 2.12 ppm, respectively. Adult longevity (days) in male and female mosquitoes exposed as larvae to 0.1 ppm silver nanoparticles was reduced by ~30% ($p<0.05$), whereas the number of eggs laid by females exposed as larvae to 0.1 ppm silver nanoparticles decreased by 36% ($p<0.05$).--PMID: 22022807 [PubMed - indexed for MEDLINE]

Commentary

This is alarming ---that NanoSilver is being used as a pesticide---the fallout on this on plant life will also have a negative impact on growth as well and this will as well get into the water table for other insects (beneficial) will also be impacted—the issue here us how do you clean this up—once it is being sprayed and released how do you collect the particles---this is not a good option – nanoparticle do not respond the same way as base metals---this will be a pollutant being added to the eco system causing chain of events---you have to really read this to understand the actual idea of what is transpiring here---if the bugs are being killed and the larva is not happening ---then what is that doing to us---when this gets into a crop---with the glyphosates chelating the minerals out and transporting the metals in like the chemtrail fall out then these nano(metals) will also be incorporated and may eventually cause a sterility in the soil since nano silver will kill off the normal bacteria

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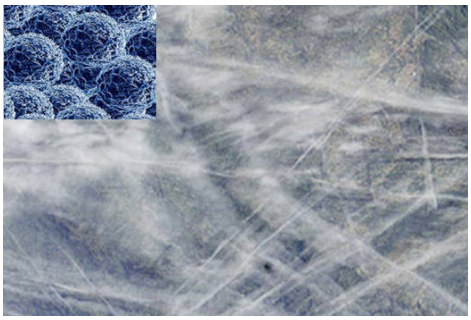
NANO CHEMTRAILS



NANO CHEMTRAILS

by William Thomas

If you did not enjoy "traditional" chemtrails raining down on you, you are not going to like the new version, which the United States Air Force promises will feature aerial dumps **of programmable "smart" molecules tens of thousands of times smaller than the particles already landing people in emergency rooms with respiratory, heart and gastrointestinal complaints**.--Under development since 1995, the military's goal is to install **microprocessors incorporating gigaflops computer capability into "smart particles" the size of a single molecule**.--**Invisible except under the magnification of powerful microscopes, these nano-size radio-controlled chips are now being made out of mono-atomic gold particles. Networked together on the ground or assembling in the air, thousands of sensors will link into a single supercomputer no larger than a grain of sand.**---Brought to you by the same military-corporate-banking complex that runs America's permanent wars, Raytheon Corp is already profiting from new weather warfare technologies. The world's fourth largest military weapons maker bought E-Systems in 1995, just one year after that military contractor bought APTI, holder of Bernard Eastlund's HAARP patents.---**Raytheon also owns General Dynamics, the world's leading manufacturer of military Unmanned Aerial Vehicles.--**Raytheon also reports the weather for NOAA through its Advanced Weather Information Processing System. According to researcher Brendan Bombaci of Durango, Colorado, these Raytheon computers are directy linked with their UAV weather modification drones. Bombaci reports that NOAA paid Raytheon more than $300 million for this "currently active, 10-year project."--She goes on to describe the Joint Environmental Toolkit used by the U.S. Air Force in its Weather Weapons System. Just the thing for planet tinkerers.

GREEN LIGHT

For public consumption, nano-weather control jargon has been sanitized. "**Microelectric Mechanical Sensors" (MMS)** and "Global Environmental Mechanical Sensors" sound passively benign. But these ultra-tiny autonomous aerial vehicles are neither M&Ms nor gems. [Space.com Oct 31/05]--According to a U.S. military flier called Military Progress, "**The green light has been given" to disperse swarms of wirelessly-networked nano-bots into the troposphere by remotely-controlled UAV drones for "global warming mitigation**."---U.S. Army Tactical Unmanned Aerial Vehicles, as well as U.S. Air Force drones "**are slated to deploy various payloads for weather warfare**," Military Progress asserts. This dual mission – to slow global warming and use weather as a weapon –

FIGHTING FOR CLIMATE CHANGE

U.S. Military Inc. is already in the climate change business big time. The single biggest burner of petroleum on this planet, its high-flying aircraft routinely rend Earth's protective radiation shielding with nitrous oxide emissions, while depositing megatons of additional carbon, sulfur and water particles directly into the stratosphere **– where they will do three-times more damage than CO_2 alone**.---Go figure. A single F-15 burns around 1,580 gallons an hour. An

Apache gunship gets about one-half mile to the gallon. The 1,838 Abrams tanks in Iraq achieve five gallons to the mile, while firing dusty radioactive shells that will continue destroying human DNA until our sun goes supernova.--A single non-nuclear carrier steaming in support burns 5,600 gallons of bunker fuel in an hour – or two million gallons of bunker oil every 14 days. Every four days, each carrier at sea takes on another half- million gallons of fuel to supply its jets.--The U.S. Air Force consumed nearly half of the Department of Defense's entire fuel supply in 2006, burning 2.6 billion gallons of jet fuel aloft.---While flying two to five-hour chemtrails missions to reflect incoming sunlight and slow global warming, a single KC-10 tanker will burn 2,050 gallons of highly toxic jet fuel every hour. The larger and older KC-135 Stratotanker carries 31,275 gallons of chemtrails and burns 2,650 gallons of fuel per hour.--The EPA says that each gallon of gasoline produces 19.4 pounds of CO2. Each gallon of diesel produces 22.2 pounds of CO2.--Total it up and routine operations by a military bigger than all other world militaries combined puts more than 48 billion tons of carbon dioxide into the atmosphere every year. Nearly half that total could be eliminated by ending the wars against Iraq and Afghanistan. [TomDispatch.com June 16/07; huffingtonpost.com Oct 29/07]

NANO RAIN

Meanwhile, the 60 year quest for weather warfare continues. Though a drone cannot carry a heavy payload, more sub-microscopic weather modification particles can be crammed into a UAV Predator than all the chemtrail slurry packed into a tanker the size of a DC-10.---According to the air force's own weather modification study, *Owning The Weather 2025*, clouds of these extremely teeny machines will be dropped into hurricanes and other weather systems to blend with storms and report real time weather data to each other and a larger sensor network.---Then these smart **particles will be used to increase or decrease the storm's size and intensity – and "steer" it to "specific targets**".---The air force report boasted that nano-chemtrails "will be able to adjust their size to optimal dimensions for a given seeding situation and make adjustments throughout the process." Instead of being sprayed into the air at the mercy of the winds aloft, as is the fate of normal chemtrails, nano versions will be able to "enhance their dispersal" by "adjusting their atmospheric buoyancy" and "communicating with each other" as they steer themselves in a single coordinated flock within their own artificial cloud**.---Nano-chemtrails will even "change their temperature and polarity to improve their seeding effects**," the air force noted. [Daily Texan July 30/07]--Rutgers University scientist J. Storrs Hall held out the military's hope that these new nano weather-warrior bots: "Interconnected, atmospherically buoyant, and having navigation capability in three dimensions – clouds of microscopic computer particles communicating with each other and with a control system, could provide tremendous capability."--Why so cheap? **Because nano particles can be potentially self-replicating. That is, they can be made to reproduce themselves until programmed to stop.--***Owning The Weather* goes on to say that the USAF will "manage and employ a weather-modification capability by the Weather Force Support Element." These weather

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Comment [101]: This goes on internally as well as soon as the frequencies activate these nanobots they will infest specific areas of the body---lung tissue—brain---intestines---muscles—they get activated and replicate—in conjunction with the genetics you are consuming this will cause extreme debilitation and depending on age will cause extreme unhealthy---

forces will use real-time updates **from swarms of the nano-size "smart sensors**" to model developing weather patterns with a super-duper computer.--Based on continually updated forecast, the weather warriors will fly follow-on missions as needed to tweak the storm. It's perfect, crows the air force. "The total weather-modification process becomes "a real-time loop of continuous, appropriate, measured interventions, and feedback capable of producing desired weather behavior."--Weather modification did not work too well with Katrina.--If the notion of inserting Autonomous Intelligence nanobots into weather systems to monitor, steer and mess with them seems risky, just wait. Around the next cloud corner are **coming swarms of airborne nano-bots to optimize wind dispersal patterns for germ warfare. Or chemtrails**.--But there's one small hitch. Nobody knows how Earth's atmosphere works. It is so big, so complex and so unpredictable, even real-time nano-snapshots are ancient history as soon as they are taken.--This is why the air force said, "Advances in the science of chaos are critical to this endeavor."-. After a decade of trying, not even a 48-hour weather forecast can be made without constant surprises.--Because they cannot be graphed in a cause-and-effect straight line, chaotic "non-linear" **weather processes can morph unexpectedly, defying predicted weather modification inputs**. --Then there's the matter of accidental genocide. I mean human health.

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Comment [102]: Already happening

Ailments and Illheath effects

The chemtrails we are too familiar with after a decade-long dose continue to inflict eye infections, nosebleeds, skin sores, muscle pain, chronic exhaustion, weakened immunity, acute asthma and allergies, short-term memory loss and heart attacks on people in more than a dozen countries. [americanskywatch.com; Chemtrails Confirmed 2008 by William Thomas]--Small particulates like the aluminum oxide found in chemtrails also kill.--Dr. Dan Woodard calls aluminum oxide a "nuisance dust". This MD says that **prolonged exposures to very high concentrations of particulates that are visible in the air "can produce pulmonary fibrosis, somewhat like the silicosis formerly seen in miners."--**"At one time it was thought to precipitate Alzheimer's disease, but more recent research has shown it is almost certainly unrelated," Dr. Woodward adds.-It's the tiny size of chemtrail fallout – one-tenth the width of the human hair – that make people very ill. The EPA warns that there is a **strong link between all tiny particles and thousands of premature deaths each year**.--Two key studies from the early 1990□s by the Harvard School of Public Health and the American Cancer Society found **strong links between high levels of small particles and a rise in death rates**. In an article headlined, "Tiny Particles Can Kill" the August 5, 2000 issue of *New Scientist* pointed to findings in six cities over 16 years showing that "city-dwellers in Europe and the U.S. are dying **young because of microscopic particles in the air."--**According to the *New York Times*, "**microscopic motes... are able to infiltrate the tiniest compartments in the lungs and pass readily into the bloodstream."** Particles in the size range called for by the Welsbach Patent describing **chemtrails are "most strongly tied to illness and early death, particularly in people who are already**

susceptible to respiratory problems." [New York Times Oct 14/06]--The Welsbach Patent calls for **megatons of 10 micron-size aluminum oxide particles to be spread in the atmosphere. The EPA calls particles this small "an extreme human health hazard" leading to 5% increased death rate within 24 hrs.--"Hearts as well as lungs can be damaged by ultra-fine particles small enough to get into the bloodstream and inflame tissues and cells,"** reports the *LA Times*. "**After they reach the heart, the particles are thought to cause a stress reaction in cells, producing inflammation that contributes to heart disease. The particles also may cause blood clots**." [Los Angeles Times Dec 29/03]--The Neurotoxicology (brain poisoning) division of the U.S. Environmental Protection Agency says **that exposure to airborne Particulate Matter "is an environmental health risk of global proportions**." [Health Risks Of Aerosoled Particulates PubMed Abstract]--The EPA explains that by penetrating deep into the lungs and circulatory system, these dust-size particles "are implicated in tens of thousands of deaths annually from both respiratory and coronary disease."-- "**SMART PARTICLES" IN YOUR BRAIN MAKE YOU BRAINDEAD-Nanoparticles might better be called smartparticles because they make a beeline for the brain as soon as they are inhaled**. Not surprisingly, **they tend to accumulate and clump in the area of the brain that deals with smell.** Too big to pass back through the blood-brain barrier, they become trapped there. [Nature.com Jan 5/04]--Dr. Celine Filippi also **observed that nanoparticles inhaled into the lungs are so mall they easily cross the lung barrier and enter the blood. "Particles in the blood can reach the liver, amongst other organs."** [globalresearch.ca Oct 21/07]

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Comment [103]: This part of the article is out dated---the nano silver can pass through the blood brain barrier ---there are reports that the silver nano has been found to congest in the brain as well

NANO CHEMTRAILS

Owning The Weather 2025 was published in 1995 and discussed only non-classified military weather modification projects. Hall's Overview of Nanotechnology also appeared in 1995, when nanotech was in its Frankenstein infancy. Since then, many sources tell us, nanotech has gone exponential. [nanotech-now.com]

Nano Chemtrails Chemtrail Mix

"They have them," he confirmed. The U.S. Air Force has occasionally added nanoparticles to the chemtrail mix to demonstrate proof of concept.--"We're way beyond science fiction," Hank confirmed. "You can hide just about anything you want in a chemtrail – including nanotubes**. Chemtrails are being altered for whatever spectrum of wavelength they're trying to bounce off of them**.

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Comment [104]: Nanotubes are usually carbon nano fibres that are 200times stronger then diamond and are usually used as some form of conduit

MORGELLONS

What about Morgellons? Is there any connection between this bizarre and frightening malady and nano experiments?"You're not going to like this. "**Morgellons is one unintended manifestation of nano spray experiments."Morgellons manifests – or presents" – as intolerable**

itching in the skin followed by alien eruptions of thin hairs or tendrils through the skin. “It’s basically the same as excreting something through a hair follicle,” Hank said.He meant a toxin – something foreign to the body.**.”If you manufacture a liquid super-cyrstalline structure, vibrate it a little and give it an electrical charge – it will form into a chain**.”--**These nanotubes will be invisible to the eye**, of course. But their tendency to clump together could eventually make them big enough to be photographed and posted on the web.--- **“Much of it is still up there,” Hank went on to explain. This is because nanoparticles are so light and small they tend bind to bind with oxygen molecules. And piggybacking on oxygen particles makes them buoyant**.--“**It travels worldwide**,” “Some of it comes down. **Whatever it’s exposed to up there it brings down here. We get exposed to it. We breathe it in, we ingest it. It accrues in the same spot every time. And attracts more of it**… ” In the liver.- And brain----“The fallout would look like a prion disease,” Hank said.-“Fallout from nanoparticles would eat holes in our brains?”-“Pretty much. **Nano particles are ionized particles that go to what attracts them…. Because of their electro-chemical properties, they are attracted to the potassium-calcium channel in the brain**.”--Think about it, he said. “If they are capable of withstanding the corrosive upper atmosphere – corrosive sunlight and all those (industrial) chemicals – what would they have to be manufactured out of? Does the body manufacture anything that can deal with that? Who will come forward and say these are good

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Comment [105]: Validating what I have said as well do not use a zapper and frequenciy devices would have to modulate on several different frequencies or a defence mechanism to offset what you throw at it

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Transformations of Nanomaterials in the Environment

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Environ. Sci. Technol., 2012, 46 (13), pp 6893–6899
DOI: 10.1021/es300839e
Publication Date (Web): May 14, 2012
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This article is part of the Transformations of Nanoparticles in the Environment special issue.
Biography

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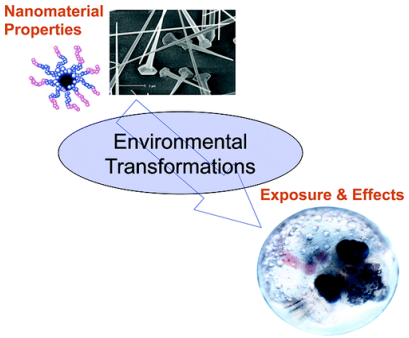
Air Pollution and Industrial Hygiene

Abstract



Increasing use of engineered nanomaterials with novel properties relative to their bulk counterparts has generated a need to define their behaviors and impacts in the environment. The high surface area to volume ratio of nanoparticles results in highly reactive and physiochemically dynamic materials in environmental media. Many transformations, e.g. reactions with biomacromolecules, redox reactions, aggregation, and dissolution, may occur in both environmental and biological systems. These transformations and others will alter the fate, transport, and toxicity of nanomaterials. The nature and extent of these transformations must be understood before significant progress can be made toward understanding the environmental risks posed by these materials.

Introduction

The nanotechnology field continues to grow rapidly and the increasing use of engineered nanomaterials (NMs) in commercial products translates into an increasing presence in the biosphere. Engineered NMs are manufactured materials having at least one dimension in the nanoscale (ca. 1–100 nm) dimension. Naturally occurring NMs are also ubiquitous in the environment, resulting from both natural processes and from anthropogenic impacts (e.g., flocculation of nanometer-scale metal oxides in acid mine drainage). The

extremely small sizes of both naturally occurring and engineered NMs results in a high percentage of surface atoms which can result in novel properties and reactivity compared to a larger size material with the same chemical composition.(1, 2) Examples of such engineered NMs include semiconductors like quantum dots which have different optical and electrical properties depending on their size,(3) and gold nanoparticles (NPs) that are typically inert but become catalytic as their size is decreased to a few nanometers.(4) NMs are becoming increasingly complex and include those with coatings that target specific cells in the body(5) or that are engineered from more than one NM for optimized utility (e.g., carbon nanotubes (CNTs) doped with quantum dots(6)). These newly emergent materials that will soon enter manufacturing supply chains are unique and xenobiotic (e.g., metal oxide NP-decorated graphene sheets(7)). The lack of a natural analog for these new materials complicates the forecasting of their fate, transport, reactivity and toxicity in environmental systems. The uncertain effects resulting from the novel properties exhibited by NMs have given rise to concerns by citizens and governments throughout the world, and a justified increase in environmental health and safety (EHS) research aimed at assessing the potential for NMs to harm the environment or human health. An overall goal of these research activities is to correlate the properties of NMs to their behavior in the environment and their effects on living organisms.(8) Assessing the environmental and human health implications of nanomaterials requires an understanding of the potential exposure routes and toxicological effects from acute and chronic exposures. To date, the predominant focus of the global research endeavor has been defining the fate, transport, and toxic properties of pristine or “as manufactured” nanomaterials. However, the high surface to volume ratio and reactivity of NMs makes them highly dynamic in environmental systems. The resulting transformations of the NMs will affect their fate, transport, and toxic properties. For example, metallic silver NPs will oxidize and may become sulfidized in the environment.**(9) Sulfidation of the particles changes their aggregation state, surface chemistry, and charge, as well as their ability to release toxic Ag^+ ions(10) and therefore their persistence and toxicity**. **Similarly, the interaction between NMs and humic substances (HS) including natural organic matter (NOM) results in a nanoscale coating of the NMs,(11) analogous to protein coronas in mammalian systems,(12) that dramatically changes their aggregation, deposition, and toxic properties.(13, 14)--**Because environmental systems are dynamic and stochastic, the physicochemical changes that accompany engineered and incidental coatings, as well as subsequent reactions in the environment, greatly complicate the understanding of risks associated with the release of NMs in the environment. We currently lack sufficient knowledge of the types, rates, and extent of transformations expected for NMs in environmental and biological systems. By extension, we also fail to understand the impact of those transformations on the fate, transport, and toxicity of NMs. To correctly forecast the environmental and human health risks associated with these materials, we must endeavor to broaden our knowledge of the transformations of NMs.

The U.S. National Research Council has recently proposed a new framework for nano EHS research.(8) This committee recommended that research should focus on understanding "**critical elements of nanomaterial interactions**", needed for assessing exposure, hazards, and hence risks posed by engineered nanomaterials (Figure 1). These critical elements include physical, chemical, and biological transformations that ultimately influence NM persistence, bioavailability/biouptake, reactivity, and toxicity.

Figure 1. Nanomaterial transformations are critical processes affecting NM interactions. Transformations include physical and chemical transformations, biologically mediated transformations, and interactions with macromolecules and biomacromolecules. Adapted from ref 8.

Oxidation and reduction (redox) reactions, dissolution, sulfidation, aggregation, and adsorption of macromolecules and molecules/ions all readily occur in the environment and in biological systems. These transformations greatly impact NM behavior**. In some cases, these transformations may enhance toxicity potential (e.g., chemical weathering of the shell of a Cd–Se quantum dot that releases toxic ions from the particle core).(15)** In other cases, these transformations have been shown to decrease effects (e.g., adsorption of NOM decreased the short-term bactericidal effects of C_{60}, Ag NPs, and Fe(0) NPs,(13, 14, 16) **but increased bioaccumulation**(17)). Some transformations can potentially limit NM persistence in the environment (e.g., dissolution of ZnO NPs).(18) There is still a great deal of uncertainty about the role that transformations have on both exposure and biological effects across the whole life cycle of NMs.----**Nanomaterials are commonly produced with an organic capping agent or stabilizer, often a small anion or polymer** (Table 1). **Transformations of the material can therefore affect the core material, the capping agent, or both.** For example, the simple coordination of ZnS nanoparticles (NPs) with water molecules can alter their crystalline phase and properties.(19) The capping agent may be bioavailable and removed by bacteria.(20) **Indeed, the rate, extent, and type of transformations possible will depend on the properties of the initial NM, its coating, and its surrounding chemical and biological environment**. **Importantly, many transformations are slow or effectively irreversible and cannot necessarily be predicted using thermodynamics**. Here, we briefly review what is known about chemical, physical, and biologically mediated transformations of NMs in natural systems and their effects on the resulting NM behavior. We also discuss state-of-the-science knowledge and instrumentation gaps preventing us from quantifying and predicting these transformations in biological and environmental media.

Table 1. Representative Nanomaterials and Capping Agents/Coatings

	typical capping agents/coatings	
nanomaterial	**inorganic and small organic molecules**	**synthetic and organic macromolecules**

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Comment [106]: So the concept that it all can come out is not entirely active and is in severe error and in the bioacumaltion is where you wind up with compromises on the bodily functions as well as environmental issues

FREEDOM 4/17/2017 10:45 AM
Comment [107]: Morphology of the components

FREEDOM 4/17/2017 10:45 AM
Comment [108]: Interesting –this would be contingent on what polymer they used—what type of protein or ligand was used as well

	typical capping agents/coatings	
nanomaterial	**inorganic and small organic molecules**	**synthetic and organic macromolecules**
zinc oxide	2-mercaptoethanol, triethoxycarprylsilane, triethanolamine, acetate	polyvinylpyrrolidone (PVP), polysaccharides,
silver	citrate, decanethiol, tannic acid, ethylenediaminetetraacetic acid (EDTA)	polyethylene glycol (PEG), PVP, gum arabic
gold	citrate, octanethiol, cetyltrimethyl ammonium bromide (CTAB), cysteine, tannic acid	biotin, bovine serum albumin (BSA), polypeptides
cerium oxide	oleic acid	PVP, poly(acrylic acid)-octyl amine
titanium dioxide	oleic acid	Poly(acrylic acid)
quantum dots (CdSe, CdS)	Silica (inorganic), zinc sulfide (inorganic), citrate, mercaptopropionic acid	PEG, aminodextran
iron oxide	dodecylamine, oleic acid	BSA, poly(acrylic acid), poly(methacrylic acid), PEG
zerovalent iron (ZVI)	Au, Pd, Pt, Ni	carboxymethyl cellulose, xanthan gum, polypropylene glycol

NM Transformations and Their Effect on NM Behavior

Chemical Transformations (Figure 2a)
Reduction and oxidation are coupled processes in natural systems and involve the transfer of electrons to and from chemical moieties. A number of NMs may be composed of or **contain constituents that undergo reduction, oxidation, or both in aquatic and terrestrial environments**. These include elemental metal NMs such as silver(21, 22) and iron.(23) Ceria NPs can contain both Ce(III) and Ce(IV) and subsequent sorption of macromolecules can alter the ratio of Ce(III)/Ce(IV) on the NP surface.(24) **The sulfur and selenium in some metal sulfides and metal selenides, major components of quantum dots, are also susceptible to oxidation that may release soluble toxic metal ions such as Cd**.(2, 25) **In some cases, oxidation may result in the accumulation of a relatively insoluble oxide surface coating on the NP that passivates the surface and reduces subsequent oxidation, while also forming metal-oxide phases with a high capacity for binding ions from solution**. In other cases, (e.g., Ag NPs), oxidation of Ag(0) to Ag(I) is

required to dissolve and release bactericidal Ag^+.(22) Natural waters and aerated soils are predominantly oxidizing environments, while carbon-rich sediments and groundwater may be depleted of oxygen and result in NM reduction. In dynamic redox environments such as tidal zones one may well encounter cycling of NMs between different redox states.---**Sunlight-catalyzed redox reactions (photooxidation and photoreduction) may prove to be very important transformation processes affecting NM coatings, oxidation state, generation of reactive oxygen species (ROS), and persistence**. The oxidation and mineralization of fullerenes dispersed in water by natural sunlight may attenuate carbon-based nanomaterials.(26) **Sunlight exposure caused the degradation of gum arabic coatings on Ag NPs and induced aggregation and sedimentation from solution.(27) Many NMs will be innately photoactive (e.g., TiO_2 and CNTs), potentially producing ROS when exposed to sunlight**.(28) **Others may be oxidized or reduced by sunlight, changing their redox state, charge, and therefore potential for toxicity**.-Dissolution and sulfidation are important processes affecting NP surface properties, toxicity, and persistence. This is especially true for NMs made from Class B soft metal cations (e.g., Ag, Zn, and Cu) because they form partially soluble metal-oxides, and because they have a **strong affinity for inorganic and organic sulfide ligands**. Class B metal NMs **commonly express toxicity through dissolution and release of toxic cations, such that persistence is reduced but toxicity is increased.** Complete dissolution may allow prediction of their impact using existing models for metal speciation and effects. However, Class B metals' affinities for electron-dense sulfur molecules make them highly reactive with sulfur-containing biomacromolecules and inorganic sulfur in sediments, soils, and air. Formation of a relatively insoluble metal-sulfide shell on the particle surface can alter the surface charge and induce aggregation.(10) **Determining the particle properties (e.g., particle size, capping agent, etc.) and environmental conditions (redox state and availability of free sulfide) that affect their dissolution and/or sulfidation rates are important for assessing their potential to release toxic metal cations, and their ultimate toxicity(29) and persistence in the environment**.(30)--Adsorption of macromolecules or organic and inorganic ligands on NM surfaces can significantly affect their surface chemistry and resulting behavior in biological and environmental systems. **For example, adsorption of polymer coatings on NPs generally decreases their attachment to silica surfaces, suggesting greater mobility in the environment and potentially less effective removal in drinking water treatment**.(31) Adsorption of biomacromolecules is a particularly important transformation and is treated separately below. Adsorption of organic ligands or metal cations or oxo-anions can occur on either the surface of the core NM or within the organic macromolecular coating of the particle. Organic ligands, such as those containing thiol groups may affect NM dissolution, charge, and stability against aggregation.(32, 33) Organics present in the atmosphere can also condense onto airborne NMs, altering their surface chemistry.(34) Understanding the effects of organic ligands and adsorbed cocontaminants on NM toxicity is needed to fully assess the potential for harm.

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Comment [109]: SO the toxic effect of silver –zinc and copper in there nano format would be in there being dissolved –and as they are being dissolved they release there toxicity

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Comment [110]: Water may need to be double or tripled filtered

Figure 2. (a) **Representative chemical transformations of metal nanomaterials and the potential impacts on their behavior and effects in the environment**. AgNPs are used to exemplify the types of transformations that may occur. The magnitude of arrows approximately correlates with potential for these processes to occur as determined from the limited data available on these processes. (b) Effects of physical transformations including aggregation and heteroaggregation on the reactivity and transport of nanomaterials. The magnitude of arrows approximately correlates with potential for these processes to occur as determined from the limited data available on these processes. (c) Biologically mediated transformations of nanomaterials and their coatings, and the subsequent impact on fate, transport, and effects. Arrows do not indicate the relative potential for these processes to occur due to the limited data currently available for that assessment. (d**) Effects of nanomaterial interactions with macromolecules such as proteins and natural organic matter. Adsorbed macromolecules can affect aggregation, nanoparticle-biointeractions, biouptake, and fate, transport, and effects in the environment**. Arrows do not indicate the relative potential for these processes to occur due to the limited data currently available for that assessment.

Physical Transformations (Figure 2b)

Aggregation of NPs reduces the surface area to volume effects on NM reactivity. This increase in aggregate size in turn affects their transport in porous media, sedimentation, reactivity, uptake by organisms, and toxicity. Over time, **aggregation of NPs into clusters is inevitable** without engineered or incidental coatings to decrease aggregation. **Aggregation may take on two forms: homoaggregation between the same NMs, or heteroaggregation between a NM and another particle in the environment**. **In most cases, the greater concentration of environmental particles compared to NMs will result in heteroaggregation**. Where aggregation occurs, the number concentration of NMs in the suspension decreases, with a concomitant increase in their effective (aggregate) size. For example, 30–70 nm diameter Fe(0) NPs rapidly aggregate in water to form micrometer-sized aggregates,(35) greatly decreasing their mobility in the subsurface and likely pathways of exposure to sensitive receptors. Heteroaggregation between NMs and comparatively larger particles (e.g., clay) **could change NM behavior** if the NM–clay heteroaggregates ultimately move more like a clay particle that the NM.(36)

Aggregation can also decrease the "available" surface area of the materials, thereby decreasing reactivity. However, the decrease in specific surface area will depend on particle number, size distribution, and the fractal dimensions of the aggregate.(37) Aggregation can therefore decrease toxicity when the toxic response is a result of a surface area-mediated reaction such as ROS generation or dissolution. **Aggregation may also serve to increase the persistence of the NM if aggregation decreases the rate of dissolution or degradation, albeit in a different location compared to the**

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Comment [111]: In other words shutting down normal biology or bioactivity

dispersed NPs. The size of a NP may also affect its bioavailability to organisms. When aggregates or heteroaggregates become too large for direct transport across the cell wall and/or membrane, uptake may be prevented. Phagocytosis and similar mechanisms may also be affected**. Conversely, heteroaggregation with soft biogenic particles might increase NM bioavailability (e.g., uptake by filter feeders who preferentially remove larger particles)**. **Delineating the effects of aggregation on uptake and any subsequent toxicity will be challenging since it is a dynamic process, uptake will be highly dependent on both the species examined and its aqueous chemical environment and metabolic state,** and because instruments for tracking NMs in situ or in vivo are lacking.

Biologically Mediated Transformations (Figure 2c)
Biological transformations of NMs are inevitable in living tissues (both intracellular and extracellular) and environmental media (e.g., soils). Redox reactions are fundamental to growth in all biological systems. **These reactions take place in the cytoplasm, cell wall, cell membrane, and extracellularly via redox-labile enzymes and cytochromes or through ancillary intracellular ROS production such as hydroxyl radicals or H_2O_2.** The redox reactions between bacteria and naturally occurring, nanoscale iron oxide are well understood.(38) Moreover, bacteria such as *Geobacter* and *Shewanella* spp. **were recently demonstrated to produce nanoscale silver particles by reduction of Ag^+ from solution**.(39) **Biologically mediated transformations of both the underlying NM core and the coatings are possible, and these transformations can affect the behavior of the NMs including surface charge, aggregation state, and reactivity, which ultimately can affect transport, bioavailability, and toxicity**. The oxidation and carboxylation of CNTs by OH radicals produced from the horseradish peroxidase enzyme has been demonstrated.(40) This oxidation increases the surface charge of the CNTs and stability against aggregation while decreasing hydrophobicity. Moreover, this biological oxidation and surface functionalization may affect the toxic potential of CNTs.(41)
Biotransformation of polymer coatings used on many NMs for biomedical applications is also feasible. Covalently bound poly(ethylene glycol) (PEG) coatings on engineered NMs, for instance, were shown to be bioavailable to microorganisms isolated from an urban stream.(20) Moreover, the biotransformation of the PEG coating caused the NMs to aggregate. Biological transformations of NMs, especially carbon-based ones, and their organic coatings may ultimately act to attenuate their concentrations in the environment or to affect transport, but it remains to be seen if these processes occur at rates that are high enough to be important. Perhaps the most critical biotransformation of NMs is adsorption of biomacromolecules on their surfaces as discussed next

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Comment [112]: Living tissues---would be anything that is living

FREEDOM 4/17/2017 10:45 AM
Comment [113]: Coatings—polymers may in fact increase the spreading of infestation in life forms

Environmental Transformations of Silver Nanoparticles- Impact on Stability and Toxicity

Environ. Sci. Technol., 2012, 46 (13), pp 6900–6914
DOI: 10.1021/es2037405
Publication Date (Web): February 16, 2012
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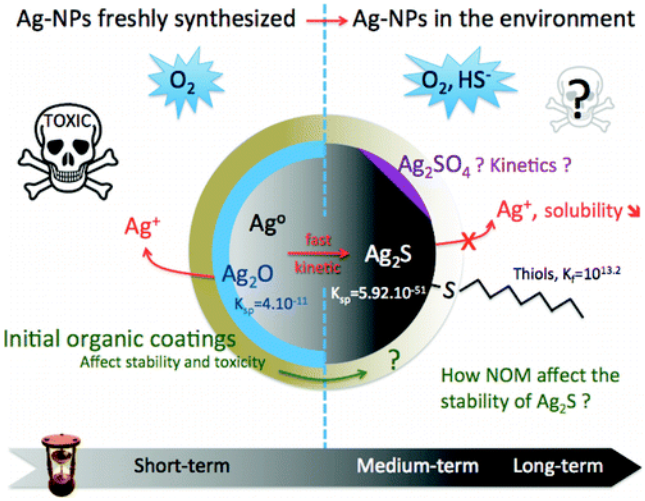
This article is part of the Transformations of Nanoparticles in the Environment special issue.

Abstract



Silver nanoparticles (Ag-NPs) readily transform in the environment, which modifies their properties and alters their transport, fate, and toxicity. It is essential to consider such transformations when assessing the potential environmental impact of Ag-NPs. This review discusses the major transformation processes of Ag-NPs **in various aqueous environments, particularly transformations of the metallic Ag cores caused by reactions with (in)organic ligands, and the effects of such transformations on physical and chemical stability and toxicity**. Thermodynamic arguments are used to predict what forms of oxidized silver will predominate in various environmental scenarios. **Silver binds strongly to sulfur (both organic and inorganic) in natural systems (fresh and sea waters) as well as in wastewater treatment plants, where most Ag-NPs are expected to be concentrated and then released**. Sulfidation of Ag-NPs results in a significant decrease in their toxicity due to the lower solubility of silver sulfide, potentially limiting their short-term environmental impact. This review also discusses some of the major unanswered questions about Ag-NPs, which, when answered, will improve predictions about their potential

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environmental impacts. **Research needed to address these questions includes fundamental molecular-level studies of Ag-NPs and their transformation products, particularly Ag_2S-NPs, in simplified model systems containing common (in)organic ligands, as well as under more realistic environmental conditions using microcosm/mesocosm-type experiments**. Toxicology studies of Ag-NP transformation products, including different states of aggregation and sulfidation, are also required. In addition, there is the need to characterize the surface structures, compositions, and morphologies of Ag-NPs and Ag_2S-NPs to the extent possible because they control properties such as solubility and reactivity.

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Nano Solutions

Chemtrails- The Consequences of Toxic Metals and Chemical Aerosols on Human Health

Ways And Means to Offset the effects

—Brain and Body Support-Supplements to Assist With Brain Restoration

Chelating Components-Ways to Eliminate the Topical and Reduce-Breatheing—To Assist in Clearing this From Lungs

Health Foods that can assist in Reduction and Repair-Soaps To use-Things to Avoid

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Chemtrails- The Consequences of Toxic Metals and Chemical Aerosols on Human Health

By Dr. Ilya Sandra Perlingieri

Global Research, May 12, 2010

Url of this article:
http://www.globalresearch.ca/chemtrails-the-consequences-of-toxic-metals-and-chemical-aerosols-on-human-health/19047

For decades, we have known that heavy metals and chemicals can cause grave physical harm. Going back to Rachel Carson's "Silent Spring," we have known and been amply warned of the serious consequences of using or being exposed to these poisons in our daily activities**. Thousands of these are well-documented carcinogens. ----**Building on Carson's ground-breaking research, **we also know that certain kinds of chemicals can and do disrupt human [and other animals'] entire immune system. Going back 30 years, researchers were investigating what became known as endocrine [hormone] disrupting chemicals and how they were affecting frogs [who sometimes had five legs or hermaphroditic characteristics], other aquatic animals, and mammals**.[F1] These animals were the proverbial canaries in the coal mine. In another pioneering book, "Our Stolen Future," authors Dr. Theo Colburn, Dianne Dumanoski, and John Peterson Myers clearly demonstrate **that 1 + 1 hormone-disrupting chemicals did not equal 2. Rather, in a nightmare of mathematical proportions, these poisons acted synergistically; and 1+1 could equal up to 1,600 times the original dose**. [F2]**We are also exposed to more than 100,000 chemicals regularly**. Most of them have never been tested for human safety. So, almost nothing has been done to reduce human exposure to a myriad of hazardous chemicals. In fact, over the past decade, **the Bush administration dismantled many environmental laws in existence for 30 years, to let corporations off the proverbial hook**. [Just look at what's unfolding in the Gulf with the BP oil spill.]---------Although this information, on the dangers of hormone disruption, is now more widely available on Internet sites, it still is not well known by the average person who gets news mostly from mainstream media.(1. With the high stres**) Most of these highly toxic chemicals are invisible; and, therefore, are easily off our collective**

radar s level created by the deliberately orchestrated financial crisis –where millions have lost their jobs and homes– a degraded/collapsing environment or serious health problems are not priorities –especially, if very little is reported in mainstream news**. This disaster scenario is part of the larger picture of what Naomi Klein writes about in her book "The Shock Doctrine." We have so many major crises, one after another, that it is hard just to keep up with one's daily routine** [F3]–let alone have time to read and consider **the toxicological health ramifications of massive amounts of thousands of heavy metals and chemicals that have poisoned our entire food chain and, thus, our own supposed "health."** We are at the very top of this wrecked food chain.[F4]---Now, however, there is another far more insidious layer of toxicity that is not being addressed at all in any mainstream, corporate-controlled news, and it is affecting our very survival. It is, however, being addressed more and more by independent researchers who have supporting evidence to back up their Internet reports. -----For more than a decade, **first the United States and then Canada's citizens have been subjected to a 24/7/365 day aerosol assault over our heads made of a toxic brew of poisonous heavy metals, chemicals, and other dangerous ingredients**. None of this was reported by any mainstream media. **The US Department of Defense [DOD] and military have been systematically blanketing all our skies with what are known as Chemtrails (also known as Stratospheric Aerosol Geoengineering).(**2) **These differ vastly from the usual plane contrails that evaporate rather quickly in the sky. Chemtrails do not dissipate. Rather, planes (fitted with special nozzles) release aerosols "lines" in the sky that do not evaporate**. Multiple planes are deployed, flying parallel (or often "checkerboard" patterns) overhead; and soon the sky is blanketed with many grayish-white lines [miles and miles long, although this is changing]. **At first, these lines are thin; but soon they expand and, in a short time, merge together. Our once-blue sky has vanished and has been replaced by a grayish-white toxic haze that blots out and greatly diminishes our usual sunshine**.-----**Military and commercial planes are involved in more than 60 secret operations.** Last year, when I flew across the country, I saw a United Airlines jet (flying below us at about 37,000 feet) **spraying a black aerosol that went for miles and miles across the sky. This clandestine program now includes aerosol-spraying planes in North America, Europe, Australia, and New Zealand [all NATO countries]**. [F5]Hundreds (if not thousands) of people have called and written their public officials to get answers. Replies from US and Canadian officials are not forthcoming; or, if they do reply, queries are dismissed. **This remains an ongoing, deliberate cover-up.** No one is held accountable, while we continued to be poisoned daily. This is not the first time, however, that citizens are being used as experimental laboratory test subjects. The US government and its military have a very long and sordid history of using us, without informed consent, in this illegal manner. As Carole Pellatt notes:--------**The U.S. military has been spraying chemical and biological weapons in open air testing over civilian populations since the 1940's. They are called**

"vulnerability tests". This is not a controversial statement. The military has admitted to this practice on many occasions and there's plenty of documentation from the government to corroborate it**. There is also documentation of intentional, experimental releases of radiation on civilian populations**. Unfortunately, this information tends to surface long after it could have saved lives, or eased the suffering of victims.(3)---Over the past decade, independent testing of Chemtrails around the country has shown a dangerous, extremely poisonous brew that includes: **barium, nano aluminum-coated fiberglass [known as CHAFF], radioactive thorium, cadmium, chromium, nickel, desiccated blood, mold spores, yellow fungal mycotoxins, ethylene dibromide, and polymer fibers. Barium**[F6] can be compared to the toxicity of arsenic.(4) **Barium is known to adversely affect the heart. Aluminum has a history of damaging brain function**. Independent researchers and labs continue to show off-the-scale levels of these poisons. A few "anonymous" officials have acknowledged this on-going aerosol spraying.(5) -----**Numerous tests have been done to verify that these poisons are off the scale in their toxicity**. **They are documented in our water, in our soil, and in our air.** For more than 10 years, researcher Clifford Carnicom has been valiantly and systematically reporting on the various detrimental aspects of these aerosols **–and what they are doing to our entire environment, as well as our blood**.(6) Various "sky watch" groups also have been carefully documenting and diligently reporting about these daily assaults.(7) --With all these poisons surrounding our every breath, it is not surprising to see a dramatic increase in illnesses. **There are numerous reports of the increase in cardiac deaths and upper respiratory illnesses (asthma, chronic bronchitis, lung cancer, and often multiple chronic illnesses**). Chemtrails toxicity has already dramatically affected our deteriorating "collective health." The significant increase in heart disease and various upper respiratory illnesses has been linked to a vast increase in "particulate matter" in our air. This can be seen by some revealing statistics:

1**. Coronary heart disease is now the leading cause of death in the US**. According to the CDC, in 2006, 631,636 died of heart disease. This means 1 out of every 5 Americans are affected.(8)

In Canada, every seven minutes someone dies of heart disease.(9)

2. **Asthma and upper respiratory illnesses. Between 100-150 million people suffer from asthma worldwide.** In the US, 16.4 million adults have asthma and 7 million children have it. Chronic bronchitis and emphysema: 9.8 million Americans were diagnosed with chronic bronchitis this past year; for emphysema the figure is 3.8 million.(10) Total: 37 million Americans afflicted.

In Canada, 2.4 million have been diagnosed with asthma.

3. Particulate matter in air pollution. Particulate matter [PM] consists of tiny particles 10 microns or less. [1 micron is about 1/70 the thickness of a single

human hair.] **These particles can lodge in the deepest part of your lungs; and over a period of time, they can damage lung function. This kind of pollution, that we breathe daily, can and does cause various upper respiratory illnesses, coronary heart disease, and premature aging and death**. Particulate matter can also exacerbate any existing illness.(11) Unanswered questions: Does hazardous particulate matter act in synergistic ways in human bodies (as do endocrine disrupting chemicals)? How does PM affect millions who already have multiple chronic illnesses?

Brain Injury

Even with the increases in preventable illnesses, the issue that has not been linked or addressed –with what Clifford Carnicom rightly calls "aerosol crimes"– **is the deterioration of cognitive function**. Our immune system is already under siege daily; and this has resulted in millions (possibly billions) of people with not just one illness, but often multiple ones. **The skin, the largest organ in our body, is a permeable membrane. This means that invisible toxins in our air, including Chemtrails and other highly dangerous chemicals, go right into our skin. Poisoned rainwater (or snow touching our skin) does the same thing. When the air we breathe is filled with a dangerous assortment of toxins, with each breath we take, these poisons assault our entire immune system. These poisons also affect our brain and, thus, our cognitive function.---Aluminum is a** [F7]**major component in these aerosols**. Although it is our planet's most abundant metal, our body has no biological need for it. Pesticide Action Network North America [PANNA] lists it as **"toxic to humans, including carcinogenicity, reproductive and developmental toxicity, neurotoxicity, and acute toxicity**."(12) **Yet, aluminum is commonly used [this is a very short list] in vaccines, deodorants and anti-perspirants, over-the-counter medications, soft drink and beer cans [aluminum leeches from the cans], baking powder, cake mixes, processed cheeses, and other food products and additives.** Over years, **aluminum accumulates in the brain, tissues, and to a lesser amount the bones**. **It causes brain degeneration, dysfunction and damage – due to the blockage and reduced blood flow and oxygen of brain arteries. The brain shrinks, as brain cells die. This causes dementia. Symptoms include: emotional outbursts, paranoia, forgetfulness and memory loss, speech incoherence, irritability, diminished alertness, changes in personality, and poor/bad judgment**. All these are on the rise, as more than 4-million Americans are afflicted. Brain deterioration and dementia take decades to cause serious and visible harm. Eventually, however, dementia is fatal. "Alzheimer's" is now being used incorrectly as a catch-all term for all kinds of dementia. Just a few days ago, the front page of the New York Times had a headline: "More with Dementia Wander from Home."(13) People afflicted with, what the Times terms "Alzheimer's" were interviewed. One person mentioned he "has a diagnosis of Alzheimer's." This is patently wrong. Alzheimer's dementia can only be accurately diagnosed after death when a post-mortem can be done.

However, heavy metals poisoning can be diagnosed through lab testing; but this is rarely done for basic check-ups.---What is not addressed in this increase in dementia is the more than 10 years of breathing **Chemtrails with nano aluminum-coated fiberglass**. Billions of tons have been sprayed on us. ---**With all these sources of aluminum added to the air we breathe with each breath, the cumulative toxicity is very high**. Even in daily events, it is obvious –to anyone who is paying attention**– that many people are behaving oddly.** While it may be considered "anecdotal" reporting, there are millions of people whose behavior is strange. There have been numerous times in just the past year when I have asked someone a question and received an answer that is totally unrelated. **There have been more and more uncontrolled outbursts in public areas: someone "snaps" for no apparent reason. Violence levels are up**. Look at all the shootings on school campuses. There are more unexplained auto accidents that never should have happened. In just one day a few weeks ago, I witnessed three traffic accidents that need not have happened. The news is full of these stories. ---Add to this already highly toxic body burden is the **US military's use of aluminum in its aerosols**. It is used because of its electrical conductivity, durability, and light weight. The US Air Force reported in 1997 that it released **"2 million, 6-7 ounce bundles of CHAFF." These are laid by military aircraft form 15-50 miles in length.(14) Another unanswered question: Why is the USAF not releasing up-to-date figures? ---**A 2002 report notes that: "over the last 25 years, the US Navy [has released from planes**] several hundred thousand pounds of aluminized chaff during flight operations over a training area on the Chesapeake Bay."(**15) If the Navy used hundreds of thousands of pounds in just this small area of the US, what could be extrapolated for the **release of possibly billions of tons of nano aluminum by all the military divisions throughout the US and Canada more recently than 2002**? **CHAFF is being stored that has lead in it**. Has that been released, without our knowledge, and added to these aerosols? What enormous, yet invisible, harm has that created for all of us?---**Dr. Hildegarde Staninger reported last year that "exposure to aerial emissions of nano composite materials resulted in cholinesterase inhibition**."(16) The [F8]**human body has three kinds of cholinesterase**: **for the brain**, **for plasma (manufactured by the liver), and red blood cells.** Some pesticides and nerve gases (such as VX, an organophosphate**)**[F9] **inhibit cholinesterase.** The chronic inhibition of this enzyme (that normally circulates in red blood cells), caused by the spraying of these Chemtrails aerosols [for weather modification, but also used for mosquito and other insect eradication], causes chronic poisoning. **This exposure causes severe neurological disorders, including paralysis in humans.----------**In a ground-breaking 2003 online essay, Dr. Kaye Kilburn, asks: "Why is Chemical Brain Injury Ignored?"(17**) His article lists 13 concealed factors that affect our willingness to believe that dangerous chemicals do affect the brain**. They include:

1. "It's all in your head" [meaning real symptoms are ignored by allopathic medicine].

2. Resistance to vulnerability [individuals, and society collectively, cannot believe the brain is at risk].

3. The acceptance of mind-altering prescription drugs [such as Paxil] that can and do affect the brain [millions are on anti-depressants –what long-term damage does that also do to cognitive thinking?].

4. Chemical brain injury is considered not to be "an imminent threat."

5. Competition from other serious threats [causing indifference or denial];

6. Delay in acknowledging health risks.

7. Economic interests [delaying tactics by big corporations are well known –delay continues profits and ignores taking responsibility –We are all expendable for corporate profits].

8. The field of neurology has been slow to consider causes [how many independent researchers are left who do not have any ties to the pharmaceutical/chemical companies?].

In all these valuable reasons for not addressing this human crisis, the one that Dr. Kilburn has not addressed **directly is the chronic assault of breathing/absorbing these now billions of tons of hazardous aerosolized chemicals and heavy metals over more than a decade without our informed consent**. When one does not look for or address

primary causes, then other issues can be blamed. This, on top of a government's silence or refusal to respond and the corporate media's complicity, make for an extremely dangerous combination that puts us all at grave and daily risk. As brain function is diminished, and other things are blamed for it**, any population is easier "to control."---**Dr. Kiburn's research clearly shows that chemicals do affect and seriously harm the brain [and, thereby, cognitive function**]. Chemicals –especially a daily onslaught of toxic chemicals over many years– can damage our ability to think clearly**. Even if we find this hard to believe, the evidence is there. Dr. Kilburn has expanded this essay into the first book to research this**: "Chemical Brain Injury" (published in 1998).** Dr. Kilburn notes----The brain's preservation represents the only possibility of survival for mankind. To find in many parts of the country and in many **individual patients that its function is eroded seriously by chemicals, chemicals that have been introduced into the environment basically in the last 50 years, is bad news indeed.(**18)---It seems almost unbelievable that millions/billions of people could look up at the sky and not notice the dramatic changes that have occurred from what it was, for instance, in the mid-1990s. **Then our sky was a gorgeous, deep blue**. Clouds were a beautiful assortment of shapes. The sun was glorious. But people under 30, may not have a real sense of recollection about looking up every day and seeing this panoramic magnificence. Most of them are too busy texting or chatting on their cell phones. There are other issues to consider, as well: **People are in their own comfort zones**; and denial is a very powerful human emotion. In the hustle and bustle (now quite out of hand, for reflective time), how many people look up at the sky? **It also takes huge courage, a very deep, internal willingness to examine politically motivated corporate controlled media spin, and search for the real answers**. **Humans like their regular routines**. To re-examine what we think we know, based on new evidence, takes a willingness to think outside the proverbial box; to want to find out the truth –not the pervasive **Orwellian doublespeak that pervades our society**. If everything in our daily routine belies what is truly going on, it requires fortitude to explore the unknown –to question the litany.----Another courageous person is Dr. R. Michael Castle who continues to address the Chemtrails toxicity issue**. He is a noted polymer chemist who has been interviewed frequently and has written articles about the extreme hazards of Chemtrails**. Dr. Castle has also written a ground-breaking document, the Universal Atmospheric Preservation Act [UAPA]. This document has been in Congress since 2008; but is tied up in committee. The only way to have this vital piece of legislation passed is to have real congressional representatives actually representing us (instead of the corporate lobbyists). See:

http://anticcorruptionsociety.files.wordpress.com/22010/04/the-unified-atmospheric-preservation-act.pdf

Given these issues, since our collapsing society has so many different levels of deceit –the financial debacle, the lies and deceit of government and the Federal Reserve blaming people for the housing/mortgage nightmare, the emerging

police state, the disasters that envelope our fragile environment– it becomes increasingly difficult just to maintain a daily routine and survive the economic depression and its daily fallout. Mainstream media does its supporting role and deceives us. Millions, like the proverbial lemmings, hasten to join the group demise. There are countless historical instances of this collective insanity. We Homo sapiens [sic, wise men?] have never learned the lessons of 5,000 years of history. **This is because each new generation of corrupt political leaders (often tied historically to previous ones) never has the real interest of their constituents as a basic part of their political practice**. Further, there is no Precautionary Principle in place.(19) It's not the way the political game of deception works. Precaution is not part of an equation that is broken from the beginning. Humans are gullible and want to believe the Orwellian deceptions.--To add to this already heavy burden, to ask uninformed, although supposedly "well educated" [What does that actually mean, given that much of our higher education has omitted much of what Prof. Peter Dale Scott calls "deep political events" that never get into our history books?] people to reconsider what they think they know about what is really going on –this takes enormous internal strength. It requires profound courage. The basis of this "courage" actually means creating new synaptical pathways in the brain. Without them, we feel scared, nervous…because those new synapses have not yet been created. It takes repeated effort, and, thus, an emerging sense of ease, to create these new synapses**.---If, however, millions of people are already on prescription pharmaceuticals to "calm them down" [long term, what is this doing to their ability to think clearly**?] and, in addition, are breathing poisoned **air rife with mind-distorting chemicals, then how clearly (if at all) is anyone able to think**? **How can anyone feel well and safe, if the very air we breathe is deliberately poisoned and is affecting our ability to think cogently**? It is already evident that no one in any official capacity is willing to tell the truth. It is like Diogenes, the ancient Greek, searching for a truthful individual. No one seems to have the desire, or courage, or authority to stop this massive poisoning, because it is the secret plan of the elite insiders to deliberate destroy everything we once knew.---Our BASIC human rights, constitutional and international laws are mere paper. These rights and laws have all been torn asunder by those in charge. It has been done by stealth. We must organize peacefully. PEACEFULLY is the operative word. If these many-pronged aerosol attacks by military and commercial planes can spray these horrific toxins on us, year after year with impunity –against all laws– then it is absolutely imperative that we organize peacefully. As Peter Dale Scott notes in Jason Bermas' new DVD "Invisible Empire": we must use the Internet and our peaceful intellectual powers to come together and shut this nightmare down. It is possible to do this.

Dr. Ilya Sandra Perlingieri *is author of the highly acclaimed book, "The Uterine Crisis."*

Notes

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Ways And Means to Offset the effects—

Brain and Body Support

Supplements to Assist With Brain Restoration

Chelating Components

Ways to Eliminate the Topical and Reduce

Breatheing—To Assist in Clearing this From Lungs

Health Foods that can assist in Reduction and Repair

Soaps To use

Things to Avoid

One thing we have to under stand is the need to reduce the over load of the exposure ---so in this I am going to suggest methods to assist in the removing of the load---the aluminum is but one thing---not the only thing to be dealing with---and at the same token as you are on your endeavor to work on yourselves ---then there is a need to corroborate your data with other people and exchange the ideas so that we can all receive the benefit---ease off on being snappy—it will be difficult due to programming and exposure---learn to be apologetic and tolerant—we are all inflicted and through this we can come up with a solution

Brain & Body Support—these are things you can do to re connect the brain or to stave off the damage---

Foods such as Eggs-Walnuts-Black Berries-Blue Berries Yogurt and Kefir (definite must these will restore the balance of bacteria to being healthy as a result if the balance is there the brain functions—80% of brain issues is directly tied to this-Cottage cheese mixed with ghee or other oils-**Saturated Fats**(ghee-butter-coconut oil-tallow-lard-palm oils-shea-coco butter) would be good oils

Evening primrose-sunflower-almond-avocado-olive (non counterfeited or convoluted) Wheat Germ Oil –when utilizing these fats make sure you use **Rosemary**—brain support, heart support, liver support-powerful anticarcinogen- hormone balancer-**Sage**-protects the acetylcholine from breaking down-lung support Hormonal support –anti carcinogen- **Iodine** (lugols-Iodoral-IOSOL-nascent) binds with mercury and aluminum to remove from the brain and body –make sure 5% or higher-use 4 drops a day minimally in divided doses-endocrine balancer-and an STD remover-**Thyme**- DHA support and promoter-powerful lung support-strong anticarinogen-powerful immune booster (stronger then oregano)-**Tumeric**- Liver support-Brain Support Anti carcinogen – anti estrogenic-**Ginger**-immune builder-digestive protector-stimulates circulation-has anti blood clotting capabilities for damages arteries as a result of build up from chemtrails-Adaptogenic-**Garlic and Onion** –High Sulphur this will also assist in the removing of the load of metal build up-will support liver-lung and is an extreme anti carcinogen –and stamina and hear support—**Pectin from fruits**—Orange and Grape fruit and Pommelo inner skin---when you peel off the outer layer the inside is the thick white---consume this with apples as a sauce to assist in the purging of the body-apple and pear sauce-again in conjunction with the peeled skins**— Eggs with Nutmeg** sprinkled on them is an excellent brain food as well

Supplements to Assist With Brain Restoration

Piracetam-reconnects the right and left side of the brain –can reverse dementia in some cases when combined with choline and dmae and aricept

Folic acid and B12 ---mix then in a powdered form in either yogurt-kefir-sour cream or cottage cheese---allow to ferment this over night and proceed to consume in morning hours –again will help slow down the degradation

Use Black Coffee—to stimulate oxygen flow---add rosemary or sage to this to increase the effect of the herbs—add 1 drop of essential oil of peppermint to increase alertness---

Chelating Components- sodium thiolsuphate-edta-serrpeptase-trypsin-ascorbic acid and NAC---Salt Baths**(critical to reduce the load entering from the surface and allowing the body to flush out of the system the build up)—**Salts required are: Epsom salt-Baking Soda-TSP-Borax-these are at ¼ cup-sea salt 1 cup-turpentine ½-1 cap –dmso 1 cap---mix in hot water as hot as you can bear---soak for 3 minutes

Niacinamide to assist in Sleep and Moods due to the metal over load the B3 in this form can reduce drastically the swings and assist with sleeping—tak with glycine—or taurine-or gaba-or tryptophan—this way these aminos will respond better

Essential oils –Rosemary-Sage-Thyme-Laurel Bay-Summer Savoury-Cinnamon-Nutmeg-Pine-Spruce-Thuja---these can all assist in either removing waste or bringing in antioxidants

Ways to Eliminate the Topical and Reduce

Bathes— These are the ingredients for this to remove excesses

Baking Soda ¼ cup

TSP-1/4 cup

Borax-1/4 cup

Epsom Salt-1/4 cup

Sea Salt 1 cup

Turpentine ½-1 CAP

Hot Water as hot as you can handle—soak 30 minutes minimally—when done observe bath water---you may see polymers and crystals floating in the tub---you may even want to get them examined by a reputable laboratory

Breatheing—To Assist in Clearing this From Lungs

Get a cool Mist humidifier (warm will work as well the cool mist just does not allow steam to flow) and add to it distilled water—Iodine (lugols or even the

tincture of iodine) 1 or 2 droppers full-essential oil of peppermint 20 drops—turpentine (about a tsp)- essential oil of thyme 10 drops and colloidal silver (100ppm or higher) 2 dropper fulls turn on in bedroom with door closed about 1 hour before sleep—(to increase the impact turn on a space heater at the same time-this will cause the vapours to be even more vapourized) you can turn of the space heater if you wish but allow the humidifier to run throughout the night-this will break up polymer build up in the nasal cavity as well as lung and cells—may see a vast improvement in oxygen uptake as well while sleeping

Another thing you can do is just add **straight 3% peroxide**-fill your container---this will definitely increase oxygen in the body again while sleeping—

You may find a lot aches and pains will diminish and as well you may find a release in the build up of the metals and polymers and biofilm that you are breathing in

Adding Aspirin to the humidifier will reduce inflammation with those who have any respiratory issues and will allow for an analgesic (pain relieving effect on the body as well) this will not hurt you since you are not using this orally then it will not cause any internal bleeding---this can be mixed with the mixes with essential oils and other elements

EXPERIMENT with different oils as well

Health Foods that can assist in Reduction and Repair

Fermented Foods need to be at the top of the list to restore the damage bacteria or renew the colon since these can restore and rebuild the colon—the dairy ferments should all have fat and be plain nothing added inside when buying---if you wish to add fruit or other supplements then do so at home where you have better control of quality and assurance then what is already pre mixed

Yogurt-Kefir-Cottage Cheese-Sour Cream- Cheese-Fermented Veggies-Fermented Fruits all should be incorporated in your eating—Never Mix with Meat proteins at the SAME TIME should be eaten separate—this can hamper animal protein digestion and assimilation---

Do Not CONSUME any SOY OR SOY DERIVED FOODS THAT ARE FERMENTED these will have been processed in aluminum and sprayed with glyphosates during the processing from the fields **no form of soy is safe** and would have used some form of fluoride as well in the process AVOID ALL SOY FOODS-FERMENTED OR OTHERWISE-

Pectin as a good source of removing build up---utilize the white part of the citrus by peeling off the skin of the citrus and then stripping of the meatier white ---use fresh or dry—then add with it an onion and a peeled apple and ¼ cup of water (distilled) and mix til mush---consume this throughout the day---

this will furthr draw out of your insides the nanoparticles and other metals and radiatin you may be acquiring from the fall out—consume 3 oz daily for children add 1 tsp of maple syrup to there mix to sweeten

Soaps To use---use simple castilles unperfumed or basic soaps made with different oils –olive-evening primrose—etc---if you have lesions or outbreaks on skin the baths will assist tremendously to get this off—but a good kerosene or turpentine rub on the outside may assist in the removing of any build up occurring--

Things to Avoid

Fish oil-all contain mercury and arsenic

Canola Oil-scars thyroid and causes dna damage and cellular degradation of the skin

Soybean Oil-destroys brain-thyroid-pancreas-intestines-oesophagus

Vegetable oil (soy)- destroys brain-thyroid-pancreas-intestines-oesophagus

Burnt Oil-break down of body

Do Not CONSUME any SOY OR SOY DERIVED FOODS THAT ARE FERMENTED these will have been processed in aluminum and would have used some form of fluoride as well in the process AVOID ALL SOY FOODS-FERMENTED OR OTHERWISE-

DO NOT CONSUME Canola oils in any form-vege oils soybean oils—these will further break down your DNA and your glands and cells and can further cause Mutations and Break down---when combined with the chemtrail fall out this would Be equated to a binary attack on the body from without and within

Any Vegetable or Fruit- consumed will need to be either peeled off ordouble peeled thoroughly due to the nanosilver and other nano metals being rained on the plants or trees—this is imperative in order to reduce the build up of these metals and to minimize the penetration into our bodies tissues and organs-fruits to be eaten are citrus and meatier fruits with the fibres like apples peaches plums pears tangelos-mandelos-oranges-grapefruit pomello's etc

Fruits to avoid –the high glycemic ones such as Bananas (**unless fermented black** will offer no potassium benefit but a sugar rush this sugar rush will cause pancrease and insulin issue when consuming grains and soy) Watermelons-Grapes-Raisons-Dates- Cherries since you cannot remove the nano and potentially other berries

Foods in Plastics-xenoestrogenic and may contain nanoparticles

Bagged in Blastic- xenoestrogenic and may contain nanoparticles

Microwave cooked anything not even water-destroys the biology of the food and dna**No Artificial or Natural Flavours—*can be anything that could further damage the body*(aborted baby extract-soy-anal glands of beaver..etc)**

Grains—no grains non are edible and have been cross contaminated with GMO or are GE in the process of making substances called
foods
Grains- Total abstinence is strongly suggested due to the cellulose and genetics and cross genetics these foods no longer break down in your body but in fact grow within causing a deterious effect on colon and stomach and pancreas—this allows this fallout to have a deeper and stronger impact further escalating the break down of neurol functions as well as physical connection within the body--
No rice or quinoa-amaranth either

Nothing sugar free or fat free—these have either added sugars or synthetics-which either overload your body causing internal damage and break down of organs –or will strip away or block any use of the nutrients they are mixed with

Any Packaged foods in metals- in metal bags or other wrappings there is a absorption of these that will get into the food and can as well exasperate the body which is already overloaded with chemtrail and other poisons (vaccine—flouridated water etc-)

Processed Sugar Brown or White-these have a drug like effect that ccan cause a addiction—and opens you up to more addictive substances

Minimize to the best of your ability over consumption- regulate your food intake to a minimal intake maximal nutrition will find that appetite will go down and body recovers

Eliminate-junk foods—all sealed in either metals or contain nanoparticles

****
ANTI NANO PAIL

http://independz.wix.com/antinano-set-up-pail

Materials for making the AntiNano Bucket-You will need 5 gallon bucket-Tape-5 amp laptop power supply-cutters and crimp -Tape-Alligator clips

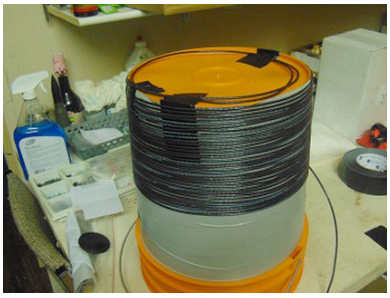
The Beginning of AntiNano Protection

The 5 gallon pail or bucket for the AntiNano effect

Start taping the Bucket with double sided duct tape sided if you do not have any double side tape then take any duct tape and flip it so that the adhesive side will be facing you

This is what the taped bucket will look like when completed the adhesive side (sticky side will be out the non sticky side will be against the bucket

The Process has just begun!



Getting Started to wrap(see now you're a wrapper a bucket wrapper) Tape the wire on the top of the bucket after you get 2 feet or so from your wire supply tape the wire on top of the bucket in a coil so it does not get in the way but allow for the tape to come of so you can release the wire at the end

AS you can see you just start to "coil" or wrap the bucket and you try to keep it as tight as you can to the proceeding wrapping if not just go back and re thread the wire around the bucket~ it will work if there is a little space so do not be conceerned to a oint of frustration- your not running a race and your not punching a clock your building a means to assist you in your health against the nano afflictions

This is progressing as the wire is being wrapped or coild or turned on the bucket you can see it is pretty uniform with some slight gaps -again do not worry about that but do try to get them as close as you can so you have a uniform or a wiring that is pretty good

This is the Bucket completely wired or coiled or wrapped all the way and you just tape the ends at the other end when you finish so that it does not unwravel and you cut another 2 ft of wire from the spool or container you are getting your wire so you have about 2 ft on each end of the bucket

Step 1

Step 2

Step 3

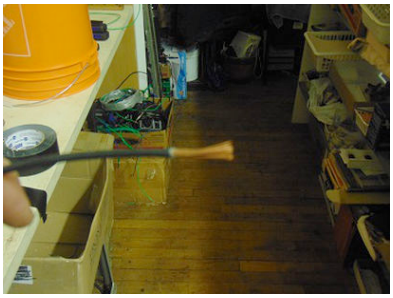
Step 4

Step 5

Step 6

Step 7

Step 8

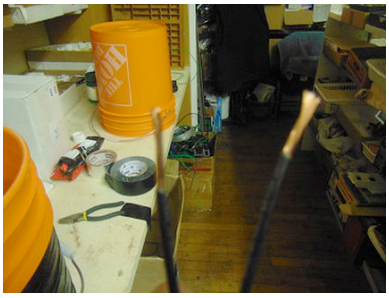


Preparing the wires to be stripped or spliced so that you will take off the insulation and have exposed wire so you can later use the ends to attach or to add whatever closures you see fit

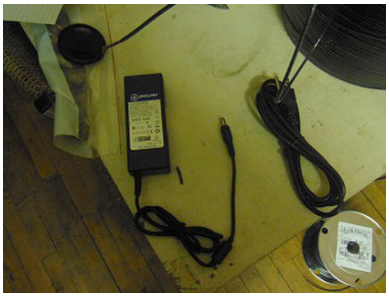
Stripped or Spliced Wire and you can see the exposure -this will require to cut into the outer insulation enough to seperatee the piece of insulation from the rest of the wire and then to pull off the severed piece til you see the exposed wire

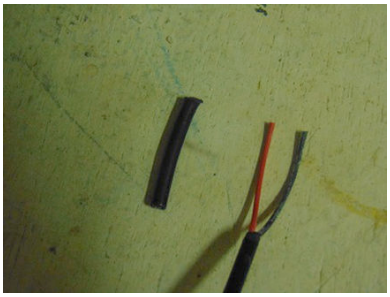
Step 9

Step 10



AS you can seee now we have bot of the wires spliced or stripped so that the exposed ends can now be connected to the power source





Here you are measuring off about a 2 inch distance (or a centimeter) after you cut off the end the you will allow some space so you can seperate the insulation from the wires inside so you can add your alligator clips

Here you see the insulation seperated and what you have is 2 wires - sometimes you will have 3 so you have to figure out then which 2 usually the back and white but it can be different so make sure by either testing the lines or find someone who knows or look up the model of your power supply and see which2 wires are the power and return

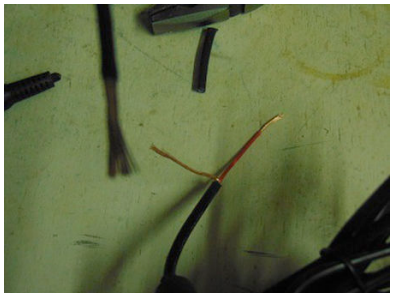
Power Supply and what you are now going to do is prepare the power supply to attach the bucket so you can produce the field to set you free

Step 11

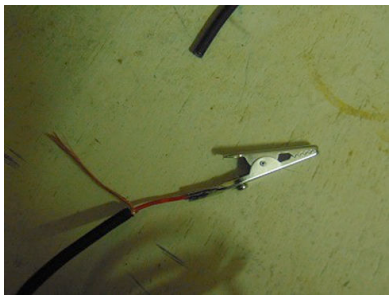
Step12

Step 14

Step 13



Wires are spliced or stripped from the power supply applying the same method of removing the insuslation from the other line you would do the same for the inside as well and leave enough exposed to make contact with the aligator clips you are going to use



Alligator clips you are going to use to connect to the power --attaching on clip to each seperate wire

Step 16

Step 15

Step 17

Step 18

Here you are going to connect the alligator clip on to 1 wire and when you get it in "crimp down" or take your cutting or plier tool and squeeze tube part of the alligator clip start with the back part to the wire where the insulation and wire meet then work your way toward the alligator clip squeezing and the compressing the sides inward as well so the connections are good and snug-if this come off the just re apply the steps and make sure they are squeezed well

The final connection of both clips now on each wire so now after you have made sure that the wires are firmly snugged into the alligator clip and you have squeezed them then beging putting insulation tape on the wires

Step 19

Here is your finished Product after you made connections with the power supply and the alligator clips then proceed to wrap the insulation tape in

such away thatt it forms a T so there wil be minimal chance of making contact and shorting the unit out

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Step 20

This is a train transformer Power supply if you use this form instead of a laptop power supply you are going to have to either put in a resistor or a capacitor to slow down the return or the unit will over heat cut off or burn out your unit~~~ and if you add either one- this will go for hours with out issue what you would do is connect the return wire to the capacitor or resistor and then back to the power supple so one end to the unit with the connection to the wire the other end a connection to the transformer direct- you will have to figure out the resistance or capacitance based on your power

Eureka!!!!!! you made it now your ready to get anti nano'd you can now connect your unit and be ready for some release

How to Use

3 ways to do this and will give both formulas

Formula 1 take 3 gallons of Vinegar(white) and add 1/8-1/4 cup of salt

And stir—connect power supply

Formula 2 – 3 gallons of Distilled water and 1-2 caps of DMSO and Citric acid 1/8-1/4 cup and Salt 1/8- 1/4 cup mix and then connect power supply

Sit with either one leg or both in the pail for 20-30 minutes

Formula 3- take TSP -1/8 cup—Salt 1/8 cup Citric Acid 1/8 cup-Distilled water 3-3.5 gallons (12 litres for the metrically orientated) mix and set the bucket it up and place foot or feet into the bucket with the coils activated 20-30 minutes

Warning Due to the nature of this device where fluids and electricity will be in the vicinity and exposure to both caution and common sense are going to be required-- DO NOT STICK WIRES IN THE BUCKET WHEN IT IS FULL OF THE MIXTURE YOU WILL ELECTROCUTE YOURSELF

~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

IF YOU MAKE THE EXTENSIONS AT 2 FEET AND WANT TO ADD ANOTHER 4 OR 5 FEET AND THEN CONNECT TO THE POWER SUPPLES WITH CLIPS THIS WOULD BENEFIT AND INCREASE SAFETY MARGINS AS WELL- THIS IS OPTIONAL

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DO NOT TOUCH THE ENDS OF A CAPACITOR IF YOU ARE UTILIZING THIS AS A MEANS OF SLOWING DOWN THE FLOW THIS WILL BUILD A CHARGE AND CAN JOLT YOU

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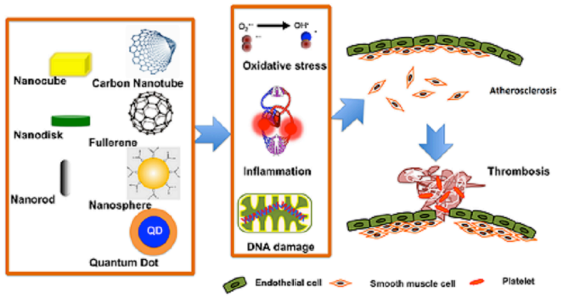
Neurotoxicity of nanoscale materials

Alokita Karmakar a, Qinli Zhang b, Yongbin Zhang a,*a

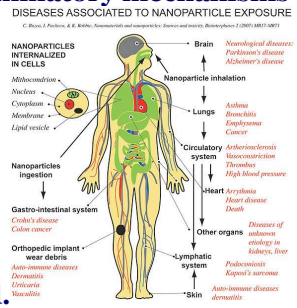


Nanotechnology Core Facility, Office of Scientific Coordination, National Center for Toxicological Research, Food and Drug Administration, Jefferson, AR 72079, USA b School of Public Health, Shanxi Medical University, 56 XinJian South

Road, Taiyuan 030001, China article info Article history: Received 30 September 2013 Accepted 28 December 2013 Available online 4 February 2014 Keywords: Bloodebrain barrier Nanomaterial Neurotoxicity Oxidative stress

Abstract

Nanotechnology has been applied in consumer products and commercial applications**, showing a significant impact on almost all industries and all areas of society.** Significant evidence indicates that manufactured nanomaterials and combustion-derived nanomaterials **elicit toxicity in humans exposed to these nanomaterials**. The interaction of the engineered nanomaterials with the nervous system has received much attention in the nanotoxicology field. In this review, the biological effects of metal, metal oxide, and carbon-based nanomaterials on the nervous system are discussed from both in vitro and in vivo studies. **The translocation of the nanoparticles through the bloodbrain barrier or nose to brain via the olfactory bulb route, oxidative stress, and inflammatory mechanisms of nanomaterials are also reviewed.**



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Introduction

People working in certain industries, for example, automobile, aerospace, various activities such as combustion, welding, and biomedical applications electronics and communications, and chemical and paint industries are at high risk of being exposed to a large amount of NPs [1e10]. As NPs persist in the environment, people living in those environments are at **higher risk of NP exposure**. Nanotechnology involves creating and applying engineered materials at the nanoscale to take advantage of these specific properties. Humans have been exposed to many nanoparticles (NPs) originating from

Copper, zinc, iron, cerium, silver, gold, iron, manganese, titanium, aluminum, silica, and other carbon-based nanomaterials are some of the NPs to which humans are exposed significantly and may cause several health-related problems **including neurotoxicity**.--As a rapidly growing emerging science, **nanotechnology has shown a significant impact on almost all industries and all areas of society**. Nanomaterials, defined by the National Nanotechnology **Initiative, have at least one dimension in the range of 1-100 nm**. Due to their small size, the properties of nanomaterials

differ from those of their bulk materials, **showing unique chemical, physical, optical, and electrical properties**.

Journal of food and drug Analysis

In recent years, a significant number of neurodegenerative diseases such as Alzheimer's disease, Parkinson's disease, or Huntington's disease have been diagnosed and treated. The **increased amount of environmental pollutants, including NPs, may be responsible for increasing the number of these neurodegenerative diseases.** The role of the bloodebrain barrier (BBB) is crucial in understanding NP toxicity in the brain. **BBB separates blood from cerebrospinal fluid in the central nervous system (CNS)**. **The BBB is an extended plasma membrane that contains tight junctions between the adjacent endothelial cells of the cerebral capillaries. The permeability properties of the BBB are of interest** [1,11]. Unlike noncerebral capillaries, the cerebral endothelium does not have vesicles for macromolecular transport. Astrocytic end feet cover most (85%) of the cerebral capillary endothelial cells and they also contain a thick basement membrane [12]. **The presence of such complex combinations of astrocytes, cerebral capillaries and basement membrane strongly supports the BBB function** [11,13], even though establishing the clear cut roles of the basal lamina and/or astrocytic end feet in maintaining BBB permeability needs further study. **When NPs reach the circulation, they may interfere with the function of the endothelial cell membrane**. **The effect of NPs on the cell membrane may be due to their direct toxicity, or indirectly, they may induce some cascade mechanism that disrupts the tight junctions in the BBB or alters the permeability of the membrane. It has been shown that intravenous, intraperitoneal, or intracerebral administration of Ag, Cu, or Al NPs (50-60 nm) reportedly disrupts the BBB, as indicated by staining with albumin-bound Evans blue [14]. Vesicular transport may also be stimulated by NPs in order to gain access to the CNS microenvironment to exert toxic effects in the CNS.** The unique size and surface modification of NPs could deliver drugs or therapeutic agents to the brain in the development of nanomedicine. Additional research is, however, necessary in order to understand fully how NPs are translocated from the blood to the brain across the BBB. **Nanomaterials could enter the human body by different routes including inhalation, dermal penetration, ingestion, and systemic administration, by which NPs may be accumulated in different tissues and organs including the brain** [15,16]**. It has been indicated that the olfactory nerve pathway may serve as a portal of entry for NPs into the CNS in humans who are environmentally or occupationally exposed to airborne NPs** [17e19]. De Lorenzo [18] showed that when silver-coated colloidal gold particles (50 nm) were intranasally instilled in squirrel monkeys, the NPs anterogradely **moved in the axons of the olfactory nerve to the olfactory bulbs. Olfactory epithelium that has been exposed to**

Owner 4/17/2017 10:45 AM
Comment [115]: NP= NanoParticles

Owner 4/17/2017 10:45 AM
Comment [116]: This is one of the reasoms never to buy any product with a nano delivey system or method or products that say colloidal but are in fact NANO~ these are nano particles irrespective if they come in a bottle or pill and that the food supply is also being sprayed with nano is indictive of another danger of accumulation and translocation not only through the BBB but through key organs and as well attached to DNA and the genetic code

Owner 4/17/2017 10:45 AM
Comment [117]: This would include everyone since we aare all either eating dfrinking or breatheing these particles and these particles can be active and activated by several methods as well making them unpredictably dangerious

Owner 4/17/2017 10:45 AM
Comment [118]: occurring or performed in the normal or forward direction of conduction or flow occurring along nerve cell processes away from the cell body
<anterograde axonal transport

manganese, cadmium, nickel, and cobalt nanomaterials can translocate the nanomaterials to the brain via olfactory neurons [20e25]. Therefore, full understanding of the neurotoxicity of these nanomaterials may lead to the design of safer therapeutics and reduce the side effects of these nanomaterials in future. Having a greater surface area than their bulk counterparts, metal oxide NPs are used in various fields such as water treatment, medicine, cosmetics, and engineering, and provide superior performance in their applications. **Unfortunately, almost no federal or state laws have specifically established regulations for the manufacture, transportation, use, sale, or disposal of nanomaterials** [26]. For metal oxide NPs, their widespread application**, small size, and large specific surface area endow them with high chemical reactivity and intrinsic toxicity, and their health effects in living creatures, especially on the nervous system**, have been of concern. **Metal oxide NPs are capable of translocating along the olfactory nerve pathway to the brain after intranasal instillation, and accumulating in the olfactory bulb, cortex, and cerebellum.** Moreover, NP deposition in the brain can **stimulate oxidative stress, inflammatory responses, and pathological changes**. These observations have provided evidence that metal oxide NPs can reach the brain and cause a certain degree of tissue damage. **Metal oxide toxicity can also be induced by dissolved metal ions from the oxides.** Brunner et al [27] studied the toxicity of NPs in human and rodent cell lines. **They divided the tested NPs into soluble and insoluble NPs, and showed that the toxicity of soluble NPs was from the soluble metal ions released from NP dissolution prior to or after the NPs entered the neural cells**. Considering the unique physicochemical properties, including small size effect, large specific surface area, and high biological surface reactivity, NPs might induce the **neurotoxicological behavior and effects in organisms**.

Owner 4/17/2017 10:45 AM

Comment [119]: Pathological · **extreme in a way that is not normal or that shows an illness or mental problem · *medical* : relating to or caused by disease**

Owner 4/17/2017 10:45 AM

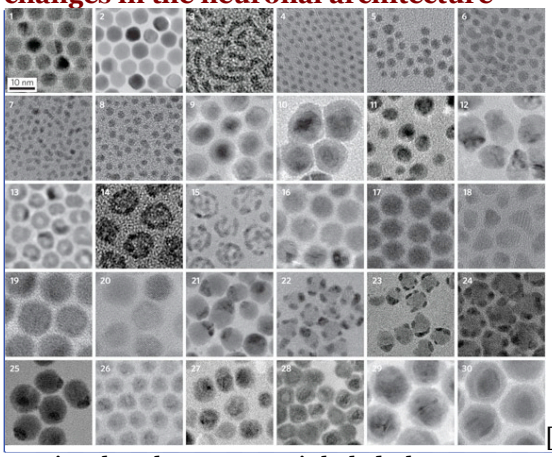
Comment [120]: The charged particles from the NANO MATERIAL will have a negative impact

2. Neurotoxicity and mechanism of nanomaterials

2.1. Titanium dioxide NPs

Among several metal-based NPs, those originating from titanium have been used widely and in large quantities. Titanium dioxide (TiO_2) is the most common compound of titanium that has found a variety of uses in our lives. TiO_2 is a white, odorless, **water-insoluble** material **that was believed** to have low toxicity [28e31]. TiO_2 is a relatively stable, nonflammable material that is found naturally in the form of various ores such as rutile, anatase, and brookite. TiO_2 can also be extracted from an iron-containing mineral ($FeTiO_3$) known as ilmenite [32e36]. TiO_2 possesses certain physiochemical properties that make it useful for multiple applications. Corrosion resistance, biocompatibility, mechanical strength, whitening property, opacity, and photocatalytic, optical, and electrical activity are some of the attractive properties that have paved the way for large-scale applications of TiO_2 [37]. The National Nanotechnology Initiative of America classifies **nanoparticulate TiO_2 particles as one of most widely manufactured NPs globally** [38]. Industrially, 80% of TiO_2,

including its **nanoparticulate form (globally), is used to produce paints, varnishes, plastic, and papers**. Besides these applications, nanoparticulate TiO_2 has major uses in developing various products such as **cosmetics, foodstuffs, toothpaste, sun blocks**, printing ink, car materials, rubber, cleaning products, materials for industrial photocatalytic applications including solar cells, **and catalysts for remediation of organic matter in wastewater** [39]. **Toxicity of nanosized TiO_2 has yet to be completely understood despite its widespread uses. Recent toxicological studies have indicated harmful effects of TiO_2 NPs in biological systems, which is of major concern [40]. It has been** recently recognized that TiO_2 may be carcinogenic to humans if inhaled [31]. As a result, it is of great importance to understand the risks and hazards including neurotoxicity associated with nanoparticulate TiO_2 exposure and its dose-dependent response [41]. Irrespective of the different forms of TiO_2, exposure route and particle size, it has been found that **TiO_2 NPs translocate to different parts of the brain [39,42e46]. The NPs accumulate in this organ and induce structural changes in the neuronal architecture**



[39,43,45]. As mentioned previously, when NPs are inhaled, they can translocate to the CNS using the olfactory nerve as a means of entry. Several studies in mice have indicated that rutile NPs can translocate to the brain and accumulate throughout the organ, primarily in the hippocampus regions [39,43,45]. Such a neuronal translocation pathway of **TiO_2 NPs may be responsible for neurotoxicity**. TiO_2 NPs **when instilled intratracheally in mice accumulate in the brain via the blood circulation and penetration of the BBB**. This type of accumulation is **responsible for inducing tissue damage** [42]. **Accumulation of nanoparticulate TiO_2 in the brain induces release and metabolism of neurotransmitters such as norepinephrine and 5-hydroxytryptamine** [39,43,45,46]. After intranasal exposure of TiO_2 NPs, enhanced levels of the above-mentioned compounds were detected [43]. However, a **decrease in response was detected when anatase TiO_2 NPs were administered intragastrically** [45]. **Reduced levels of homovanillic acid, dopamine, 5hydroxyindole acetic acid, and 3,4-dihydroxyphenylacetic acid were**

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Comment [121]: Where direct exposure will enter the system

Owner 4/17/2017 10:45 AM
Comment [122]: Translocation into lymph node, blood and urinary excretion of INP1 (left) and INP3 (right) using real-time NIR fluorescence imaging. Each point represents the mean ± s.d. of *n* = 3 animals. SBR, signal-to-background ratio. (b) Frozen sections obtained from resected organs of INP1-administered Sprague-Dawley rats at 1 h after instillation. From top to bottom are representative images of lung, lymph node, kidney (arrow: cortex; arrowhead: calyces), and liver. Mu, muscle; LN+, posterior mediastinal lymph node; LN−, negative para-aortic lymph node. Scale bars, 5 mm. Shown are color video and NIR fluorescence of intact specimens (left two panels, respectively) along with representative histological images from the same organ/tissue (H&E, NIR, right two panels, respectively). Green dotted circle, bronchiole; ... [7]

Owner 4/17/2017 10:45 AM
Comment [123]: Norepinephrine is synthesized and released by the central nervous system, and also by a division of the autonomic nervous system called the sympathetic nervous system. In the brain, norepinephrine is produced in closely packed brain cell neurons or nuclei that are small yet exert powerful effects on other brain areas. The most important of these nuclei i... [8]

Owner 4/17/2017 10:45 AM
Comment [124]: Norepinephrine is synthesized from the amino acid tyrosine by a series of enzymatic steps in the adrenal medulla and postganglionic neurons of the sympathetic nervous system. While the conversion of tyrosine to dopamine occurs predominantly in the cytoplasm, the conversion of dopamine to norepinephrine by dopamine β-monooxygenase occ... [9]

Owner 4/17/2017 10:45 AM
Comment [125]: 5-hydroxytryptamine (5-HT) is a monoamine neurotransmitter. Biochemically derived from tryptophan,[9] serotonin is primarily found in the gastrointestinal tract (GI tract), blood platelets, and the central nervous system (CNS) of animals, including humans. It is popularly thought to be a contributor to ... [10]

detected when TiO2 NPs were administered intranasally or intragastrically [43,46]. Enhanced catalase and acetylcholinesterase activity was detected during intranasal instillation of rutile [39] and intragastric administration of anatase TiO2 NPs [46]. Acetylcholine, glutamic acid, soluble protein carbonyl, and nitric oxide content were also increased by such NP treatments. When anatase TiO2 NPs were intraperitoneally injected, increased nitric oxide **but decreased acetylcholine and glutamic acid were detected** [44]. Hu and colleagues [46] **showed that the levels of sodium, potassium, magnesium, calcium, iron, and zinc in the brain were changed after nanoparticulate TiO2 exposure**. **In that study, the treated mice had impaired spatial recognition memory, which could be linked to the disturbed homeostasis of neurotransmitters, trace elements, and enzymes in the brain** [46]. **Proteomic analysis showed differentially expressed proteins in the brain in response to TiO2 NP exposure, even though no NPs were detected in the tissue** [47]. Oxidative-stress-related damage with a consequent change in the balance between oxidative and antioxidative activities was observed both in vitro [48e50] and in vivo [39,42,44,45,47]. **Levels of malondialdehyde, an oxidative marker, increased after intranasal instillation [39,44] of TiO2 NPs. A similar effect was also found with intraabdominal injection and intratracheal instillation of TiO2 NPs in mice** [42]. **Reactive oxygen species (ROS) such as superoxide [42], hydrogen peroxide [42,45], and hydroxyl radical [42] were also found to be increased in animals treated with TiO2 NPs. Increased cytokine levels, which are indicative of inflammatory effects in the brain**, were detected in animals treated with TiO2 NPs [44,51]. TiO2 NPs (P25 Degussa TiO2 and rutile forms) when injected intraperitoneally in mice induce **an increase in lipopolysaccharides, and alter the mRNA levels of interleukin IL-1b and tumor necrosis factor (TNF)-a, as well as IL-1b protein. Lipopolysaccharide induction** was necessary to cause this phenomenon, which suggests the importance **of a trigger element or a possible synergistic role in tissue responses to nanoparticulate TiO2**. The embryotoxic role of TiO2 was also studied by maternal intravenous injection of TiO2 NPs, which yielded no characterized TiO2 NPs [52], and by subcutaneous injection of TiO2 NPs in the anatase form [53e55]. In the case of subcutaneous injections, TiO2 **accumulation was found in the offspring cerebral cortex and olfactory bulb.** A large number of olfactory bulb cells were found to be positive for markers of apoptosis [53]. **Altered gene expression was detected for prenatal TiO2 NP exposure**, which was involved in **cell death, brain development, and the response to oxidative stress in newborn pups** [54]. Finally, the influence of prenatal **TiO2 NP exposure on the dopaminergic system was established as increased levels of homovanillic acid, dopamine, 3,4dihydroxyphenylacetic acid, and 3-methoxytyramina hydrochloride in the prefrontal cortex and neostriatum of exposed mice** [55]. **These findings indicate that TiO2 NPs can be carried from the mother to the fetal brain, which ultimately has a toxic effect on fetal brain development, leading to**

several nervous system disorders. More in-depth studies are necessary in order to understand fully the toxic effect of TiO2 NPs on neurons in various stages of life, including during pregnancy and early stages of development.

Zinc oxide NPs

Like TiO2, another metal-based **NP is zinc oxide (ZnO)**, which has broad uses and applications. **ZnO is also white, thermally stable**, and a naturally occurring material. **It can be used to develop sunscreens, biosensors, food additives, cement, rubber, ceramics, pigments, plastic, catalysts, and electronic materials. ZnO shows antibacterial activities** and in recent years studies have also focused on the effect of **nanoparticulate ZnO on various microorganisms** [56,57]. In recent years, ZnO toxicity has been demonstrated both in vitro and in vivo in various mammalian cells. Dissolved Zn from the NPs is responsible for the toxicity. **ROS were detected in these studies and may have been responsible for the inflammatory effects associated with ZnO toxicity.** The neurotoxic effect of ZnO has not been studied much. In one of the early works, **neurotoxicity of different-sized ZnO NPs (10-200 nm) in mouse neural stem cells (NSCs) was investigated**. As determined by cell viability studies, ZnO NPs showed dose-dependent toxic effects towards NSCs. However no size dependent toxic effects on NSCs were found in this study [58]. **Using confocal microscopy, transmission electron microscopy, and flow cytometry, apoptotic cells were detected and analyzed in this toxicity study.** Like previous studies, **the results indicate that ZnO NP toxicity originates from the dissolved Zn O in the culture medium or inside the cells** [58]. The effects of ZnO NPs on voltage-gated sodium and potassium pumps and action potential generation have been studied by Zhao et al [59]. The study on isolated rat **hippocampal CA3 pyramidal neurons demonstrated that ZnO NP solution was able to generate neuronal injury by inducing depolarization through activation of voltage-gated sodium channels, and led to higher Naþ influx and intracellular accumulation of Naþ and Ca2þ, release of glutamate, and neuron excitability**. ZnO **NPs are also able to induce neuronal apoptosis by depleting intracellular K level due to increased ion efflux** [59]. An in vivo toxicity study involving rats showed **that intraperitoneal ZnO altered synaptic plasticity, which changed spatial learning and memory ability** [60]. In that study, 20-80-nm ZnO NPs (4 mg/kg body weight) twice weekly for 8 weeks were administered to rats. ZnO NPs synthesized using the solegel method and starch as a template have been tested for in vitro cytotoxicity in neuro2A cells. **A dose-dependent toxicity profile was obtained, whereas nontoxic effects were seen at a concentration < 6 mg/mL [61]. More studies have shown that the antibacterial activity or adverse effects of ZnO NPs are partly due to the generation of ROS** [62-69], or **causing membrane damage through the direct NPecell membrane interaction or generation of ROS** [56,65], or release of Zn2þ ions in the ZnO NP

Owner 4/17/2017 10:45 AM

Comment [126]: This explains the use of copper and the requirements of copper needing to be increased to assist in the displacing of the ZNP which is also accumalating within the cells

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Comment [127]: This would explain the over reaction of people and there moods and anxiety issues as well due to the turning on the glutamate activity this is where Taurine and Glyscine would come in and Magnesium and Potassium to offset the nanotoxicolgy going on in the brain

Owner 4/17/2017 10:45 AM

Comment [128]: Strips the potassium by increasing sodium ion channels so you would need more potassium to offset this and to maintain brain and heart functions

suspensions [27,67]. **Studies in mammals have suggested that oral exposure of ZnO NPs causes an increase in blood viscosity and pathological lesions in the stomach, liver, kidney, pancrea, and spleen** [70]. However, the potential hazards of high concentrations of manufactured nanoscale ZnO on the CNS need further investigation.

Manganese oxide NPs

Manganese is an important metal. It is a trace element and necessary for survival. In plants in photosystem II, a manganese-containing metal cluster is responsible for oxygen generation from water activity and there are several enzymes that use manganese for their activity [71]. Manganese has found several other uses in our lives. Manganese is a major component of making different types of steel and cast iron [72]. Manganese chloride is used in batteries, disinfectants, dyes, paint driers, and dietary supplements. Oxides of manganese, such as manganese oxide (MnO), are used in colored glass, ceramics, paints, textile printing, fertilizers, and in food supplements and additives. Manganese dioxide (MnO_2) is used in batteries and may also be generated from the welding of manganese alloys. Use of manganese-containing welding rods is a major source of occupational exposure to welders. **Manganese tetroxide (Mn_3O_4) may be generated in situations where other oxides of manganese are heated in** air [73]. Methylcyclopentadienyl manganese tricarbonyl is used as an antiknocking agent in some unleaded gasolines. The compound is **released to the environment during fuel combustion in the form of manganese sulfate, phosphate, and oxides**. Farm workers who work with Maneb (manganese ethylenebis-dithiocarbamate) **may also be exposed to a significant amount of manganese** [74]. **As manganese is known for its neurotoxicity**, toxicity studies associated with manganese-containing nanomaterials provide a useful test case in the evaluation of nanomaterial toxicity [75]. The occupational disease associated with **manganese exposure and toxicity is known as manganism**. **The disease in later stages resembles Parkinson's disease** [76]. **It has been found that if manganese is inhaled in water-soluble and water-insoluble forms, it is translocated to the brain, crossing the BBB via the olfactory nerve pathway [77]. It has been found that, among many metals, manganese is preferentially taken up via the olfactory nerve route** [21,78]. **After nasal exposure to manganese oxide NPs (MnO, MnO_2, Mn_2O_3, and Mn_3O_4), the concentration of manganese in the olfactory bulb, striatum, frontal, and other brain regions is increased**. Macrophage inflammatory protein-2, glial fibrillary acidic protein, and neuronal cell adhesion molecule mRNA is also increased in the olfactory bulb. **The results indicate that the olfactory neuronal pathway is efficient for translocating inhaled manganese oxide as solid ultrafine particles to the CNS and can result in inflammatory changes** [24]. Although absorption of manganese in the lungs is dependent on particle size and solubility [24,79], **for neuronal manganese uptake and further translocation into the CNS, dissolution of manganese is not necessary**. As mentioned earlier, major sources of ultrafine manganese oxide

Owner 4/17/2017 10:45 AM
Comment [129]: Indicating that nanomanganese is transferable irrespective of size volume or density

particles include the iron and steel industries, battery production, ferroalloy production, and power plant and coke oven combustion emissions [80]. **Use of glass, paints, and ceramics may also provide major sources of manganese oxide. Methylcyclopentadienyl manganese tricarbonyl is presently used in gasoline, mainly in Canada and Australia [81,82], and decomposition and oxidation of 3-(4,5dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) during combustion may release manganese oxide of nanoparticulate size into the environment**. In all of these cases, the most likely route of **human exposure is through inhalation**. Toxicity of various manganese oxide nanomaterials has been investigated in a neuronal precursor cell model. The Promega Cell Titer Aqueous One Solution Cell Proliferation (MTS) assay was used to evaluate mitochondrial function in living cells and the lactate dehydrogenase (LDH) assay was used to quantify the **release of the enzyme as a result of damage to the cell membrane**. Both assays indicated that **manganese toxicity was dependent on the type of manganese oxides and their concentration. State of cell differentiation also contributed to varying NP toxicity.** Manganese oxide **NPs are responsible for the generation of ROS and cell death due to apoptosis, as revealed by flow cytometry. During cell division, exposure to manganese oxide NPs results in elevated levels of the transcription factor nuclear factor NF-kB**. Such enhanced levels of NF-kB mediate the cellular inflammatory response [83]. In another study, Hussain et al [84] investigated the effect of **manganese oxide NPs (40 nm) on dopamine production in PC12, neuronal phenotype cells. Manganese oxide NPs induced depletion of dopamine and its metabolites dihydroxyphenylacetic acid and homovanillic acid in PC12 cells, with a similar mechanism as Mn2þ** [84]. In an in vivo study, adult male Wistar rats were exposed to **MnO2 NPs of w23 nm diameter**. The experiment was a model study to understand the inhalational risks associated with MnO2 NPs. MnO2 NPs were instilled into the trachea for several weeks in daily doses of 2.63 mg/kg and 5.26 mg/kg. The endpoints of functional neurotoxicity (open field behavior and electrophysiology) and general toxicity (body and organ weights) were investigated. Animals treated with MnO2 did not gain weight after 6 weeks exposure. **High levels of manganese were detected in brain and blood samples of the treated animals after 9 weeks exposure**. **The open field behavior of treated rats showed decreased ambulation and rearing, and increased local activity and immobility were observed**. Electrophysiological studies of animals treated for 9 weeks **indicated a shift in spontaneous cortical activity to higher frequencies,** lengthened cortical **evoked potential latency, and slowed nerve conduction**. Many of these neurofunctional and general parameters were significantly correlated with the tissue manganese levels**. It can be concluded that the instilled manganese in the NP form was absorbed and the NPs were responsible for the neurotoxic effects** [85]. The acute oral toxicity of MnO2 NPs and MnO2 bulk particles in female albino Wistar rats was investigated [86]. MnO2 NPs (45 nm) exhibited higher absorption and tissue distribution compared with MnO2 bulk particles. **The histopathological analysis revealed that MnO2 NPs**

Owner 4/17/2017 10:45 AM

Comment [130]: NF-κB (nuclear factor kappa-light-chain-enhancer of activated B cells) is a protein complex that controls transcription of DNA, cytokine production and cell survival. NF-κB is found in almost all animal cell types and is involved in cellular responses to stimuli such as stress

Owner 4/17/2017 10:45 AM

Comment [131]: In the brain, dopamine functions as a neurotransmitter—a chemical released by neurons (nerve cells) to send signals to other nerve cells. The brain includes several distinct dopamine pathways, one of which plays a major role in reward-motivated behavior. Most types of reward increase the level of dopamine in the brain~ Other brain dopamine pathways are involved in motor control and in controlling the release of various hormones. These pathways and cell groups form a dopamine system which is neuromodulatory.

Owner 4/17/2017 10:45 AM

Comment [132]: Things that would inhibit Manganese These Substances may Interfere with Manganese

Minerals

Excessive consumption of Calcium may interfere with Manganese. references
. Excessive consumption of Copper may inhibit the absorption of Manganese. references
. Excessive consumption of Iron may inhibit the absorption of Manganese. references

Lead may interfere with the body's absorption of Manganese.
. Excessive consumption of Magnesium may reduce the absorption of Manganese. references
. Phosphorus may interfere with the absorption of Manganese. references
. Potassium may interfere with the absorption of Manganese.

Excessive consumption of Zinc may inhibit the absorption of Manganese.

Polyphenols

Tannins bind to Manganese and may inhibit the absorption of Manganese.

Pharmaceutical Drugs

... [11]

caused alterations in the liver, spleen, and brain. The neurotoxicity of 45-nm MnO2 NPs in the brain and red blood cells, as determined through acetylcholinesterase activity, was significantly inhibited at doses of 1000 mg/kg and 500 mg/kg. MnO2 NPs (45 nm) **disrupted the physicochemical state and neurological system of the animals through alterations in ATPases** via the total NaþeKþ, Mg2þ, and Ca2þ levels in the brain. Toxicity of Mn3O4 NPs was investigated in ST-14 rat striated neuroblasts, a neuronal precursor cell model, using the MTS assay to evaluate mitochondrial function in living cells and the LDH assay to quantify the release of the enzyme as a result of damage to the cell membrane [87]. Both assays showed that the toxicity of Mn was dependent on the type of manganese oxide NPs and their concentration, as well as the state of cell differentiation. Following exposure to manganese oxide NPs, ROS were generated, and flow cytometry experiments **suggested that cell death occurred through apoptosis. During exposure to manganese oxide nanomaterials, increased levels of the transcription factor NF-kB (which mediates the cellular inflammatory response**) were observed.

Silver NPs

death and oxidative stress in human skin carcinoma and fibrosarcoma cells [94]. The same group have also reported that **Ag NPs can enter cells, causing DNA damage and apoptosis in liver cells and fibroblasts** [95]. **Cell viability is decreased when alveolar macrophages and lung epithelial cells are treated with Ag NPs** [96]. In vitro studies have shown Ag NP toxicity in neural-like cell lines, such as PC12 cells, which is a rat cell line with a neuronal-like phenotype [97]. **It has been shown that Ag NPs could come across through and be accumulated in brain microvessel vascular endothelial cells. An in vitro BBB model composed of primary rat brain microvessel vascular endothelial cells, it has been shown crossing and accumulation capability of silver nanoparticles** [98]. **Ag NPs can induce inflammation and affecte the integrity of this BBB model, and be readily translocated to the brain** [99]. **Ag NPs can also induce BBB damage, astrocyte swelling, and neuronal degeneration [100]. Ag NPs can translocate to the brain using the nasopharyngeal system as a gateway during inhalation exposure** [17]. In vivo studies by Liu and coworkers have shown the effects of Ag NPs on hippocampal synaptic plasticity and spatial cognition in rats. Their studies have revealed that intranasally administered **Ag NPs induce impairment of hippocampal function** [101]. These results suggest that **Ag NPs cause neurotoxicity in humans and other animals.** More recently, a significant finding indicated **that 7nm Ag NPs decreased motor activity and body weight in a time- and dose-dependent manner after intravenous injection, suggesting that the nervous system may be targeted by Ag NPs** [102]. Yin and coworkers tried to establish the mechanism of Ag NP neurotoxicity both in vitro and in vivo using rat cerebellar granule cells. Their studies indicated that **Ag NPs, depending on the caspase-activation-**

Owner 4/17/2017 10:45 AM

Comment [133]: Brain Damage—Another Hoax on the benefit of nano silver

Owner 4/17/2017 10:45 AM

Comment [134]: The hippocampus is a small organ located within the brain's medial temporal lobe and forms an important part of the limbic system, the region that regulates emotions. The hippocampus is associated mainly with memory, in particular long-term memory. The organ also plays an important role in spatial navigation.
Damage to the hippocampus can lead to loss of memory and difficulty in establishing new memories. In Alzheimer's disease, the hippocampus is one of the first regions of the brain to be affected, leading to the confusion and loss of memory so commonly seen in the early stages of the disease.

mediated signaling, drastically decreased the survival of primary neuronal cells through apoptosis coupled to oxidative stress [103]. Silver is a bright, silvery white, soft metal that has been used for thousands of years. Silver ornaments, utensils, and art work have been around for a long time. Silver has monetary value and silver coins and jewelry are considered as valuables. Silver is used in large quantities as catalysts, mainly in the production of ethylene oxide. It is also used industrially for conductors, mirrors, and photographic applications. One of the interesting properties of silver is its antibacterial and antifungal activity. As a result, the use of nanoparticulate silver is one of the fastest growing areas of commercial NP applications [88]. Due to their excellent antibacterial properties, silver NPs have been used in food services, building materials, textile industry, medical instruments, personal care products, and washing machines [89]. Silver NPs (Ag NPs) are used as room sprays, deodorants, wall paints, and laundry detergents, and are also used for indoor air purification and water detoxification [90,91]. **As a result of these widespread uses and exposure of silver NPs to humans, it is likely that Ag NPs enter the body and accumulate in various tissues and organs** [92]. **Previous research has indicated that Ag NPs can accumulate in several organs, which includes the kidney, liver, testis, lung, and brain** [93]. In vitro studies have shown that **Ag NPs are capable of inducing toxicity in cells derived from a variety of tissues, including liver, skin, vascular system, lungs, and reproductive organs**

Iron oxide (FeO, Fe2O3, Fe3O4) NPs

Iron oxide or superparamagnetic iron oxide nanoparticles (SPIONs) have become one of the most favorable and exciting choices in both the industrial and biomedical fields, due to their superparamagnetic property and other physicochemical characteristics unique to nanomaterials. SPIONs (Feridex) are small NPs composed of a Fe3O4 (magnetite) or Fe2O3 (maghemite) core. Although maghemite is naturally ferromagnetic, **with the decreasing size (< 30 nm), it becomes superparamagnetic.** Their potential application ranges from biomedical imaging (magnetic resonance imaging, positron emission tomography, or ultrasound as contrast agent), gene and drug delivery, tissue regeneration, hyperthermia in cancer treatment, catalysis, and magnetic storage [104]. They are extensively used specifically for brain imaging or braintargeted drug and gene delivery, due to their ability to move across the BBB [105]. SPIONs are metal oxide NPs that have been clinically approved, **although recently they have been taken off the market** [106,107]. In spite of their desirable traits, there is a critical need to investigate their toxicity both in vivo and in vitro. SPIONs have already been shown to have **potential toxicity that can lead to altered gene expression, actin modulation, interference with cell cycle regulation and signaling pathways, excessive ROS generation, and disruption of iron homeostasis** [108]. According to the recent findings, environmental factors are a major contributor to the development of neurodegenerative diseases, such as Parkinson's disease and Alzheimer's disease [109]. Peters et al [110] **have emphasized the significance of oxidative**

stress generated by NPs in the brain, along with the evaluation of the possible connection between long-term NP exposure and neurodegenerative disease. With increased use of Fe3O4 NPs in industry and biomedical sciences, **the risk related to occupational exposure has escalated considerably.** Involvement of ultrafine particulate materials in polluted air **leads to protein fibrillation**. Fibrillation of specific proteins, for example, Ab42 and a-synuclein, may play a role in the development of Alzheimer's disease and Parkinson's disease [111]. SPIONs have further been shown to form a corona with plasma proteins. **This corona can lead to several toxic side effects because the initial cellular interaction of magnetic nanoparticle (MNP) changes lead to downstream modification of cellular and tissue interaction** [112,113]. In 2007, Pisanic et al [114] used PC12 cells as a quantifiable in vitro model system to study the toxic effect of anionic Fe3O4 MNPs in a dosedependent manner. In that study, it has been established that when PC12 cells were exposed to the anionic MNPs at an increasing concentration ranging from 0.15mM to 15mM iron, they lost their **viability and were unable to generate normal neurite growth in the presence of nerve growth factor**. They have concluded that the anionic **MNPs were possibly interfering with transcriptional regulation and protein synthesis, for example, Growth associated protein (GAP)-43 leading to cellular death and phenotypic changes**. In 2009, Wang et al [115] discussed the ability of submicron level Fe3O4 NPs to be transported to the brain via the olfactory nerve pathway, leading to oxidative-stress-related damage in the brain. **They also discussed changes in the ultrastructure of the olfactory bulb nerve cells.** Recently, Wu et al [116] have focused on the neurotoxicity of iron oxide NPs in the rat brain (in vivo). The study investigated the effect of uptake and retention of Fe3O4 NPs in rat brain hippocampus and striatum, including oxidative injuries. T**he olfactory bulb, striatum, and hippocampus seemed to be the main sites for Fe3O4** NP deposition after intranasal instillation [117]. **Approximately 80% of NPs were still found in the striatum at 7 days after instillation and about 50% were found in both the striatum and hippocampus after 14 days. The striatum in the instillation groups exhibited comparatively more susceptibility to oxidative stress, as indicated by increased levels of H2O2 and decreased Glutathione peroxidase (GHS-PX) activity in the control group at 7 days after exposure. The group also investigated the effect of Fe3O4 NPs in PC12 cells in vitro. The PC12 cells showed dose-dependent cytotoxicity, as measured by LDH release and MTT assay, demonstrating membrane disruption and mitochondrial enzyme activity, respectively.** The oxidative stress was also evident **by the reduced GSH-PX and superoxide dismutase activity**, and increased ROS level and **lipid peroxidation**. Fe3O4 NPs also had a substantial cytotoxic effect on PC12 cells by modulating the cell cycle and inducing apoptosis. JNK is usually activated by oxidative stress and **modulates apoptosis, neurodegeneration, cell cycle control, and cellular proliferation** [118]. The cells also exhibited phosphorylation of p53 protein at ser15 position and elevated levels of bax and bcl-2 proteins upon exposure to NPs. It has been demonstrated that intranasally

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Comment [135]: biophysicochemical properties of NPs, which define their affinity for protein monomers, unfolded monomers, oligomers, critical nuclei, and other prefibrillar states--- • Amyloidosis • Systemic - Build - up of amyloid deposits • Organ - specific amyloidosis • E.g. Alzheimers disease, Parkinsons disease… • Protein re/mis - folding and aggregation • General feature of all proteins ('the other side of folding')? • From native/soluble to non - native/cytotoxic, massive insoluble

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Comment [136]: these are the highest or most potent antioxidants in the immune system and when iron nano is added is showing a drastic reduction in them due to the fact the system is trying to rid the density of the iron and as a result is overloaded and depleted

instilled **Fe2O3 NPs are transported into the brain via the olfactory route [119], and additional investigation has been made of the size-related effect. After a single intranasal exposure of 21-nm Fe2O3 NPs, there was a significant increase in iron content in almost all the brain regions, from the olfactory bulb, hippocampus, cerebral cortex, and cerebellum to the brainstem** [120]. However, a single intranasal exposure of 280-nm Fe2O3 NPs led to a significant increase in iron content only in the olfactory bulb and hippocampus, with no significant alteration of iron content in other brain regions. At 30 days after instillation of 280nm Fe2O3 NPs in mice, the iron content in the olfactory bulb and hippocampus also increased but was lower than that in mice treated with 21-nm Fe2O3 NPs. **It is widely known that brain iron accumulation is associated with the oxidative stress induced by the formation of the highly reactive OH via the Fenton reaction [121e123]**. The excess iron in the brain suggests an association with the oxidative stress response. The generation of ROS is a well established paradigm to explain the toxic effects of NPs [40]. It has been demonstrated that intranasal exposure of iron oxide NPs causes a certain degree of oxidative stress response in mouse brain [119]. Significant oxidative stress responses in the brain of mice have also been observed after intranasal exposure of 21-nm and 280-nm Fe2O3 NPs [124]. **Alterations of iron and zinc levels in the brain are more evident in mice exposed to nano-sized than submicron-sized Fe2O3**. Furthermore, the strong positive correlation between the iron and zinc levels in the sub-brain regions may contribute to the understanding of zinc homeostasis in the brain after Fe2O3 particle inhalation. **The biomarkers of oxidative stress, activity of nitric oxide synthases, and release of monoamine neurotransmitters in the brain have been studied as well [115]. It was shown that significant oxidative stress was induced by the two sizes of Fe2O3 NPs**. The activities of GSH-PX, copper, zinc superoxide dismutase, and constitutive nitric oxide synthase were significantly elevated and the total glutathione and glutathione/glutathione disulfide ratio were significantly decreased in the olfactory bulb and hippocampus after treatment with nano- and submicron-sized Fe2O3 particles. **The nano-sized Fe2O3 generally induced greater alteration and a more significant dose effect response than the submicron particles did**. Transmission electron microscopy showed that nano-sized Fe2O3 treatment induced some **ultrastructural alterations in nerve cells, including neurodendron degeneration, membranous structural disruption, and increased lysosomes in the olfactory bulb, dilation in the rough endoplasmic reticulum, and increased lysosomes in the hippocampus.** The results indicated that intranasal exposure of Fe2O3 NPs could induce more severe **oxidative stress and nerve cell damage in the** brain than the larger particles did. **Fe3O4 NPs also exert cytotoxic effects by influencing the cell cycle and apoptosis** [116]. For example, cells are arrested at the G2/M phase after 24 hours exposure to NPs. Arrest at the G2/M phase provides time for these cells to instigate DNA repair and delay cell death. **However, cells with impaired DNA repair processes enter apoptosis**. The study indicates that Fe3O4 NPs are deposited and retained in the striatum after intranasal instillation,

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Comment [137]: You would almost think that the nanotech is by design made to induce brain damage and brain trauma ~ disconnect from the body and to create the inability to think or remember

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Comment [138]: NANO Iron causes severe nerve damage –reduces the glutathione and SOD

and the NPs may then cause oxidative damage in the striatum. The results of in vitro studies on dopaminergic neurons have demonstrated that Fe_3O_4 NP exposure decreases cell viability and induces marked oxidative stress. Furthermore, Fe_3O_4 NPs mediated apoptosis signaling pathway included JNK and c-Jun phosphorylation, p53 phosphorylation, Bax upregulation, Bcl-2 downregulation, and apoptosis.

Copper and copper oxide NPs

Copper is one of the essential trace elements for energy production in biological systems. **Copper is a requirement for the synthesis of different enzymes, including cytochrome c oxidase, superoxide dismutase, tyrosinase, lisyl oxidase, and cupro-protein** [125,126]. **Copper is also responsible for stimulating the production of neurotransmitters such as epinephrine and norepinephrine in the brain and can be found there at a high concentration** [127]. However, at higher than normal levels, unbound copper become toxic to the human body because it disrupts homeostasis. Its redox activity can give rise to ROS, leading to oxidative stress and modification of protein, lipid, and nucleic acid [128,129]. Compounds of copper such as copper oxide (CuO) NPs have found a broad use in various areas. CuO NPs are used in inks, lubricants, coatings, semiconductors, heat transfer fluids, antimicrobial preparations, and intrauterine contraceptive devices [130]. Copperbased NPs are used as lubricant additives because they reduce friction and wear, and worn surfaces can be repaired by an addition of copper NPs in lubricants. As more copper NPs are currently in use, it is likely that human exposures to copper NPs will increase gradually. **Due to their nanolevel size, CuO NPs are capable of crossing the BBB and pose a threat to the CNS**. Studies have shown that copper **NPs can cause BBB dysfunction, swelling of astrocytes, and neuronal degeneration once introduced into the bloodstream** [1,131]. Li et al [132] showed neurotoxicity of CuO NPs in a dose-dependent manner in H4 neurogiloma cells using an automated image analysis technique. **Primary cultures of dorsal root ganglion of neonatal rat pups were investigated to measure neurotoxicity of copper NPs of varying size and concentration** by Prabhu et al [133]. After exposured to 10-100mM copper NPs (40 nm, 60 nm, and 80 nm) for 24 hours**, the neurons started to develop vacuoles and became detached from the substratum**. **They also exhibited disruptive neurite growth.** LDH and MTT assays have also shown the significant toxicity of **copper NPs, and the smaller size is associated with higher toxicity**. The whole-cell patch-clamp technique was used to study the influence of CuO NPs on voltage-dependent potassium current in acutely isolated rat CA1 pyramidal neurons of the hippocampus [134]. Although the CuO NPs did not have a significant effect on the outgoing potassium current, they did inhibit the delayed rectifier potassium current at a relatively high concentration. CuO NPs shifted the inactivated curve of rectifier potassium current negatively but did not show any significant effect on transient outgoing potassium current. These blockades of the potassium current might inhibit the normal functional activity of

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Comment [139]: A vacuole (/ˈvækjuːoʊl/) is a membrane-bound organelle which is present in all plant and fungal cells and some protist, animal[1] and bacterial cells.[2] Vacuoles are essentially enclosed compartments which are filled with water containing inorganic and organic molecules including enzymes in solution, though in certain cases they may contain solids which have been engulfed. Vacuoles are formed by the fusion of multiple membrane vesicles and are effectively just larger forms of these.[3] The organelle has no basic shape or size; its structure varies according to the needs of the cell.
The function and significance of vacuoles varies greatly according to the type of cell in which they are present, having much greater prominence in the cells of plants, fungi and certain protists than those of animals and bacteria. In general, the functions of the vacuole include:
•Isolating materials that might be harmful or a threat to the cell
•Containing waste products
•Containing water in plant cells
•Maintaining internal hydrostatic pressure or turgor within the cell
•Maintaining an acidic internal pH
•Containing small molecules
•Exporting unwanted substances from the cell
•Allows plants to support structures such as leaves and flowers due to the pressure of the central vacuole
•In seeds, stored proteins needed for germination are kept in 'protein bodies', which are modified vacuoles.[4]
Vacuoles also play a major role in autophagy, maintaining a balance between biogenesis (production) and degradation (or turnover), of many substances and cell structures in certain organisms. They also aid in the lysis and recycling of misfolded proteins that have begun to build up within the cell. Thomas Boller [5] and others proposed that the vacuole participates in the destruction of invading bacteria and Robert B Mellor proposed organ-specific forms have a role in 'housing' symbiotic bacteria. In protists, vacuoles have the additional function of storing food which has been absorbed by the organism and assisting in the digestive and waste management process for the cell.[6]

nerve cells. In another study, Trickler et al [135] **has determined the effect of copper NPs on induction of proinflammatory mediators, followed by their influence on rat brain microvessel endothelial cells**. **At a low dosage, the copper NPs enhanced cellular proliferation, whereas at a high concentration, they started to express toxicity. NP exposure increased prostaglandin E2 release. Extracellular levels of TNF-a and IL-1b were considerably higher in the exposed cells**. **This resulted in the disruption of cerebral microvasculature by increasing its permeability**. According to Karlsson [136], nano-CuO is highly toxic when compared with other metal oxide NPs. However, few studies have investigated the direct effects of nano-CuO on neurotoxicity and the potential mechanisms involved in these effects. A study explored the potential neurotoxicity of nano-CuO on ion channels of neuron, voltage-dependent sodium current (INa) in rat hippocampal slices with whole cell patch-clamp technique [137]. **The results showed that nano-CuO inhibited the peak amplitude of INa, which might have decreased intracellular Naþ concentration due to decreased Naþ influx**. This could inhibit the exchange of Naþ for Ca2þ by NaþeCa2þ exchangers [138]. **The exchanger was shown to generate inward current during the repolarization phase of the action potential [139], thus, the effect on INa could contribute to the change in action potential shape by nano-CuO. It is well established that voltage-gated sodium current (VGSC) plays a role in neurotransmitter release** [140]. Thus, the effects of nano-CuO on INa also mean that modulation may produce functional effects on neurotransmission in the CNS. It has been shown that nano-CuO produces a hyperpolarizing shift in the activation curve. The S4 segment in a subunit of VGSCs contains 4-8 positively charged residues at three residue intervals. They serve as voltage sensors and initiate the voltage-dependent activation of VGSCs by moving outward under the influence of the electric field [141,142]. **The results suggest an effect on the S4 segment of the activation gating, resulting in conformational changes of the channel**. The findings also confirm that the effects of nano-CuO on hippocampal neurons are mediated through activation of ROSeINaeaction potential signaling cascades and are independent from the G-protein pathway. These results show the primary mechanisms underlying nano-CuO-induced INa amplitude inhibition and improve our understanding of nanoCuO neurotoxicology. To determine the potential neurotoxicity of CuO NPs, human SH-SY5Y neuroblastoma and H4 neuroglioma cells were exposed at a concentration range of 0.01-100 mM for 48 hours [132,143]. The data indicated that exposure of CuO NPs induced differential toxic effects in both SH-SY5Y and H4 cells, and the cells had dose-dependent toxic responses to the CuO NPs. The toxic effects of CuO NPs were also investigated in a semiadherent, fast-growing, mouse neuroblastoma cell line (N2A cells), to provide a better understanding of the toxicological risks of CuO NPs in future nanotechnology developments [144]. N2A cells were less sensitive to CuO NP effects than other cultured cells were. The lower sensitivity may have been due to the agglomeration of CuO NPs in the culture medium, which resulted in an average particle size > 300 nm. **Agglomeration of CuO NPs reduced surface-specific effects specific to nanoscale materials, and increased**

the contribution of particle solubilization in the toxic response induced in N2A cells. **Agglomerated CuO NPs induced both cytotoxic and genotoxic effects in N2A cells**.

Aluminum oxide (alumina, Al2O3) NPs

In recent years, the areas of nanotechnology and nanomedicine have expanded rapidly, aluminum oxide (alumina) NPs, having good electric and abrasive properties, are widely used as abrasive agents or insulators in motor vehicles, electronics, energetics, exterior coatings, personal care products, scratch-resistant coatings, alloys, and sensors [145]. **This has led to increased human exposure to aluminum oxide NPs (nano-alumina).** An in vivo study in ICR mice aimed to investigate the effects of nano-alumina, with a focus on the effects on neurobehavioral defects and possible mechanisms of action. It showed that **nano-alumina induced apoptosis via increased caspase-3 gene expression and impaired spatial learning behavior, which suggests that mitochondrial impairment plays a key role in the neurotoxicity of nano-alumina** [146]. The research could help to understand the underlying mechanisms of toxicity of nano-alumina, particularly with respect to neurobehavioral function. The authors declared that **impairment of the mitochondria played an important role in the neurotoxicological effects of nano-alumina and might be a direct cause of neurobehavioral defects**. The possible neurotoxic effects of nano-alumina and bulk alumina have been compared in nematodes [147]. The relatively large surface area of nano-alumina compared with bulk alumina might also explain the differences in toxicity between nano-alumina and bulk alumina. **The decrease in locomotive behavior in nematodes chronically exposed to nano-alumina was associated with both an increase in ROS generation and disruption of ROS defense mechanisms.** Drosophila was used as another model organism to explore the effects of nano-alumina on the CNS [148]. The rhythmic and electrophysiological activities in the antennal lobe of Drosophila were recorded using patch clamps. Fifteen minutes after application of nano-alumina, **the average frequency of spontaneous activity was significantly decreased** compared with that of the control groups. The results indicate that **nano-alumina might have adverse effects on the CNS** in Drosophila. The hypothesis that nano-alumina can affect the BBB and induce endothelial toxicity has been proposed [149]. In the first series of experiments, **human brain microvascular endothelial cells were exposed to nano-alumina and control NPs in dose- and time-responsive manners**. Treatment with nano-alumina markedly **reduced human brain microvascular endothelial cell viability, altered mitochondrial potential, increased cellular oxidation**, and decreased tight junction protein expression as compared to treatment with control NPs. Alterations of **tight junction protein levels were prevented by cellular enrichment with glutathione**. In the second series of experiments, rats were infused with nano-alumina at a dose of 29 mg/kg and brains were stained for expression of tight junction proteins. **Treatment with nano-alumina resulted in marked**

fragmentation and disruption of integrity of claudin-5 and occludin. The results indicate that the **cerebrovasculature could be affected by nano-alumina**. In addition, the data indicate that alterations of mitochondrial function might be the underlying mechanism of nanoalumina toxicity. As far as the assessment of toxicological properties of nanoparticles is concerned, it is important to know whether cultured neural cells take up NPs, and if so, what mechanisms are involved [150]. **Ultrastructural examination has shown that nano-alumina penetrates the cell membrane and that some particles are engulfed by the cells and mainly accumulated in the cytoplasm**. It has been demonstrated that the NPs entering the cells are likely to have an effect on cellular function. **Bulk-alumina-treated cells show apoptotic characteristics,** whereas **nano-alumina-treated cells demonstrate both apoptotic and necrotic morphological changes**. Photomicrographs **show that the vesicles with individual particles and aggregates remain in the cytoplasm and the nucleus**. According to transmission electron micrographs, **NPs form aggregates inside the lysosomal vesicles and their internalization in lysosomal bodies is arranged in a perinuclear fashion.** The presence of an elevated amount of lysosomes might reflect enhanced phagocytosis of exogenous particles. Microglia and astrocytes are dominant glial and major immune cells in the CNS. They are sensitive to changes in the microenvironment of the CNS and are rapidly **activated in almost all conditions that affect normal neuronal functions**. Activation of microglia and astrocytes in the cortex and hippocampus following peripheral administration of nanoalumina have been analyzed in SpragueeDawley rats [151]. There was significant glial activation induced in rat brain after nano-alumina administration.

Silicon dioxide (silica) NPs

Silica (SiO_2) NPs have been developed for mechanical polishing, additives to food and cosmetics, and have various applications in biomedical fields, including diagnosis, optical imaging, targeted drug delivery for the CNS, cancer therapy, and controlled drug release for genes and proteins. In particular, being considered more biocompatible than other imaging NPs, silica NPs are emerging as ideal materials for medical applications. For applications of potential drug delivery, imaging, and diagnostics in the CNS, silica NPs are also being modified or used for coating or stabilization of other optical materials. However, to date, little is known concerning the potential adverse effects on the brain associated with exposure to silica NPs**. Research has indicated that silica NPs via intranasal instillation enter the brain and show a distinct pattern of biodistribution, and are especially deposited in the striatum**,  except for the olfactory bulb [152]. **Such an accumulation could result in oxidative stress, inflammatory changes, and functional damage of the striatum**. In addition, silica NPs appeared to

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Comment [140]: Functionally, the striatum coordinates multiple aspects of cognition, including motor and action planning, decision-making, motivation, reinforcement, and reward perceptio

induce **depleted dopamine in the striatum, and the main contribution was downregulation of tyrosine hydroxylase protein**. In vitro studies on dopaminergic neurons have demonstrated **that silica NPs have marked cytotoxic effects and oxidative stress activity against PC12 cells** [152]. Furthermore, activation of the p53 pathway is involved in the mechanism of the silica-NP- induced G2/M arrest and apoptosis**. Additionally, the decrease in dopamine levels is most likely attributable to the reduction of dopamine synthesis.** The authors **have claimed that although extrapolation** of the animal effects to humans remains a challenge, **their results for the neurotoxic effect on rat brains could be suggestive of human exposure**, because different species may respond differently to the same substance. **Another study demonstrated that exposure to 300 ppm silica NPs in differentiating cells showed less cytotoxicity than in undifferentiated cells** [153]. Silica NPs at 100 ppm had no significant effect on the viability of either undifferentiated or differentiating neuroblastoma (SH-SY5Y) cells. Neurite outgrowth in differentiating cells after 48 hours exposure to 100 ppm silica NPs was not significantly changed. **Thus, silica NPs appeared to have no effects in the early initiation of neurites.** Although the production of ROS was not induced, **neurotoxicity induced by silica NPs may be the result of increased DNA damage, apoptosis, and cell cycle arrest in undifferentiated and differentiating cells, which is independent of neuronal differentiation of SH-SY5Y cells.**

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Comment [141]: This would explain the possibility of a T4 deficiency since the tyrosine and selenium and iodine are part of this nd dopamaine uptake and use

Carbon-based nanomaterials

Owing to their unique chemical and physical properties, carbon-based nanomaterials have a potential use in a variety of biomedical applications, including early diagnosis of cancer, imaging, targeted photothermal therapy, drug delivery, and tissue engineering. **Based on the shape, organic carbon-based nanomaterials are categorized as carbon nanotube, fullerene, graphene, or carbon NPs. Carbon nanotubes are onedimensional forms of graphitic material and are present in many forms, depending on the number of graphene sheets used: single-walled carbon nanotubes, double-walled carbon nanotubes, and multi-walled carbon nanotubes with diameters of 1-2 nm and lengths of 0.05-1 mm.** Graphene has similar chemical composition and crystalline structure with a flat sheet with a single layer or multilayer graphene with several layers. The fullerenes (C60) are named after Richard Buckminster Fuller as buckminsterfullerene, or the "bucky ball". **This allotrope of carbon consists of 60 carbon atoms joined together to form a cage-like structure**. C60 is soluble in aromatic solvents (e.g., toluene or benzene), but insoluble in water and alcohol. However, C60 can be functionalized (e.g., with eOH, eCOOH, or eNH2) to increase its hydrophilicity. By contrast, aqueous fullerene aggregates can be generated by mixing pure C60 in water or through solvent extraction. Some fullerenes have been shown to inhibit human immunodeficiency virus (HIV) activity through inhibiting an HIV-associated protease, an essential enzyme for

viral survival. It has been reported that some fullerenes can interact with biological membranes to elicit antimicrobial action, antitumor activity, enzyme inhibition, DNA photo cleavage, and neuroprotective activity via antioxidant actions. At present, fullerenes are commercially used in products including fuel cells, semiconductors, and product coatings, for example, bowling ball surfaces. **Studies of carbon nanomaterials have indicated the potential neurotoxic effects after inhalation or systemic exposure**. Oberdorster and co-workers [17] showed that inhalation ¨ of elemental 13C NPs of 36 nm by rats, **which were exposed for 6 hours whole-body exposure**, **led to a significant and persistent increase in the accumulation of 13C NPs in the olfactory bulb, and the NP concentration gradually increased**. A recent study has shown that **different shapes of carbon nanomaterials elicit different toxicity in neuronal culture models**. Specifically, pure graphene is less toxic than highly purified single-walled carbon nanotubes in a concentration dependent manner after 24 hours exposure of PC12 cells, involving the apoptosis pathway [154]. Subsequently, the impact of surface functionalization on the toxicity of carbon nanotube has been demonstrated using the same culture model. Carbon nanotubes with surface-coating polyethylene glycol are **less toxic** than uncoated carbon nanotubes, by measuring mitochondrial function and membrane integrity. A mechanistic study has shown **that oxidative stress is involved in this toxic pathway, with surface coating playing an important role [155]. It has been reported that 14-nm carbon black particles might translocate to the olfactory bulb through olfactory neurons, resulting in the activation of microglial cells, which induces proinflammatory cytokines and chemokines, suggesting an inflammatory response** [156]. Additional systematic evaluations and mechanistic in vivo studies are needed to understand the effect of surface coating on the biocompatibility of these carbon-based nanomaterials prior to use in humans.

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Comment [142]: Does nor imply safe just means not as dangerious or is less hazardous

Future perspectives

Physical and chemical characterization is considered to be the key element in assessing the neurotoxicity of nanomaterials. The nanomaterials used in the study require a comprehensive physicochemical characterization before during, and after the biological testing models are exposed to nanomaterials. **As mentioned previously, the size, size distribution, purity, shape, crystal structure, composition, surface coating, surface charge, and surface reactivity may result in a different distribution, accumulation, and transport of the nanomaterials to the target organs, as well as across the BBB**. Research findings are meaningless for hazard identification in the absence of adequate evaluation of the physical and chemical properties of nanomaterials. For example, **impurities that contaminate the nanomaterials being tested may contribute most to neurotoxicological responses**. **The dissolution of metal ions from metal oxide nanomaterials may play an important role in neurotoxicity. The size or surface charge of nanomaterials**

might change the biokinetics of the nanomaterials, resulting in different pharmacological or toxicological actions in biological systems. **However, batch-to-batch inconsistency is a major challenge when nanomaterials are produced by different manufactures/laboratories. The exposure dose level should be carefully considered when laboratory animals or in vitro models are exposed to nanomaterials**. The practically exposure level to human should be used as a reference when calculateing the relevant dose exposed to the animals or in vitro models. This will support studies for understanding the dosimetry in the nervous system. **The characteristics of the nanomaterials should also be considered in physiologically based pharmacokinetic modeling to better predict the environmental hazard of the nanomaterials**. To date, the data gap of well-designed neurotoxicity assessment of nanomaterials still exists, and further in vivo studies will be considered as an urgent demand in the future**. Appropriate dose eresponse research should be considered in neurotoxicological studies**. Recent inhalation studies have shown that the surface area or particle number, instead of the nanomaterials mass, is considered as the major dosimetry unit in term of the dose-response relationship**. Cellular or target organ dose will provide a better understanding of the neurotoxicological responses, because the physical properties might change quickly in the biological system under the experimental conditions**. Sensitive and specific methods need to be developed to quantify the nanomaterials, including metal NPs or carbon-based nanomaterials. The nanomaterials may interfere with the enzymatic assay during the measurement of neurotransmitters (such as acetylcholine or dopamine) using traditional methods. Therefore, the traditional approaches using chemicals should be carefully validated because they are used in nanoneurotoxicological studies.

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Comment [143]: The other issues with nano is the integration with other nano partiles which can integrate and alter the initial chemical structure and as a result when falling to the ground or environmental exposure or a mixture of air pollutamts already in the atmosphere engage with biological and pathological will also play a roll in the impact of the damage and morphology of the nano with the genetic code or dna of other organic life

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Comment [144]: The reason why this is so is because of the unregulated policy is due to the 1.6 trillion profits and the fact this is a total weaponized tech

heat stroke following chronic intoxication of Cu or Ag engineered nanoparticles. A comparative study with other neuroprotective agents using biochemical and

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Mechanisms of Nanoparticle-Induced Oxidative Stress and Toxicity

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Received 9 May 2013; Accepted 16 July 2013

Academic Editor: Nikhat J. Siddiqi

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Abstract

The rapidly emerging field of nanotechnology has offered innovative discoveries in the medical, industrial, and consumer sectors. The unique physicochemical and electrical properties of engineered nanoparticles (NP) make them highly desirable in a variety of applications. **However, these novel properties of NP are fraught with concerns for environmental and occupational exposure**. Changes in structural and physicochemical properties of **NP can lead to changes in biological activities including ROS generation, one of the most frequently reported NP-associated toxicities**. **Oxidative stress induced by engineered NP is due to acellular factors such as particle surface, size, composition, and presence of metals, while cellular responses such as mitochondrial respiration, NP-cell interaction, and immune cell activation are responsible for ROS-mediated damage**. **NP-induced oxidative stress responses are torch bearers for further pathophysiological effects including genotoxicity, inflammation, and**

fibrosis as demonstrated by activation of associated cell signaling pathways. Since oxidative stress is a key determinant of NP-induced injury, it is necessary to characterize the ROS response resulting from NP. Through physicochemical characterization and understanding of the multiple signaling cascades activated by NP-induced ROS, a systemic toxicity screen with oxidative stress as a predictive model for NP-induced injury can be developed.

1. Introduction

The growing field of nanotechnology has transformed many sectors of the industrial field with their breakthrough applications in the areas of biotechnology, **electronics, medicinal drug delivery, cosmetics, material science**, aerospace engineering, and biosensors. Manufactured nanomaterials (NM) have gained commercial interest in a variety of consumer products. Their novel physicochemical, thermal, and electrical properties facilitate their application in clothing, medicine, and cosmetics thereby increasing the probability for human and environmental contact with these NM [1–3]. Of all the NM, **carbon nanotubes (CNT) and metal-based nanoparticles (NP) have generated considerable commercial interest owing to their remarkable intrinsic properties such as high tensile strength and conductivity**, which in turn meet the needs of the specific application for which these NP are designed [4, 5]. Their widespread use raises concerns of their inadvertent exposure in humans and the consequent deleterious health effects [6]. As compared to the growing commercial interest of NM, modest research effort has been invested in evaluating the potential adverse effects of these engineered NM**. The sheer multiplicity of the physicochemical parameters of NM such as size, shape, structure, and elemental constituents makes the investigation of their toxic effects complex and challenging** [7]. **Some of the paradigms for NP-mediated toxicity include oxidative stress, inflammation, genetic damage, and the inhibition of cell division and cell death** [8–11]. Most work to date has suggested **that ROS generation** (which can be either protective or harmful during biological interactions) and **consequent oxidative stress are frequently observed with NP toxicity** [3, 9]. **The physicochemical characterization of NP including particle size, surface charge, and chemical composition is a key indicator for the resulting ROS response and NP-induced injury since many of these NP intrinsic properties can catalyze the ROS production [**6]. NP-mediated ROS responses have been reported to **orchestrate a series of pathological events such as genotoxicity, inflammation, fibrosis, and carcinogenesis**. For instance, CNT-induced oxidative **stress triggers cell signaling pathways resulting in increased expression of proinflammatory and fibrotic cytokines** [12]. Some **NP have been shown to activate inflammatory cells such as macrophages and neutrophils which can result in the increased production of ROS** [13–15]**. Other NP such as titanium dioxide (TiO_2), zinc oxide (ZnO), cerium oxide (CeO_2), and silver NP have been shown to deposit on the cellular surface or inside the subcellular organelles and induce oxidative stress signaling cascades that eventually result in oxidative stress to the cell** [16]. The mechanism for ROS generation is different for each NP and to date the exact underlying cellular mechanism for ROS generation is incompletely understood and remains to be elucidated. Most of the metal-based NP elicit free radical-mediated toxicity via Fenton-type reactions [4, 17], whereas **mitochondrial damage plays a major role in CNT-mediated ROS generation** [18]. However, it is inaccurate to assume that ROS generation is a prerequisite to NP-induced toxicity since a few studies have reported the direct toxicity of NP without causing ROS [19]. Nevertheless, ROS generation is a major

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Comment [145]: this would be the means of entry and exposure where by at the 1-50 NM would pass through an object or person and immediately translocate throughout the body~ after years of research on nanoparticles nano materials and nanotoxicology and nano biology there is more data on what damage this can do and the extent of the damage

event during NP-induced injury that needs to be thoroughly characterized in order to predict NP-induced toxicity. This review will focus on oxidative stress as a mechanism for understanding NP-induced toxicity. For this paper, we have considered metal-based NP and CNT in the light of oxidative stress. The relationship between different NP characteristics and resulting oxidative stress is discussed.

1.1. Generation of ROS

ROS, key signaling molecules during cell signaling and homeostasis, are **reactive species of molecular oxygen. ROS** constitute a pool of oxidative species including superoxide anion ( ), hydroxyl radical (OH•), hydrogen peroxide (H_2O_2), singlet oxygen ($^1O_2$), and hypochlorous acid (HOCl). ROS are generated intrinsically or extrinsically within the cell. Molecular oxygen generates , **the primary ROS via one-electron reduction catalyzed by nicotinamide adenine dinucleotide phosphate (NADPH)** oxidase. Further reduction of oxygen may either lead to H_2O_2 or OH• via dismutation and metal-catalyzed **Fenton reaction**, respectively [20, 21]. **Some of the endogenous sources of ROS include mitochondrial respiration, inflammatory response, microsomes, and peroxisomes, while engineered NM, environmental pollutants act as exogenous ROS inducers**. Physiologically, ROS are produced in trace amounts in response to various stimuli. Free radicals occur as essential byproducts of mitochondrial respiration and transition metal ion-catalyzed **Fenton-type reactions [20]. Inflammatory phagocytes such as neutrophils and macrophages induce oxidative outburst as a defense mechanism towards environmental pollutants, tumor cells, and microbes**. **A variety of NP including metal oxide particles induce ROS as one of the principal mechanisms of cytotoxicity** [22]. **NP have been reported to influence intracellular calcium concentrations, activate transcription factors, and modulate cytokine production via generation of free radicals** [12, 23].

1.2. Oxidative Stress

Antioxidant metabolite	Solubility	Concentration in human serum (μM)[79]	Concentration in liver tissue (μmol/kg)
Ascorbic acid (vitamin C)	Water	50 – 60[80]	260 (human)[81]
Glutathione	Water	4[82]	6,400 (human)[81]
Lipoic acid	Water	0.1 – 0.7[83]	4 – 5 (rat)[84]
Uric acid	Water	200 – 400[85]	1,600 (human)[81]
Carotenes	Lipid	β-carotene: 0.5 – 1[86] retinol (vitamin A): 1 – 3[87]	5 (human, total carotenoids)[88]
α-Tocopherol (vitamin E)	Lipid	10 – 40[87]	50 (human)[81]
Ubiquinol (coenzyme Q)	Lipid	5[89]	200 (human)[90]

Abundance of ROS can have potentially damaging biological responses resulting in oxidative stress phenomenon. **It results from an imbalance between the production of ROS and a biological system's ability to readily detoxify the reactive intermediates or repair the resulting damage**. To overcome the excess ROS response, **cells can activate enzymatic and nonenzymatic antioxidant**

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Comment [146]: Niacin or Niacinamide or B 3

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Comment [147]: ***several metals have a special oxygen transfer properties which improve the use of hydrogen peroxide. Actually, some metals have a strong catalytic power to generate highly reactive hydroxyl radicals (.OH). Since this discovery, the iron catalyzed hydrogen peroxide has been called Fenton's reaction***

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Comment [148]: List of all the enzymatic and non-enzymatic antioxidants along with their functions and cellular localization. Enzymatic antioxidants Enzyme code Reaction catalyzed Subcellular location
Superoxidedismutase(SOD)1.15.1.1O•−+ 2 O•− 2 + 2H+ → 2H2O2 + O2 Peroxisomes,Mitochondria,Cytosol,andChloroplast Catalase(CAT)1.11.1.62H2O2 → O2+ 2H2O PeroxisomeandMitochondria Ascorbateperoxidase(APX)1.11.1.11H2O2 + AA → 2H2O + DHA Peroxisomes,Mitochondria,Cytosol,andChloroplast Monodehydroascorbatereductase (MDHAR) 1.6.5.42MDHA + NADH → 2AA + NAD Mitochondria,Cytoplasm,andChloroplast Dehydroascorbatereductase (DHAR) 1.8.5.1DHA + 2GSH → AA + GSSG Mitochondria,Cytoplasm,andChloroplast Glutathionereductase(GR)1.6.4.2GSSG + NADPH → 2GSH + NADP+ Mitochondria,Cytoplasm,andChloroplast Guaiacol peroxidase(GPX)1.11.1.7H2O2 + DHA → 2H2O + GSSG Mitochondria,Cytoplasm,Chloroplast,andER **Non-enzymatic Antioxidants Function Subcellular location**
AscorbicAcid(AA)DetoxifiesH2O2 via actionofAPXCytosol,Chloroplast,Mitochondria,Peroxisome, Vacuole,andApoplast ReducedGlutathione(GSH)Actsasadetoxifyingco-substrateforenzymeslike peroxidases,GRandGST Cytosol,Chloroplast,Mitochondria,Peroxisome, Vacuole,andApoplast α -TocopherolGuardsagainstanddetoxifiesproductsofmembrane LPO Mostly inmembranes Carotenoids Quenchesexcessenergyfromthephotosystems,LHCsChloroplastsandothernon-greenplastids FlavonoidsDirectscavengersofH2O2 and 1O2 and OH• Vacuole ProlineEfficientscavengerofOH• and 1O2 and prevent damages duetoLPO Mitochondria,Cytosol,andChloroplast

systems [24]. The hierarchical model of oxidative stress was proposed to illustrate a mechanism for NP-mediated oxidative stress [4, 9]. According to this model, cells and tissues respond to increasing levels of oxidative stress via antioxidant enzyme systems upon NP exposure. During conditions of mild oxidative stress, transcriptional activation of phase II antioxidant enzymes occurs via nuclear factor (erythroid-derived 2)-like 2 (Nrf2) induction. At an intermediate level, redox-sensitive mitogen-activated protein kinase (MAPK) and nuclear factor kappa-light-chain enhancer of activated Bcells (NF-□B) cascades mount a proinflammatory response. However, extremely toxic levels of oxidative stress result in mitochondrial membrane damage and electron chain dysfunction leading to cell death. Some of the key factors favoring the prooxidant effects of engineered NM include either the depletion of antioxidants or the increased production of ROS. Perturbation of the normal redox state contributes to peroxide and free radical production that has adverse effects on cell components including proteins, lipids, and DNA [23]. Given its chemical reactivity, oxidative stress can amount to DNA damage, lipid peroxidation, and activation of signaling networks associated with loss of cell growth, fibrosis, and carcinogenesis [16, 25, 26]. Besides cellular damage, ROS can result from interactions of NP with several biological targets as an effect of cell respiration, metabolism, ischemia/reperfusion, inflammation, and metabolism of various NM [22]. Most significantly, the oxidative stresses resulting from occupational NM exposures as well as experimental challenge with various NP lead to airway inflammation and interstitial fibrosis [27–30].

1.3. Nanoparticle-Induced Oxidative Stress

Nanomaterials of varying chemical composition such as fullerenes, CNT, and metal oxides have been shown to induce oxidative stress [20, 31]. The key factors involved in NP-induced ROS include (i) prooxidant functional groups on the reactive surface of NP; (ii) active redox cycling on the surface of NP due to transition metal-based NP; and (iii) particle-cell interactions [22, 25]. From a mechanistic point of view, we discuss the sources of ROS based on the physicochemical parameters and particle-cell interactions.--Several studies demonstrate the significance of reactive particle surface in ROS generation [20, 32]. Free radicals are generated from the surface of NP when both the oxidants and free radicals bound to the particle surface. Surface bound radicals such as SiO• and present on quartz particles are responsible for the formation of ROS such as OH• and [17, 25]. Ambient matter such as ozone and nitrogen dioxide (NO_2) adsorbed on the particle surface is capable of inducing oxidative damage [16]. Reduced particle size results in structural defects and altered electronic properties on the particle surface creating reactive groups on the NP surface [27, 33]. Within these reactive sites, the electron donor or acceptor active sites interact with molecular O_2 to form which in turn can generate additional ROS via Fenton-type reactions [3]. For instance, NP such as Si and Zn with identical particle size and shape lead to diverse cytotoxicity responses due to their surface properties. ZnO being more chemically active than SiO_2, led to increased formation resulting in oxidative stress. Free radicals are either directly bound to the NP surface or may be generated as free entities in an aqueous suspension [17]. Dissolution of NP and subsequent release of metal ions can enhance the ROS response [25]. For instance, aqueous suspensions of quartz particles generate H_2O_2, OH•, and $1O_2$ [17, 20, 32].--Apart from surface-dependent properties, metals and chemical compounds on the NP surface accelerate the ROS response [34].

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Comment [149]: here we have to look at Antioxidants that reduce ROS and Support the mitochondria- Vitamin C and E -Cq10 - Alpha lipoic Acid- Zinc + copper -Zinc + Manganese-Vitamin A- Selenium

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Comment [150]: silicon dioxide ~ same stuff in grains and pills and other foods

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Comment [151]: these would be the metals themselves in the concentration in the cells tissues or organs or skeletal system due to there density or area they cover would also cause a high level of metal poisoning on there own

Transition metals including iron (Fe), copper (Cu), chromium (Cr), vanadium (V), and silica (Si) are involved in ROS generation via mechanisms such as Haber-Weiss and Fenton-type reactions [25]. **Fenton reactions usually involve a transition metal ion that reacts with H2O2 to yield OH• and an oxidized metal ion**. For example, the **reduction of H2O2 with ferrous iron (Fe2+) results in the formation of OH• that is extremely reactive and toxic to biological molecules** [21]. **Cu and Fe metal NP have been reported to induce oxidative stress (and OH•) via Fenton-type reaction** [26], **while the Haber-Weiss-type reaction involves a reaction between oxidized metal ion and H2O2 to induce OH•** [21, 35]. **NP including chromium, cobalt, and vanadium can catalyze both Fenton and Haber-Weiss-type reactions** [26]. **Glutathione reductase, an antioxidant enzyme, reduces metal NP into intermediates that potentiate the ROS response**. In addition, some metal **NP (Ar, Be, Co, and Ni) promote the activation of intercellular radical-inducing system such as the MAPK and NF-κB pathways [36].** In addition to the prooxidant effect of NP, **ROS are also induced endogenously where the mitochondrion is a major cell target for NP-induced oxidative stress**. Once **NP gain access into the mitochondria, they stimulate ROS via impaired electron transport chain, structural damage, activation of NADPH-like enzyme system, and depolarization of the mitochondrial membrane** [37, 38]. **For instance, cationic polystyrene nanospheres induce mediated apoptosis in murine macrophages based on their ability to target mitochondria [38].** **Cellular internalization of NP has been shown to activate immune cells including macrophages and neutrophils, contributing to ROS/RNS** [22, 25]. This process usually involves the activation of NADPH oxidase enzymes. **In vivo particle exposures such as silica activate the rich pool of inflammatory phagocytes within the lung causing them to induce oxidative outburst** [39]. **NP with smaller particle size are reported to induce higher ROS owing to their unique characteristics such as high surface to volume ratio and high surface charge. Particle size determines the number of reactive groups/sites on the NP surface** [34, 37, 40]. **The pulmonary responses induced by inhaled NP are considered to be greater than those produced by micron-sized particles because of the increased surface area to particle mass ratio [28]. Larger surface area ensures that the majority of the molecules are exposed to the surface than the interior of the NM** [3]. **Accordingly, nano-sized SiO2 and TiO2 and MWCNT induce greater ROS as compared to their larger counterparts** [41]. Additionally, **a study with cobalt/chromium NP exposure demonstrated particle size dependent ROS-mediated genotoxicity** [42].

2. Oxidant Generation via Particle-Cell Interactions

Besides being self-oxidative in nature, NP react with cells and induce their prooxidant effects via intracellular ROS generation involving mitochondrial respiration and activation of NADPH-like enzyme systems [43]. **NP can activate the cellular redox system specifically in the lungs where immune cells including alveolar macrophages (AM) and neutrophils act as direct ROS inducers**. Professional phagocytic cells including neutrophils and AM of the immune system induce substantial ROS upon internalization of NP via the NADPH oxidase enzyme system [44]. The phagocytic oxidative outburst is attributable to some of the NP physicochemical properties. In case of silica and quartz particles, inflammation-induced ROS was associated with the surface-based radical-generating properties of the

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Comment [152]: This is what a Fenton Reaction is will come out Fenton reactions usually involve a transition metal ion that reacts with H2O2 to yield OH• and an oxidized metal ion

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Comment [153]: in other words breaks down the mitochondria ` . basically the structural integrity of the body

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Comment [154]: Anti Aging0 RNA-DNA -Mitochondria and ATP and Telomer Support
Liposomal Formulation with: CQ10-B2-B3-B4-B5-IP6 -GSE-Zinc and Copper- Lysine-BHT-Rosemary e/oThis Formula is designed to support Cellular ATP as well as support Cell and Tissue~ renew DNA and Slow Down Telomere Break down and even reverse

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Comment [155]: in NP or NM Size does matter the smaller it is and the more area or density it can cover the more the charge on the cells

particles [45]. Additionally, NP from the residual oily fly ash and diesel exhaust activate the pool of inflammatory phagocytes resulting in massive ROS release [46]. Furthermore, adsorption of chemicals such as organic matter onto the NP surface may drive the inflammation-induced oxidative stress [24].

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Comment [156]: Organic Matter + NP = increased inflammation and ROS

2.1. Lung Injury Caused by Nanoparticle-Induced Reactive Nitrogen Species

Besides oxidative damage, NP exposure within the lung is reported to induce reactive nitrogen species (RNS). Particle deposition in the lung causes recruitment of inflammatory cells that generate ROS, clastogenic factors, and cytokines either harming or stimulating resident lung cells [31]. Inflammatory phagocytes are an important source of RNS/ROS generation within the lung. Owing to their inducible nitric oxide synthase (iNOS) activity, phagocytes can produce a large amount of genotoxic RNS, including nitric oxide (NO•) and the highly reactive peroxynitrite (ONOO−). ONOO− formed by the reaction of NO• and causes DNA fragmentation, lipid oxidation, and protein dysfunction consequently contributing to particle-induced lung injury [47]. In vivo exposure to SiO_2 and quartz NP elicited an RNS response characterized by increased iNOS and NO• within the lung as a result of phagocyte influx [48, 49].

2.2. Mechanisms of ROS Production and Apoptosis within Metal Nanoparticles

Apoptosis has been implicated as a major mechanism of cell death caused by NP-induced oxidative stress [50–52]. Among the different apoptotic pathways, the intrinsic mitochondrial apoptotic pathway plays a major role in metal oxide NP-induced cell death since mitochondria are one of the major target organelles for NP-induced oxidative stress [38]. High levels of ROS in the mitochondria can result in damage to membrane phospholipids inducing mitochondrial membrane depolarization [53]. Small proportion of electrons escapes the mitochondrial chain and interacts with molecular oxygen to form which later gives rise to H_2O_2 or partially reduces to the damaging OH•. NP can catalyze the generation either by blocking the electron transport chain or accelerating electron transfer to molecular oxygen [54, 55]. Various metal oxide NP including Zn, Cu, Ti, and Si elicit ROS-mediated cell death via mitochondrial dysfunction [56–59].

3. Introduction to Transition Metals

Transition metal oxide particles have been used to revolutionize several fields including catalysis, sensors, optoelectronic materials, drug delivery, automobile, and material science engineering. Apart from industrial scale applications, metal NP are increasingly used in a variety of consumer products such as cosmetics, sunscreens, textiles, and food products. Among the transition metal oxides, titanium dioxide, cupric oxide, and zinc oxide have gained attention owing to their commercial usage [60]. Metal oxide particles can undergo surface modification for better stability and binding to other substrates. Such widespread applications are attributable to their electrochemical and physical properties reflecting their small sizes and reactive surfaces. For example, a relatively inert metal or metal oxide may become a highly effective catalyst

when manufactured as NP. Their fixed particle mass, high aspect ratio, and particle surface bioreactivity tailor them to meet the needs of specific application. **However, a high surface-to-volume ratio makes NP reactive and exposes them to environmental stressors, particularly free radical generation** [61, 62]. Besides, **the nanoscale dimensions enhance their cellular uptake and interaction with biological tissues**. Metals can generate free radicals via the Fenton-type reactions that react with cellular macromolecules and induce oxidative stress [63]. **The toxicity of metallic NP including Zn, Ti, Si, Fe, and Ce has been characterized by increased ROS generation and oxidative stress and apoptosis** [61, 64–66]. The oxidative stress mediated outcomes of various metal NP are summarized in Table 1.
Table 1: List of studies describing the ROS-dependent effects of metal-based NP.
4. Prooxidant Effects of Metal Oxide Nanoparticles

To overcome the overwhelming ROS production**, cells trigger either a defensive or an injurious response eliciting a chain of adverse biological responses. Free radicals are potentially damaging to cellular macromolecules including lipids, proteins, and nucleic acids. DNA is one of the major targets for oxidative stress and represents the first step involved in mutagenesis, carcinogenesis, and aging. ROS/RNS cause oxidative DNA damage in the form of DNA strand breaks, DNA protein cross-links, and alkali-labile sites [67, 68], and given their characteristic nature free radicals appear as one of the likely carcinogens** [25, 69]. Testing the genotoxic potential is essential for carcinogenic risk assessment of NP. **Genotoxic effects may be produced either by direct interaction of particles with genetic material or by secondary damage from particle-induced ROS. Transition metal NP induce chromosomal aberrations, DNA strand breaks, oxidative DNA damage, and mutations** [70]. **OH•, one of the highly potent radicals, is known to react with all components of DNA causing DNA single strand breakage via formation of 8-hydroxyl-2′-deoxyguanosine (8-OHdG) DNA adduct [71, 72]. 8-OHdG is a biomarker of OH•-mediated DNA lesions**. NP exposure significantly elevated 8-OHdG levels both in vivo [73] and in vitro [74], **demonstrating their mutagenic behavior**. A recent study **comparing metal oxide NP including Cu, Fe, Ti, and Ag reported ROS-mediated genotoxicity characterized by micronuclei and DNA damage in vivo** [75]**.--Along with chromosomal damage, free radicals also interact with lipids and proteins, abundantly present in biomembranes, to yield lipid peroxidation products associated with mutagenesis. Polyunsaturated fatty acids are subject to oxidation giving rise to lipid hydroperoxides as the initial step in ROS generation** [25, 76]. **Prooxidant metals such as Cu and Fe react with these lipid hydroperoxides to induce DNA damaging end-products malondialdehyde (MDA) and 4-hydroxynonenal that act as inflammatory mediators and risk factors for carcinogenesis. Exposures to metal oxide NP of Ti, Cu, Si, and Fe were reported to induce tissue damage, abnormal cellular stress response via lipid peroxidation** [77–79].---Alterations within the antioxidant defense system pose as a risk factor for carcinogenesis [68]. **Glutathione, (GSH) a potent free-radical scavenger, is responsible for maintaining the cellular redox state and protecting cells from oxidative damage** [80, 81]. **NP-triggered free radicals reduce GSH into its oxidized form glutathione disulfide (GSSG), thereby contributing to oxidative stress, apoptosis, and sensitization to oxidizing stimuli** [82, 83]. Apart from GSH, **NP-induced ROS modulate the antioxidant activities of ROS-metabolizing enzymes including NADPH-dependent**

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Comment [157]: Genetic Damage through access of exposure to either free radical damaging exposure either through the materia or the effect of the material

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Comment [158]: The individual components of the extracts—e.g., sulfhydryl and flavonoid compounds, gallic acid, ellagic acid, mucic acid, citric acid, reducing sugars, tannin—are observed to have an additive interaction with the major constituents chlorophyll and ascorbic acid, when modulating the effects of the clastogens. (chromosome damage)--- Desmutagens are factors which inactivate mutagens or prevent their interaction with DNA, whereas Boantimutagens modulate DNA replication and repair, preventing mutation fixation

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Comment [159]: USE NAC if you are going to activate this due to the fact NAC directly goes into Gluthatione without any re building

flavoenzyme, catalase, glutathione peroxidase, and superoxide dismutase [84].--It is well established that uncontrolled generation of ROS triggers a cascade of proinflammatory cytokines and mediators via activation of redox sensitive MAPK and NF-κB signaling pathways that control transcription of inflammatory genes such as IL-1β, IL-8, and TNF-α [21]. Oxidative stress plays a key role in NP-induced airway hypersensitivity and respiratory inflammation [85]. A study involving coexposure of metal oxide NP with a bacterial endotoxin demonstrated exaggerated lung inflammation and pulmonary edema [86]. Additionally, studies with different metal oxide NP have demonstrated ROS-mediated inflammatory response. For instance, SiO_2 and TiO_2 NP induce an elevated inflammatory response through the underlying mechanism of ROS generation [64, 85, 87]. Pulmonary inflammation may induce changes in membrane permeability, facilitating NP distribution beyond the lung and indirectly affecting cardiovascular performance [88].--Metal ion-induced free radicals can activate oncogenes such as Ras [25]. Excess amounts of NP have been associated with skin, bladder, liver, lung, and respiratory tract cancers [7]. Transition metals in trace amounts are introduced during the manufacture and preparation of CNT. Given their oxidizable nature, studies suggest that metals including Fe, Co, and Ni are more toxic and fibrogenic upon their interaction with CNT as compared to pure CNT [89–93]. Vanadium pentoxide (V_2O_5), a transition metal byproduct of petrochemicals, is associated with fibrosis via generation of H_2O_2 and other ROS [94]. Occupational exposures to combustion-derived NP such as welding fumes consisting of metals such as Fe, Mn, Si, Cr, and Ni induce fibrogenic responses [95]. Metal containing welding fume NP elicited ROS-dependent lipid peroxidation and inflammation in vivo [96, 97].

5. Cellular Signaling Affected by Metal Nanoparticles

The prooxidant effects of NP result in the activation of signaling pathways, transcription factors, and cytokine cascade contributing to a diverse range of cellular responses. The regulation of redox homeostasis entails signaling cascades such as HIF-1, NF-κB, PI3 K, and MAPK which control proliferation, metastasis, cell growth, apoptosis, survival, and inflammation [7, 12]. At an intermediate level of oxidative stress, proinflammatory pathways are activated in an attempt to maintain the redox equilibrium. The inflammatory cascade involves profibrotic mediators such as TNF-□, IL-1□, and TGF-□ which have been implicated in the pathogenesis of fibrosis. Cells are known to counteract the overwhelming oxidative stress response via increased cytokine expression such as interleukins and TNF-□, activation of kinases, and inhibition of phosphatases thereby influencing the phosphorylation cascade. Protein phosphorylation is involved in the regulation of critical cellular responses including mitogenesis, cell adhesion, oncogenic transformation, and apoptosis. Thus, ROS response appears to be closely related to factors driving carcinogenesis [98].

5.1. NF-□B -The NF-κB group of proteins activates genes responsible for defense mechanisms against cellular stress and regulates miscellaneous functions such as inflammation, immune response, apoptosis, and cell proliferation.

Prooxidant H_2O_2-mediated NF-□B activation through the classical IKK-dependent pathway is well established. ROS such as OH•, HOCl, and $^1O_2$ and RNS such as ONOO□ activate NF-□B via the release of I□Bs resulting in the nuclear translocation of NF-□B [99, 100]. Once inside the nucleus, NF-κB induces

transcription of proinflammatory mediators resulting in inflammation and oxidative stress. During NP-mediated lung injury, ROS activate NF-□B to modulate the production of proinflammatory TNF-□, IL-8, IL-2, and IL-6 from macrophages and lung epithelial cells [101]. **Several metal oxide NP such as Zn, Cd, Si, and Fe exert their toxic effects via ROS-dependent NF-κB activation** [62, 102, 103].

5.2. **AP-1**

Activator protein (AP)-1 is a transcription factor activated in response to oxidants, cytokines, growth factors, and bacterial and viral infections. It is responsible **for regulation of cell proliferation, differentiation, and apoptosis, thereby it is a key factor in carcinogenesis** [104]. **Activation of AP-1 under oxidative conditions is believed to be mediated via phosphorylation of protooncogene c-jun** [68]. **Metal NP including Cr, Ni, and Fe have been shown to activate AP-1 via ROS generation** [60]

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5.3. **MAPK**

MAPK are serine-threonine protein kinases that control a diverse range of cellular responses including proliferation, gene expression, differentiation, mitosis, cell survival, and apoptosis. **MAPK consist of growth factor-regulated extracellular signal-related kinases (ERK) and the stress-activated MAPK, c-jun NH2-terminal kinases (JNK), and p38 MAPK**. **Once ROS production exceeds the capacity of the antioxidant proteins, free radicals may induce oxidative modification of MAPK signaling proteins (e.g., RTK and MAP3 K), thereby leading to MAPK activation**. ROS may activate MAPK pathways via inhibition and/or degradation of MAPK phosphatases (MKP) [105, 106]. Finally, the site of **ROS production and the concentration and kinetics of ROS production as well as cellular antioxidant pools and redox state are most likely to be important factors in determining the effects of ROS on activation of MAPK pathways** [107]. Ag-NP activate JNK protein signaling and apoptosis in a variety of cells [50], **whereas CeO2 NP trigger p38 MAPK signaling in bronchoalveolar cells** [64].

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Comment [160]: Cerium(IV) oxide

5.4. **PTP**

Protein tyrosine phosphatases (PTP) are key regulatory components in signal transduction pathways **involved in cell growth, differentiation, proliferation, and transformation**. The highly reactive cysteine residues of PTP are predisposed to oxidative stress in the form of H2O2, free radicals or changes in intracellular thiol/disulfide redox state [98, 108**]. Metal NP including Zn2+ and V5+ may be critical in redox regulation of PTP via the inhibition of MAPK and EGFR [109, 110].**

5.5. **Src**

Src kinases belong to the nonreceptor tyrosine kinase family involved in the regulation of cell growth. Mild oxidative stress is sufficient to activate Src kinase which later triggers a cell signaling cascade [111]. **This may explain the low dose of metal NP-induced lymphocyte cell death via ROS-dependent activation of Src kinases** [112].

6. Carbon Nanotubes

One of the most promising materials in the field of nanotechnology is CNT, and their widespread applications are attributable to the diverse physical, chemical, and electrical characteristics they possess. CNT are high aspect ratio nanomaterials (HARN) having at least one of their dimensions in the order of 100 nm or less according to the British Standards Institute Report [113]. **CNT are made of either single-walled (SW) or multiwalled (MW) graphite layers**. With unique properties such **as high tensile strength and conductivity**, they have been explored in the areas of electronics, biotechnology, medicinal drug delivery, cosmetics, material science, and aerospace engineering. **CNT structure facilitates their entry, deposition, and residence in the lungs and pleura, resulting in incomplete phagocytosis and clearance from the lungs** [5]. **Owing to their biopersistent and nonbiodegradable nature, and particularly their resemblance to needle-like asbestos fibers**, CNT are believed to **induce biologically harmful effects [89]. Physicochemical parameters such as particle size, surface modification, presence of metals, surface reactivity, and surface charge are responsible for the prooxidant effects of CNT**. Frustrated phagocytosis of CNT has been implied in CNT-induced oxidative stress.

7. Carbon Nanotube-Induced Oxidative Stress

One of the most frequently reported toxicity endpoints for CNT is the formation of ROS which can be either protective or harmful during biological interactions. **Oxidative stress may be caused directly by CNT-induced ROS in the vicinity or inside the cell or could arise more indirectly due to the effects of internalized CNT on mitochondrial respiration** [114] **or in depletion of antioxidant species within the cell** [64]. Moreover, NADPH-mediated ROS are critical for SWCNT-induced pulmonary responses [91]. **The most likely mechanism for CNT-induced oxidative stress and lung toxicity involves mitochondrial dysfunction**. Incomplete phagocytosis of CNT, presence of transition metals and specific reactive groups on the CNT surface are key drivers of ROS generation. **Metal impurities such as Fe, Co, and Ni introduced within the CNT during their synthesis are key factors driving CNT-mediated ROS response** [115, 116]. **CNT-induced oxidative stress mediates important cellular processes including inflammation, cell injury, apoptosis, and activation of cellular signaling pathways such as MAPK and NF-κB which are implicated in the pathogenesis of lung fibrosis** [31, 117]. For instance, SWCNT dependent OH• generation leads to activation of molecular pathways MAPK, AP-1, NF-□B, **and Akt associated with cell proliferation and tumor progression in vitro** [93]. Several studies demonstrate SWCNT-induced oxidative stress [118–120]. Similarly, MWCNT exposures have been reported to induce ROS both in vitro and in vivo [18, 121–123]. Interestingly**, oxidative stress is reported to be a mechanism for biodegradation of CNT**. SWCNT undergoes oxidative biodegradation via myeloperoxidase, a prooxidant enzyme involved in host defense responses [120]. Table 2 summarizes the different studies that report ROS-dependent effects of CNT.

tab2

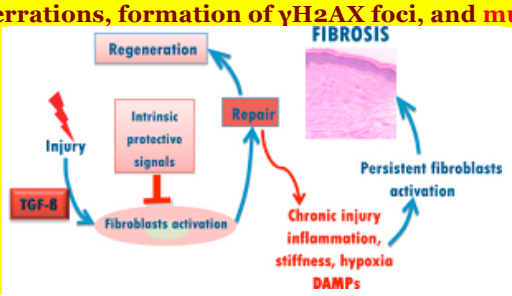
Table 2: List of studies describing the ROS-dependent effects of CNT.

8. Role of ROS in CNT-Induced Inflammation

ROS and inflammation demonstrate an interdependent relationship in the case of exposure to NP. Inflammatory cells such as macrophages and neutrophils induce enormous **ROS release in order to get rid of the NP**. However, NP exposure-mediated oxidative stress leads to activation of RTK, **MAPK, Akt, and NF-κB contributing to the proinflammatory cascade** [124]. Accordingly, CNT-induced ROS were reported to elicit pro-inflammatory transcription factors such as NF-☐B, AP-1 and MAPK in vivo. This was found to be an inflammation dependent response [93]. **MWCNT treatment in macrophages mediates ROS-dependent activation of NF-κB pathway, thereby inducing the expression of chemokines and cytokines such as TNF-α, IL-1β, IL-6, IL-10, and MCP-1 [18]. Likewise, MWCNT-induced nitrosative stress in vivo is associated with pulmonary inflammation** [125].

9. Role of ROS in CNT-Induced Genotoxicity

CNT elicit genotoxic effects through direct interaction with DNA or indirectly via CNT-induced oxidative stress and inflammatory responses. CNT-induced sustained oxidative stress can result in DNA damage and abnormal cell growth, possibly leading to carcinogenesis and fibrogenesis [126, 127]. A plethora of studies demonstrate the genotoxic potential of both MWCNT and SWCNT [128–131]. **ROS can activate cellular signaling pathways resulting in cell cycle arrest and apoptosis**. CNT induce a multitude of genotoxic responses including **DNA strand breakage, oxidation, micronuclei induction, chromosomal aberrations, formation of γH2AX foci, and mutant frequencies** [132]. **Oxidative stress-dependent DNA breakage and repair and activation of signaling pathways including poly-ADP-ribose polymerase** (PARP), AP-1, NF-☐B, p38, and Akt were reported in human mesothelial cells exposed to SWCNT [93]. **CNT induce ROS-dependent lipid peroxidation both in vitro and in vivo** [133, 134]. **A number of studies account for mitochondrial membrane depolarization, damage, and oxidative stress upon CNT exposure** [92, 135, 136]. Unlike the traditional prooxidant effect of NP, **CNT have been reported to sequester ROS which in turn is associated with their structural defects** [83]. This quenching is reported to be related to the genotoxic and inflammatory effects observed with CNT [137].



10. Role of ROS in CNT-Induced Fibrosis

Increased ROS has been implicated in lung inflammation and fibrosis. The inflammatory cascade is reported to contribute to oxidative stress mediated lung injury [138]**. Exposure to CNT results in expression of genes responsible for inflammation and fibrosis via the activation of cell signaling pathways and**

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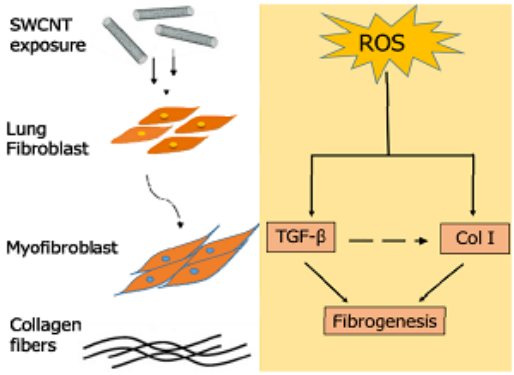
Comment [161]: Fibrogenesis implies just this fibre growth or initiating Fibre growth

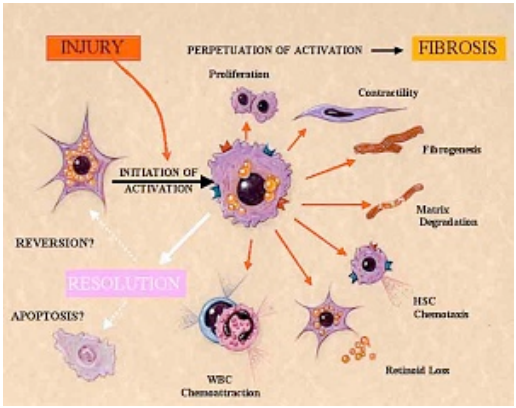
transcription factors including NF-κB, STAT-1, MAPK, and RTK [31]. ROS-dependent p38-MAPK has been shown to be responsible for CNT-induced collagen and angiogenic responses



[118]. Additionally, SWCNT induce fibrogenic effects via ROS-mediated NF-☐B activation [139], whereas MWCNT induce fibroblast to myofibroblast differentiation via ROS-dependent NF-☐B activation [18].

11. **Oxidative Stress as an Underlying Mechanism for NP Toxicity**

Findings from several studies have pointed out that ROS generation and oxidative stress occur as an early event leading to NP-induced injury. Oxidative stress corresponds with the physicochemical reactivity of NP including **metal-based particles as well as the fibrous CNT. Oxidative stress related to NP exposure involves mitochondrial respiration, mitochondrial apoptosis, activation of NADPH oxidase system, alteration of calcium homeostasis, and depletion of antioxidant enzymes; all of which are associated with tissue injury.** NP-driven ROS response contributes to activation of cell signaling pathways, inflammatory cytokine and chemokine expressions, and specific transcription factor activation. **Activation of these cellular mechanisms is closely associated with transcription of genes involved in inflammation, genotoxicity, fibrosis, and cancer**.



Thus, the pathological consequences observed during NP exposure could be attributable to ROS generation. It is essential to incorporate these adverse biological responses as a screening tool for toxic effects of NP. **For instance, over-expression of antioxidant enzymes is indicative of the mild oxidative stress, whereas mitochondrial apoptosis occurs during conditions of toxic oxidative stress**. The hierarchical model of ROS response provides a scale to gauge the adverse health effects upon NP exposures. A NP exposure study must collectively involve rigorous characterization of NP and assign in vitro and in vivo oxidative stress markers as toxicity endpoints as a predictive paradigm for risk assessment [6, 9, 12]. Figure 1 summarizes the key findings regarding the oxidative effects of NP and resulting toxicity. 942916.fig.001-Figure 1: Prooxidant pathway for NP-induced toxicity: various NP exhibit oxidative stress dependent toxicity. **Upon NP exposure, ROS generation is capable of inducing oxidative DNA damage, strand breaks, protein denaturation, and lipid peroxidation thereby demonstrating the mutagenic and carcinogenic characteristics associated with NP**. **Excess free radical production leads to mitochondrial membrane damage causing necrosis and cell death**. Phagocytes including neutrophils and macrophages generate massive ROS upon incomplete phagocytosis of NP through the NADPH-oxidase enzyme system whereas NP-induced ROS triggers an inflammatory cascade of chemokine and cytokine expression via activation of cell signaling pathways such as MAPK, NF-□B, Akt, and RTK**. Furthermore, oxidative stress mediated stimulation of these cellular mechanisms results in transcription of genes responsible for fibrosis, EMT, and carcinogenesis. NP-elicited ROS is at the center stage for majority of the ensuing adverse outcomes**.

12. Conclusion

This paper reviews the cellular mechanisms of NP-induced oxidative stress and toxicity. We focus on the toxicity of metal oxide NP and CNT with respect to the oxidative stress paradigm. The principal factors for NP-induced oxidative stress involve (a) the oxidative properties of the NP themselves and (b) oxidant generation upon interaction of NP with cellular material. The direct prooxidant effects of NP are attributable to their physicochemical properties including surface reactivity, particle size, surface charge, chemical composition, and the presence of transition metals. Therefore, it is necessary to ensure extensive characterization of the physicochemical properties for safer design and manufacture of NP. Whereas, ROS mediated via NP-cell interaction involve mechanisms including immune cell activation, mitochondrial respiration, and NADPH oxidase system. **Apart from ROS, NP also arbitrate RNS-mediated injury. Given their chemical reactivity, metal-based NP induce oxidative damage to cellular macromolecules such as proteins, lipids, and DNA via Fenton-type and Haber Weiss-type reactions**. The key pathophysiological outcomes of oxidative insults during metal NP exposures **involve cell membrane damage, lipid peroxidation, protein denaturation, and alteration of calcium homeostasis**. Furthermore, the findings in the review article suggest that CNT-induced oxidative stress is indicative of the pulmonary toxicity of CNT. Metal-based NP and fibrous CNT-mediated ROS result in activation of cell signaling pathways, transcription factor activation, cytokine mediator release, and apoptosis. **The persistent activation of these signaling cascades has some clinical ramifications. Redox imbalance via engineered NP exerts undesirable pathophysiological outcomes such as genotoxicity, inflammation, fibrosis, and carcinogenesis**. It is of utmost importance to understand the molecular and cellular mechanisms of NP-induced

oxidative stress which in turn will yield novel strategies to mitigate the toxicity of engineered NP. Moreover, it necessitates the establishment of stringent procedures for testing the oxidative potential of manufactured NP prior to their commercialization. Identifying the major cellular targets for NP-induced ROS will facilitate safer design and manufacture of NM in the market place.

Abbreviations

ROS: Reactive oxygen species
NP: Nanoparticles
NM: Nanomaterials
RNS: Reactive nitrogen species
CNT: Carbon nanotubes
H_2O_2: Hydrogen peroxide
: Superoxide anion
OH•: Hydroxyl radical
$^1O_2$: Singlet oxygen
HOCl: Hypochlorous acid
ONOO□: Peroxynitrite
AM: Alveolar macrophages
NADPH: Nicotinamide adenine dinucleotide phosphate
Nrf2: Nuclear factor (erythroid-derived 2)-like 2
MAPK: Mitogen activated protein kinase
NF-□B: Nuclear factor kappa-light-chain enhancer of activated B cells
iNOS: Inducible nitric oxide synthase
IL-1□: Interleukin-1beta
ERKs: Extracellular signal-related kinases
GSH: Glutathione
GSSG: Glutathione disulfide
8-OHdG: 8-Hydroxyl-2□-deoxyguanosine
AP-1: Activator protein-1
STAT-1: Signal transducer and activator of transcription-1
RTK: Receptor tyrosine kinases
PTP: Protein tyrosine phosphatases
HARN: High aspect ratio nanomaterials
PARP: Poly-ADP-ribose polymerase
TNF-□: Tumor necrosis factor-alpha
TGF-□: Transforming growth factor-beta
EMT: Epithelial-mesenchymal transition.

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NanoPlastic

Polystyrene degrades into nanoplastics.
The formation of nanoplastic particles increase over time.
Results suggest a continuous process of plastic surface erosion.

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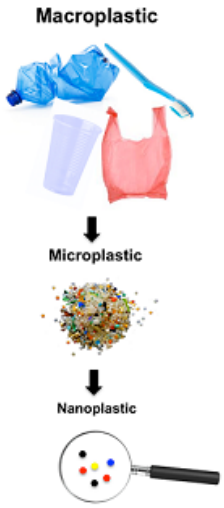
Comment [162]: The release of plastic materials into the environment is recognised as an important pollution related issue (Sutherland et al., 2010 and UNEP, 2011). Once in the environment plastics undergo abiotic and biotic weathering processes that cause their degradation and fragmentation into increasingly smaller particles, commonly termed microplastics (MPs; often defined as fragments <5 mm). A number of environmental monitoring studies have quantified the environmental occurrence of MPs in surface waters (Faure et al., 2015), coastal sediments (Browne et al., 2011), beach sands (Liebezeit and Dubaish, 2012), freshwater sediments (Castañeda et al., 2014), and deep-sea environments (Woodall et al., 2014). MPs are also known to effectively sorb organic pollutants from surrounding water (Mato et al., 2001, Endo et al., 2005 and Van et al., 2012). Therefore, internalized MPs might not only lead to direct physical injury, but also to a chemical exposure of the organism through the ingestion of pollutant loaded MPs (Ryan et al., 1988 and Saal et al., 2008).
Recent publications have also suggested that MPs will subsequently degrade into nano-sized plastic particles (see Andrady, 2011, Lambert et al., 2013 and Mattsson et al., 2015). The environmental impacts of nanoplastics will be different to those presented by microplastics, because of their smaller size makes tissue penetration and accumulation in organs a possibility (Mattsson et al., 2015). This is a potentially important issue given the current concerns regarding the environmental behaviour and ecotoxicity of engineered nano-materials (Lambert et al., 2014). Therefore, the aim of this work was to test the hypothesis that nanoplastic particles are formed during plastic degradation processes and that the concentrations will increase over time.



The amount of nano- and microplastic in the aquatic environment rises due to the industrial production of plastic and the degradation of plastic into smaller particles. Concerns have been raised about their incorporation into food webs. Little is known about the fate and effects of nanoplastic, **especially for the freshwater environment**. In this study, effects of **nano-polystyrene (nano-PS)** on the growth and photosynthesis of the green alga *Scenedesmus obliquus* and the growth, mortality, neonate production, **and malformations of the zooplankter** *Daphnia magna* were assessed. **Nano-PS reduced population growth and reduced chlorophyll concentrations in the algae**. Exposed *Daphnia* showed a **reduced body size and severe alterations in reproduction. Numbers and body size of neonates were lower, while the number of neonate malformations among neonates rose to 68% of the individuals**. These effects of **nano-PS were observed between 0.22 and 103 mg nano-PS/L. Malformations occurred from 30 mg of nano-PS/L onward. Such plastic concentrations are much higher than presently reported for marine waters as well as freshwater, but may eventually occur in sediment pore waters**. As far as we know, these results are the first to show **that direct life history shifts in algae and *Daphnia* populations may occur as a result of exposure to nanoplastic**.

Distribution and effects of plastic pollution that results from insufficient resource efficiency in a world that consumes 100 million tonnes of plastic annually. Plastics are of concern both for their chemical toxicity associated with the toxic additives and monomers often found in plastic products, and the adverse ecological and toxicological effects caused by the solid materials themselves

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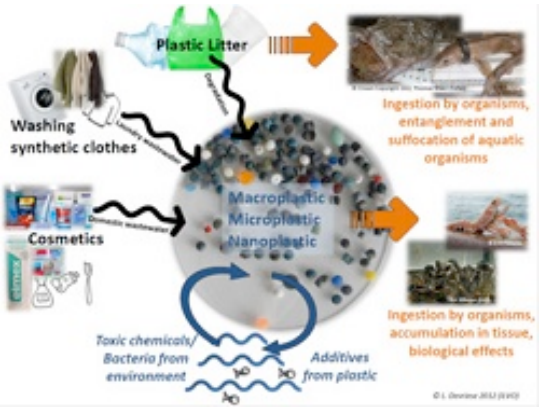
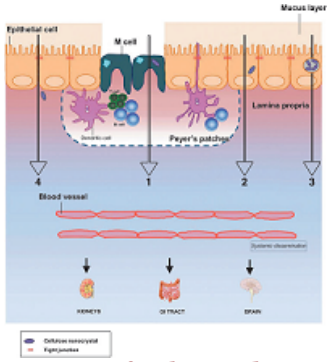
Comment [163]: During the first half of the project we successfully developed new molecular-models for two common hydrophobic polymers, namely polypropylene (PP) and polyethylene (PE).{ *The sum of propylene glycol and glycol ethers was associated with increased asthma prevalence in preschool-age children*} Our models are coarse-grained – that is, they do not include the explicit description of all the atoms of the polymer chains, but describe them as sequences of coarser beads, each one representing a group of atoms. Our coarse-grained models retain some of the chemical specificity of the original polymers, for example their degree of hydrophobicity. The advantage of coarse-graining is to allow for the sampling of much longer length and time scales in molecular simulations.
We are now using our new models to study the interaction of polymer nanoparticles with lipid membranes. We are considering both homogeneous membranes, constituted by a single type of lipid molecules, and laterally heterogeneous membranes, made of a mixture of different lipids. The latter, while posing more challenges from a technical point of view, are more realistic models of plasma membranes, whose lipid composition is extremely rich. Our preliminary results in the homogeneous membranes suggest that both PE and PP penetrate the membrane core. While the PE chains have a strong tendency to aggregation in the membrane core, where they form compact bulges, PP chains have the opposite behavior and dissolve well in the membrane hydrophobic region. In heterogeneous membranes, in presence of two different lipid phases (a properly liquid phase, often referred to as liquid-disordered phase, and a more gel-like phase, known as liquid-ordered) both polymers show the tendency to accumulate at the phase boundaries. While we are still collecting data on these aspects of the nanoplastics-membrane interactions, we can already claim that the polymers induce membrane alterations that could potentially disturb membrane functioning.



Wastewater treatment plants have been identified as a potential source of microplastics, as many plastic particulates can be found both in sewage sludge and the treated effluents- It is expected particularly in areas where biosolids are applied to agricultural lands that there will be elevated microplastic levels since these materials are extremely slow to mineralise.



Microplastics might be a vector for hazardous substances because they can sorb persistent, bioaccumulating and or toxic chemicals (e.g. POPs, endocrine disrupters). Thus, plastic particles may facilitate the entrance of these substances into the food chain, potentially threatening human health both chemically and by particle toxicity.

***Glycol ethers*. Glycol ethers, a chemical class with > 80 compounds, are used in a broad array of cleaning applications because of their combined hydrophilic and lipophilic nature**. They are often used in paints, varnishes, and cosmetics and have been detected in a variety of household products (Kwon et al. 2008; Plaisance et al. 2008). Biomonitoring methods are currently being developed, so large-scale studies are limited**. In human studies, exposure to glycol ethers has been associated with low sperm mobility**

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Comment [164]: *Impact of Endocrine Disrupting NPs on Reproductive Health*

Hormones play a key role in influencing the development of the reproductive system and subsequently in controlling its activities once developed. For this reason, most of the research carried out on EDCs in the last two decades has focused its attention on reproductive health. With regard to the male reproductive system, numerous *in vitro* and *in vivo* study findings have demonstrated that EDCs can exert a number of detrimental effects such as malformed reproductive tissue, poor semen quality (low sperm counts, low ejaculate volume, high number of abnormal sperm, low number of motile sperm), prostate diseases, testicular cancer, and other recognized abnormalities of male reproductive tissues [3,28]. There is also evidence that EDCs may interfere with female reproductive development and function causing adverse effects such as fibrocystic disease of the breast, polycystic ovarian syndrome, endometriosis, uterine fibroids and pelvic inflammatory diseases, breast and reproductive organ tissue cancers and declining sex ratio [3,29]. Recently, the results of studies conducted to assess the potential toxic effects of NPs, have suggested that some of these may pose risks to male and female reproductive health by altering normal testis and ovarian structure, spermatogenesis and sperm quality, oogenesis, follicle maturation and sex hormone levels.

(Cherry et al. 2008), **hematological effects** (Starek et al. 2008), **and asthma and allergies** (Choi et al. 2010).

In the present study, we analyzed all samples for 2-butoxyethanol and 2,2-methoxyethoxyethanol, and in a later second sampling round, we analyzed 14 additional samples for six other glycol ethers. **We detected glycol ethers in 3 conventional cleaners, face lotion, polish/wax, sunscreen, and in alternative shaving cream, pillow protector, and sunscreen samples**. Of the 5 conventional samples with detectable 2-butoxyethanol, only the carpet cleaner was labeled as containing 2-butoxyethanol. **When analyzed and detected, other glycol ethers were not listed on labels**. Although we detected phenoxyethanol in conventional and alternative sunscreen samples, we did not detect this chemical in some conventional and alternative samples comprising products labeled as containing this compound; levels may have been < LOD.

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Comment [165]: Limit Of Detection

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Application of dental nanomaterials- potential toxicity to the central nervous system nervous system

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Received 26 December 2014

Accepted for publication 16 March 2015

Published 14 May 2015 Volume 2015:10(1) Pages 3547—3565

DOI https://dx.doi.org/10.2147/IJN.S79892

Checked for plagiarism Yes

Review by Single-blind

Peer reviewer comments 5

Editor who approved publication: Dr Lei Yang

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Republic of China

Abstract: **Nanomaterials are defined as materials with one or more external dimensions with a size of 1–100 nm**. Such materials possess typical nanostructure-dependent properties **(eg, chemical, biological, optical, mechanical, and magnetic)**, which may differ greatly from the properties of their bulk counterparts. **In recent years, nanomaterials have been widely used in the production of dental materials, particularly in light polymerization composite resins and bonding systems, coating materials for dental implants, bioceramics, endodontic sealers, and mouthwashes**. However, the dental applications of nanomaterials yield not only a significant improvement in clinical treatments **but also growing concerns regarding their biosecurity**. The brain is well protected by the blood–brain barrier (BBB), which separates the blood from the cerebral parenchyma. **However, in recent years, many studies have found that nanoparticles (NPs), including nanocarriers, can transport through the BBB and locate in the central nervous system (CNS).** Because the CNS may be a potential target organ of the nanomaterials, it is essential to determine the neurotoxic effects of NPs. In this review, possible dental nanomaterials and their pathways into the CNS are discussed, **as well as related neurotoxicity effects underlying the in vitro and in vivo studies**. Finally, we analyze the limitations of the current testing methods on the toxicological effects of nanomaterials. This review contributes to a better understanding of the nano-related risks to the CNS as well as the further development of safety assessment systems.

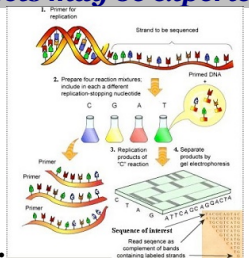
Keywords: dental, nanomaterials, central nervous system, toxicity, testing methods, risk assessment

A Letter to the Editor has been received and published for this article.

Introduction

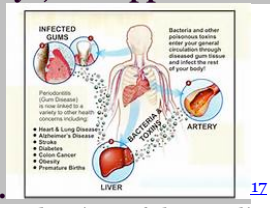
Nanomaterials are defined as materials composed of unbound particles or particles in an aggregate or agglomerate state with one or more external dimensions with a size ranging from 1 nm to 100 nm.[1] Such materials possess typical nanostructure-dependent properties **(eg, chemical, biological, optical, mechanical, and magnetic),** which distinguish them from their bulk counterparts. Because of their new and unique properties, nanomaterials are becoming ubiquitous in various products, such as sunscreens, cosmetics, medical supplies, clothing, and building materials. The global demand for nanomaterials and nano-enabled devices is expected to approach US$1 trillion by 2015.[2] The overwhelming increase in the amount of nanotechnology-related products has had major impacts on both society and the environment.--The benefits of nanomaterials to modern medicine have been particularly tremendous. **In recent years, nanomaterials have been widely**

used in the production of dental materials, **including light polymerization composite resins**[3,4] **and bonding systems, coating materials for dental implants,**[5] **bioceramics, endodontic sealers,**[6] **and mouthwashes**.[7] However, in addition to yielding significant improvements in clinical treatments, the applications of dental nanomaterials **have also created growing concerns regarding their biosecurity**. Because **the nanomaterials are similar in size to DNA molecules, proteins, viruses, and biological molecules, some of their biological effects may lie in the interaction mechanisms between living things and the environment**, which has not yet been distinctly understood. In fact, nanoparticles (NPs) **are a type of mesoscopic system that possesses a special surface effect, a small size effect, and a macroscopic quantum tunneling effect**. When reduced to the nanoscale, many benign materials may exhibit appreciable cellular toxicity. For example, TiO_2, a common substrate material for dental implants, was previously classified as being biologically inert in humans and animals and has been used as a negative control particle in a variety of toxicological studies. **Nevertheless, several possible adverse effects of TiO_2 NPs on human health have been recently discovered**.[8,9] Additionally, in vitro data have also demonstrated **the cellular toxicity of zinc oxide nanomaterials (nano-ZnO), which have been developed for numerous anti-infection applications.**[10] **---Indeed, nanomaterials are not inherently benign; they can affect biological behaviors at different levels, including the cellular, subcellular, and protein levels**. After exposure, some **nanomaterials readily travel throughout the body, deposit in target organs, penetrate cell membranes, lodge in the mitochondria, and trigger injurious responses**. In recent years, many studies have demonstrated that **nanomaterials can accumulate in the heart, liver, spleen, lungs, and kidneys of animals**.[11,12] The brain is different from other organs, as the blood–brain barrier (BBB) can prevent the majority of substances from entering the brain. **However, existing research has shown that nanomaterials have relatively easily crossed the BBB into the brain, and the crossing of the BBB by nanomaterials was attributed to their small sizes and high surface activities**. Furthermore, **these nanomaterials may even translocate into the brain by the olfactory and sensory nerves**.[13,14] All of these findings have suggested that the ***central nervous system (CNS) could be damaged and a range of pathogenic effects may be experienced upon exposure to nanomaterials*.**



Researchers have conducted many in vivo and in vitro studies to explore the interactions **between the nanomaterials**

and biological macromolecules, cells, organs, and tissues, and the majority of these studies have found that the effects of the biological toxicities of the nanomaterials may be induced by the mechanisms of oxidative stress and inflammatory reactions.[15,16] However, one problem that has arisen is whether the traditional methods and techniques utilized in the analysis of the toxicities of the nanomaterials are accurate and reliable. Further questions have arisen regarding whether **the unique physicochemical properties of the nanomaterials have introduced new mechanisms of injury and whether these new mechanisms will lead to new pathologies**. Even if the nanomaterials do not introduce new pathologies, **there could be new, novel mechanisms of injury that require special tools, assays, and approaches to assess their toxicities.**



[17] ---This review is mainly intended to provide a detailed introduction of the applications of dental nanomaterials along with **their potential neurotoxic effects**. Possible dental **nanomaterials and their pathways into the CNS are stated first, and the neurotoxic effects and related mechanisms behind the in vitro and in vivo studies are further discussed.** Finally, we highlight the limitations of the current investigative methods and provide some suggestions on some aspects of future researches. We hope this review will contribute to a better understanding of the nano-related risks to the CNS and the further development of safety assessment systems.

Possible commercial dental nanomaterials

Alongside the industrialization process of nanotechnology, dental nanomaterials have been widely utilized, and the opportunities for people to come into contact with nanomaterials have improved greatly. Nanotechnology-based materials have led to great improvements in clinical treatments and have driven the innovation of numerous conventional dental materials. Major applications of nanomaterials in the dental field are described in this section, and a summary of these examples is provided in **Composite resins and bonding systems** Schematic of the blood–brain barrier and the associated components of the neurovascular unit. **Note:** Reprinted from *Adv Drug Deliv Rev*, 64(7), Chen Y, Liu L. Modern methods for delivery of drugs across the blood–brain barrier. 640–665., Copyright (2012), with permission from Elsevier.[46]Neurotoxicity on the in vitro BBB model

in recent years, nanomaterials have been reported to be able to overcome the BBB and to produce biologic effects on the CNSI.[92,93] In many situations, the microvascular endothelial cells of the human brain are used as an in vitro BBB model, such as hCMEC/D3 cells,[94] **human brain**

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Comment [166]: Prosthodontics is an important branch of the oral medicine. With the improvement of people's living standards and the promotion of oral health knowledge, prosthodontics increasingly received widespread attention. Prosthodontics is mainly for dental defects, treatment after tooth loss, such as lays, crowns, and dentures, also including the use of artificial prostheses for periodontal disease, temporomandibular joint disease, and maxillofacial tissue defects

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Comment [167]: Nanomaterials can be divided into four categories of nanopowder, nanofiber, nanomembrane, and nanoblock

microvascular endothelial cells,[95] **and human cerebral endothelial cells**.[96] Rat is another common experimental animal due to its availability of resources and pathological models.[97,98] In addition to vascular endothelial cells, astrocytes play a key role in the induction and maintenance of the integrity of the BBB.[99] Thus, the two types of coculture models that have been widely utilized are as follows[100,101]: **(1) brain microvascular endothelial cells + astrocytes and (2) peripheral vascular endothelial cells + astrocytes. Other studies have also utilized "endothelial cells + microglia"**[102] **and "endothelial cells + pericytes" cocultured systems.**[97] At present, cell-based BBB models are the most extensively used because they are easy to obtain and maintain and they are highly effective for the screening studies of drugs and nanocarrier systems. In a recent review by Wong et al[103] **the authors summarized different types of BBB models, including isolated brain capillaries, cell-based/free models, and dynamic in vitro models** Despite these attempts to mimic in vivo conditions, each of the in vitro BBB models possesses their own advantages and disadvantages, and none of them are completely ideal. More effective in vitro BBB models must be developed for the evaluation of the deliveries of therapeutic agents in further investigations.

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Comment [168]: How the test were done to determine the effect or impact of nano materials

Figure 4 In vitro cell culture models for the studies on drug and NP transport through the BBB.
Note: Reprinted from *Adv Drug Deliv Rev*, 64(7), Wong HL, Wu XY, Bendayan R. Nanotechnological advances for the delivery of CNS therapeutics. 686–700., Copyright (2012), with permission from Elsevier.[103]
Abbreviations: NP, nanoparticle; BBB, blood–brain barrier.Different in vitro studies have focused on evaluating various aspects, such as pharmacology, transport, migration, and the metabolic activity of the BBB. **Research has also been focused on DNA damage, the morphological and functional changes of the mitochondria, endoplasmic reticulum, lysosomes, and other organelles, and the transportation mode of internalization, transcytosis and exocytosis**. ***NPs have been demonstrated to be taken up by mammalian cells by such mechanisms as pinocytosis, endocytosis dependent on caveolae and lipid raft composition, and phagocytosis.***[104] The intracellular sites of the localization of NPs vary depending on the cell type and applied method**. NPs have entered the endothelial cell monolayer and have accumulated along the endo-lysosomal pathway, which affected the normal morphology and function of the BBB itself.** For example, Brun et al[98] observed an **accumulation of TiO_2 NPs in the endothelial cells of the brain by using an in vitro cell-based rat BBB model**. ***An intense inflammatory response associated with a modulation of the endothelial cell functioning of the brain was also observed. Therefore, an impaired transport capacity resulting from the dysfunction of the endothelial cells of the BBB might constitute the first step in the neurodegeneration process***.--Although numerous studies have considered the effects of nanomaterials to the BBB itself, a distinct lack of knowledge exists with respect to the biological effects of NP accumulation within

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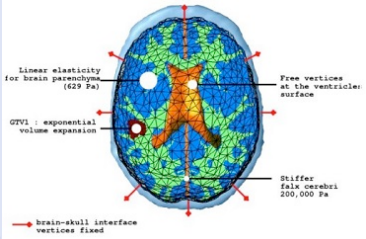
Comment [169]: Brain Damage~ see other links on site in regard to 10 different nanometals that can cause brain lobotomy at different parts of the brain—see "Neurotoxicity of nanoscale materials in the June script of the show 2016 " to get a more indepth report on how this causes compromise and debilitation to everyone

the BBB of the neighboring cells in the CNS, particularly over the long term. Wiley et al[105] **observed that transferrin-containing gold NPs reached and accumulated in the brain parenchyma following an intravenous injection in mice through a receptor-mediated transcytosis pathway**. Raghnaill et al[106] **also reported an accumulation over time, but there was no degradation of 100 nm PS COOH NPs within the lysosomes of the BBB model in vitro**. Thus, possible **long-term toxicity must be considered, and this toxicity may result from the accumulation of known "toxic" and "nontoxic" NPs**.



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Comment [170]: The brain parenchyma is the functional tissue in the brain. It's comprised of two types of cells that are used specifically for cognition and controlling the rest of the body. The remaining brain tissue is known as *stroma*, which is the supportive or structural tissue. Damage or trauma to the brain parenchyma often results in a loss of cognitive ability or even death.

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Comment [171]: This is saying that they do not break down and are accumulating in the brain

Neurotoxicity on glial cells or neuroglia

Among all of the neuroglial cells, astrocytes and microglia have received the most attention. The **astrocyte** is thought to induce the barrier phenotype of cerebrovascular endothelial cells during development through the release of soluble factors, such as vascular endothelial growth factor. **Recently, many studies have attempted to determine the specifics of the NP–astrocyte interactions**. **For instance, the ZnO NP–astrocyte interaction was reported to induce an oxidative stress that could trigger cell apoptosis by activating the JNK signaling pathway in cultured primary astrocytes.**[107] A similar finding was observed in the interactions between **superparamagnetic iron oxide NPs and astrocytes**.[108] Mixed glial cultures have often been established from the cerebral cortices of neonatal Sprague-Dawley rats and purified astrocytes. Another common cell line is human glial cells (U87 astrocytes).[109] As the sentinels of the CNS, microglia are the first cells to respond to a disruption of the brain homeostasis and the entry of foreign particles or infectious agents. **Once activated, microglia can generate ROS and reactive nitrogen species (RNS) and even elicit an inflammatory response**. In most cases, the macrophage cell line was adopted to evaluate its activation and inflammatory reaction. **In a neurotoxicity study of Si NPs performed by Choi et al[77] even low levels of NPs were capable of increasing ROS and RNS production and inducing cytokine release. These changes had an adverse effect on the microglial function and surrounding neurons. This result was consistent with other toxicity studies that have been conducted more recently**.[110,111] **It was also determined that exposure to Fe_2O_3 NPs did not cause a significant release of inflammatory factors even though cell phagocytosis and a generation of ROS and NO were observed**.[112] This finding indicated that **microglial activation may also act as an alarm and defense system in**

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Comment [172]: astrocyte
[as´tro-sīt]
a neuroglial cell of ectodermal origin, characterized by fibrous or protoplasmic processes; collectively called ASTROGLIA or MACROGLIA.

the processes of the exogenous NPs invading and accumulating in the brain.

Neurotoxicity on neurons

Neuronal cell lines commonly used for in vitro studies include the following: 1) rat PC12 neuronal cells,[113,114] which have been derived from a **pheochromocytoma of the rat adrenal medulla**; PC12 cell lines are commonly **used for the neurobiological and neurochemical assessment of NP-induced neurotoxicity; and 2) a human SHSY5Y neuroblastoma cell line**,[115,116] which is perceived as an appropriate cell model for the assessment of neurotoxicity because it possesses many biochemical and functional properties of neurons.[117] Additionally, primary culture cell lines have also been used in the **evaluation of the neurotoxicity of NPs; these lines include human cortical neuronal cells (HCN-1A), rat dopaminergic neurons (N27), rat primary neuronal cells,[118] embryonic rat striatum or cerebellar granule cells,[119] and hippocampal CA1 and CA3 neurons**.[120,121]--It has now been confirmed that **some nanomaterials can exploit the endocytotic pathways both to cross the BBB endothelium in vivo and to enter the neurons or glial cells in vitro.**[122] For instance, Vilella et al[66] discovered that **there was an uptake of NPs in hippocampal neurons that were prepared from rats at embryonic day 18 or 19. Aside from intracellular accumulation, there was also evidence that different metal oxide NPs affect the membrane potentials of neurons and increase the neuronal firing rate by changing the responses of the potassium channels**.[90] **This finding was consistent with a toxicity study of nano-CuO on CA1 hippocampal neurons performed** by Xu et al.[123] Furthermore, this **toxic effect may have a physiological impact on animal behavior, which was demonstrated in rats by testing their spatial cognition capabilities.**[81] Recently, the impact of nanomaterials on the CNS, particularly the hippocampal neuronal cells, has been illustrated in a comprehensive review by Yang et al.[124]

Studies on cell-to-cell communication

The CNS is composed of a dense network of neurons and glial cells that are highly interconnected. Therefore, cell-to-cell communication is an important factor in maintaining a functional organization. In recent years, **tunneling nanotubes (TNTs) were reported as a new principle of cell-to-cell communication**. **As a form of membrane continuity, TNTs may be efficient communication tunnels that facilitate information and material exchange. Such communication may even occur over a relatively long distance**.[125] **Considering that NPs can be transported intra- and intercellularly within vesicles after internalization by the vesicle, this cell-to-cell transport may be mediated by TNT-like structures in glial and neuronal cells in vitro.** Furthermore, the transport was dependent on F-actin and was increased by the induction of TNT-like

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Comment [173]: Pheochromocytoma is a relatively rare tumor of the adrenal glands or of similar specialized cells outside of the adrenal glands

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Comment [174]: Telling you here it creates a tunnel or a connection to get the particles from point A to Point B and can increase the signals of communication

structures.[126] Nevertheless, the influence of nanomaterials on cell-to-cell communication in the CNS remains unclear; thus, more in-depth studies are warranted.

Other problems and future research prospects

Alongside the rapid advances in the development of nanotechnology-based materials, it has become **imperative to elucidate the toxicity of NPs.** However, the **safety evaluation systems of nanomaterials lag far behind their emerging development and applications**. Although researchers have obtained some important information, the risks of NP exposure are not understood sufficiently well to enable the development of a science-based risk assessment. Because investigation into the possible harmful effects of NPs has only been conducted for a few years, it is not surprising that many studies suffer from shortcomings. Therefore, better testing and evaluation systems are urgently needed.

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Comment [175]: nanocomposites composed of nanomaterials and traditional metals, ceramics, resin, or other matrix materials have been widely used in prosthodontics because their properties, such as modulus elasticity, surface hardness, polymerization shrinkage, and filler loading, were significantly increased after the addition of the nanomaterials

Limitations of the testing methods

Cytotoxicity in vitro is typically estimated with colorimetric tests.[127] However, Monteiro-Riviere et al[128] determined that MTT and neutral red assays, two classical dye-based assays, may produce invalid results in the testing of cell viability when applied with nanomaterials due to their interactions and/or adsorption of the dye/dye products. **Furthermore, carbon nanomaterials can interact with assay markers to cause variable results in classical toxicological studies**. This finding is consistent with the results of Griffiths et al.[129] For these reasons, such interactions in cytotoxicity assays must be considered. Another challenge of the testing methods lies in the accurate detection of nanomaterials in biological objects**. At present, flow cytometry, induced coupled plasma mass spectroscopy, confocal microscopy, the radioactive tracer technique, and transmission electron microscopy in combination with energy-dispersive X-ray spectroscopy are commonly used to study the cellular uptake of NPs**.[130,131] However, there is not a single method that is satisfactory in obtaining precise information for all types of nanomaterials. **Therefore, a combination of the utilization of different testing methods is suggested to provide more accurate results**. Furthermore, different assays should be employed according to the certain types of NPs, as well as in addition to imaging techniques.[132]

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Comment [176]: Dental materials of dentures can be divided into mainly three categories: resin, ceramic, and metal. They are important to fabricate dental prosthesis, which directly contacts with the oral mucosa and is under long-term use in the oral environment

Limitations of the experimental models

Under in vivo conditions, nanomaterials could yield different effects compared with in vitro experiments.[133] Although the observations from in vivo studies are **more representative of the situations in living organisms**, in some cases, these studies may provide inaccurate results. The challenges are largely related to the experimental models (animals), which are difficult to control and could be affected by various unpredictable factors. Additionally, other considerations,

such as **dosimetry, the optimization of the dispersion of NPs, the evaluation of the interactions between the nanomaterials and cells, and their biodistributions, create more challenges for in vivo toxicity assessments**.[134]--Compared with animal studies, in vitro studies are less expensive, ethically ambiguous, and most importantly, easier to control and reproduce. The first step toward understanding **how NPs will react in the body often involves cell culture studies. An increasing number of in vitro cytotoxicity studies of different nanomaterials using various cell lines, incubation times, and colorimetric assays have been published**. However, many problems still exist in the studies performed under in vitro conditions. First, the appropriate selections **of a set of sensitive cell lines** and in vitro assays measuring the different cytotoxicity endpoints are essential to ensure the accurate identification of nanomaterial cytotoxicity.[135] However, for a certain NP, the selection of the most appropriate cell line is still difficult. To some extent, more sensitive cell models are required to determine the cytotoxicity of a certain type of NP. **One example is with the use of nano-ZnO. The majority of the toxicity studies specific to ZnO NPs have relied on the use of immortalized cell lines, which display altered sensitivities to foreign materials/chemicals due to their changes in metabolic processes and significant genetic instabilities. Nevertheless, the toxicity of ZnO NPs on normal primary human cells and their potential immunomodulatory effects are often neglected**. Furthermore, the cytotoxic response varies with different types of cell lines and nanomaterials, making it difficult to develop predictive models because of the lack of detailed and systematic investigations.[136] Finally, the toxic effects of NPs on rat cell lines (a common in vitro model) may not be able to accurately reflect the effects in humans.---In vitro investigations will not be able to completely determine the in vivo situations until further in vivo analyses have been performed to confirm their findings.[133] In a recent review by Donaldson et al[137] the authors stated that cells in culture did not experience the range of pathogenic changes that might occur under in vivo conditions, which were partially related to the issues of translocation, toxicokinetics, and coordinated tissue responses. Some other studies have also cast doubt on the results obtained from in vitro models, especially in models in submersed conditions when NPs were suspended in media that could impact the dispersion and dissolution.[15,138]

Until now, the understanding of nanomaterial neurotoxicology has been extremely limited. In-depth studies are warranted, particularly when considering the recent emphasis on the use of nanocarriers for drug delivery in the brain.[139] Here, we have provided some suggestions on the research prospects that require further detailed investigations.

As indicated by Laurent et al the effects of the **protein corona on NP–cell interactions are often ignored at the nano–bio interface**.[140] Because in vitro biological studies typically use low amounts (10% dilution or less, depending on cell types) of animal-derived serum, which is present in in vivo studies, NP coronas are likely to form at different protein-to-NP ratios between

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Comment [177]:]. Nanomaterials can be divided into four categories of nanopowder, nanofiber, nanomembrane, and nanoblock, in which development of nanopowder is longest, and its technology is most mature

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Comment [178]: In this section they are basically soft pedaling the danger here ~ irregardless of the soft cell line use if there is going to be a reaction where the cells terminate-mutate or become assimilated through a mutation then there is no questioning the validity of the aspects to the danger of these component

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Comment [179]: Usually the cell lines of the rats are more durable and are more adaptable then the human cell line and because there is no model to accurately predict the effect which is really very unscientific to make such a statement the fact remains they have a toxic effect irrespective of what tissue or cell line you chose ~ the need to figure out what the method of the toxicology or the triggering effect is another issue

Owner 4/17/2017 10:45 AM

Comment [180]: biological integration is usually unsatisfactory, and some patients are prone to allergies, causing skin, mucous membrane inflammation [22, 30, 31]. Satisfactory biological integration of the implant surfaces with the surrounding host tissues is one of the most important elements for long-term success of dental implants

the in vitro and in vivo studies.[141] In this sense, in vitro models that evaluate the NPs for brain-related diseases are supposed to use the corona-coated NPs to reflect the real in vivo situations,[142] as the protein corona may cover the designed functional groups and significantly reduce the ability of NPs to cross through the cell barriers.[143,144] Another consideration with respect to the protein corona arises from the evaluation of its structural evolution over time. NPs will interact with tissues and cells in living organisms, including passing through cellular membranes and being transported to the final subcellular locations. Therefore, the detailed changes of the nanomaterial corona at these stages and their implications require further study. Additionally, as described in the limitations of the in vitro models, more appropriate cell lines should be developed. Takhar and Mahant[145] recently suggested the possibility of using transgenic cell lines carrying human genes, which may be more predictive to situations involving humans than the traditional rat cells.

With regard to animal studies, the effects of the life stages should be considered. First, fetal life and early childhood are vulnerable periods. These life stages are of great importance for the rapid growth of whole organism, cell differentiation, and organogenesis, and in the case of the brain, are involved in critical processes in neurodevelopment. If toxic exposures occur at these stages, they could alter the trajectory of the development of the brain, which may have minor effects in the early years and profound implications later in life.[146] Currently, growing evidence from animal research has confirmed that the CNS is highly vulnerable to chemical injury during development.[147] Therefore, particular attention should be given to determine the influence of nanomaterial exposure at these developmental stages.

Aging may also represent an important factor in the susceptibility of NP-induced neurotoxicity. **Aged brains have demonstrated an increase in cytokine and microglial activation and are more vulnerable to environmental insults, particularly in pro-inflammatory stimuli**,[148,149] **including various NPs**. **In recent years, it has been predicted that many neurodegenerative diseases can result from the cumulative exposure throughout a lifetim**e.[150] This finding was consistent with the observations in another toxicity research conducted by Qin et al.[151] **In this animal study, chronic neuroinflammation in response to a single intraperitoneal injection of lipopolysaccharide, a potent inflammatory stimulus, in young adult mice only culminated in dopaminergic neurotoxicity in aged animals**. **Other associated factors, such as the sex and genetic background, should also be investigated**.[152,153] **Recently, it was reported that the differential expression of the enzyme paraoxonase 2 (PON2) between male and female brains may be responsible for a number of sex differences with regard to neurotoxicity**.[152] **Gene–nanomaterial interactions also played an important role in NP-induced neurotoxicity, as genetic polymorphisms may modulate individual susceptibilities to nanomaterials**. Given the prominent role of oxidative stress, genetically based

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Comment [181]: based on a gene code a person could have a more susceptible break down on regard to nano inducments and as the nanao accumulates as a result of the genetics of the person could cause more then usual sympotoms

differences in antioxidant enzymes may predispose certain individuals to significant air pollution neurotoxicities.[153]

A continuous exposure may result in the significant accumulation of NPs in a secondary target organ. Therefore, it is important to obtain data on the retention characteristics of NPs in both primary and secondary target organs, as well as NP elimination pathways. **No data on NP elimination in the CNS are available yet**. It is conceivable that the CSF, via its connections to the nasal lymphatic system and to the circulation of blood, could be an excretory pathway for the brain, and this topic should be investigated in future studies. Indeed, from his review on CSF barriers, Segal[154] concluded that the CSF may act as not only a compartment for the distribution of substances to different brain regions but also an elimination route for waste products into the blood circulation because the brain has no lymphatics. However, this is a single study and need to be complemented by more systematic research on nanomaterial elimination.

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Comment [182]: Primarily because the nano particles will translocate and polymorph with other things in the body and can get trapped and accumulate in every major organ and skeletal tissue

Summary

Nanomaterials have made major contributions to modern dentistry in various areas, including composite resin and bonding systems, coating materials for dental implants, and dental restorations. The wide applications of these dental nanomaterials have created more exposure opportunities to these NPs in both dental staff and patients. Because the CNS may be a potential target organ of nanomaterials, it is essential to determine the neurotoxic effects of NPs. Although the impact of NPs on the CNS has received considerable attention in recent years, the data and findings obtained from the in vivo and in vitro studies are still limited. The limitations of the present testing methods and the experimental models also make it difficult to establish a science-based evaluation system. Better testing and evaluation systems are urgently needed. In conclusion, more efforts are required to ensure the safe use of nanomaterials.

Acknowledgments

This work was supported by the National Natural Science Foundation of China (31070857, 50973045, 51172283, 81400557), and the Project on the Integration of Industry, Education and Research of Guangdong Province, People's Republic of China (2012B091000147).

Disclosure

The authors declare no conflicts of interest in relation to this paper.

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The matrices of traditional composite resins have generally been comprising various types of inorganic fillers. Applications of nano-sized fillings in the resin matrices have overcome some of the mechanical limitations and have significantly improved their clinical performance. Commonly used nanomaterials include nano-ZnO,[3,4] nano-silica,[18,19] nano-calcium phosphate and calcium fluoride (nano-$Ca_3(PO_4)_2$ and CaF_2, respectively),[20] and nano-TiO_2.[21] In addition to composite resins, the utilization of nanomaterials in dental adhesives has also effectively improved their bonding strengths and mechanical properties. For instance, polyhedral oligomeric silsesquioxanes hybrid nano-composites have polymerized with silicon-based nanomaterials to form a novel type of bonding material that possessed a large mechanical strength and good thermostability.[22] Furthermore, the antibacterial properties of the bonding agents could be greatly improved by the inclusion of nano-sized silver and calcium phosphate.[23] Other possible additions have included nano-hydroxyapatite (nano-HAp)[24] and nano-silica.[25]

Root filling materials

Root canal filling materials are supposed to effectively kill the bacteria in the periodical lesions, densely seal the apical zones, and promote healing. However, the brittleness of the root canal often increases after treatment due to the large size of the traditional HAp. The mismatch of the elastic modulus between the root dentin and fillings has also resulted in percolation. In contrast, nano-HAp represents a unique advantage in this aspect because its structure is similar to natural inorganic bone. Nano-HAp was able to induce osteogenesis[26] and further improve the bacteriostatic and antibacterial effects of the root fillings.[27] Considering its good bioactivity, nano-HAp was also used as an optimum replacement material in the repair of bone defects.[28,29] For example, Yang et al[30] demonstrated that nano-HAp-coated silk scaffolds effectively guided the regeneration of periodontal and bone tissue. Similarly, porous ZrO_2/HAp composite scaffolds were also reported to possess excellent mechanical properties and cellular/tissue compatibilities.[31]

Bioceramics and associated dental prosthesis

Nanostructured bioceramics, which are constructed by a plasma-coating or chemical deposition process, generally possess enhanced mechanical properties, such as a better resistance to crack propagation[32] and an increased fracture toughness and Vickers hardness.[33] Additionally, the utilization of nano-sized Ti-, Zn-, and Ce-oxide has greatly improved the mechanical properties of a maxillofacial silicone elastomer.[34] Nano-sized silver may be an effective addition to denture-based materials to improve their antifungal properties and biocompatibility.[35] Thus, the applications of nanomaterials have the potential to effectively improve the comprehensive properties, including the mechanical, chemical, and biological properties, of different types of conventional dental materials.

Coating materials for dental implants

Good osseointegration at the implant–bone interface is essential for the success of dental implantation, but, unfortunately, this still remains a significant challenge. In recent years, a number of studies have reported the increased success rates of implants through the applications of a nano-coating on the surface, a nano-ceramic, and artificial nano-bone materials. For example, nano-porous alumina,[36] nano-zirconia/nano-$Ca_3(PO_4)_2$,[37] nano-ZnO,[5] and nano-HAp[38] have been utilized to increase the surface bioactivities of dental implants to achieve superior osseointegration. The advantages of the nanoscale modifications of dental implant surfaces have been presented in a recent review by Mendonca et al.[39]

Target delivery and imaging in tumor chemotherapy

A key problem in the use of chemotherapy for oral malignant tumors is how to improve the local concentrations of the drug while reducing the systemic side effects. To solve this problem, novel NP-based drug delivery strategies have been studied where the NPs are the drug carriers that can transport the anticancer drugs to the tumor sites, which further increases the therapeutic efficacy. For example, Lebold et al[40] applied mesoporous thin silica films with nanoscale pores as drug carriers that were incorporated with doxorubicin, a widely used anticancer drug. The mesoporous silica nanomaterials demonstrated a sustained and controlled release of the anticancer drugs. Mu and Feng[41] discussed the advantages of manufacturing polymeric NPs (vitamin E d-alpha-tocopheryl polyethylene glycol 1000 succinate) for the controlled release of paclitaxel and other anticancer drugs. Another application of targeted therapy with novel NPs involves tumor imaging. Superparamagnetic iron oxide with special surface modifications has been utilized to guide the laser ablation of maxillofacial cancer because these manufactured NPs are magnetic resonance-active and can be selectively heated up for simultaneous imaging.[42] Similarly, liposomal nanocarriers also possess special advantages in their use for tumor radiography and imaging due to their good encapsulation of drugs and gadolinium.[43]

Aside from the aforementioned therapeutic uses, many nanomaterials, such as nano-TiO_2 and nano-ZnO, have been utilized in everyday dental items, including toothpastes and mouthwashes.[7,44] Considering the various applications of dental nanomaterials listed above, we should admit the outstanding contributions of nanomaterials to modern medicine. In the meantime, however, the risks of nanomaterials to human health have also significantly increased accompanied with more exposure opportunities.

Possible pathways for entering the CNS

Based on the principles of toxicology, nearly all substances are potentially toxic to humans, and the key lies in the dose and method of exposure. The people who most likely come into contact with dental nanomaterials are the production, research, and development staff, as well as the dental staff and patients. In clinical situations, most of the dental nanomaterials were directly applied in the oral cavity or maxillofacial region, allowing the nanomaterials to easily enter into the bloodstream (or lymph fluid) via absorption through oral mucosa or through the digestive tract after swallowing. In addition, opportunities for exposure to nanomaterials may also occur with the utilization of dental tools. At present, tungsten carbide (WC) nanowires, which are a new form of nano-WC, have been applied in the production of carbide micro-drills, including in dental drills and burrs. Thus, dental staff and patients may face abrasive NPs directly during a grinding or polishing process, especially considering that many dental prosthetic materials also contain nano-metals (eg, Co, Cr, Au, Ag, Ti), resins (Si), and ceramics (eg, Zr, Al, Li, Mg, Fe). Once these NPs are absorbed into our bloodstreams, they can be distributed to different organs, including the liver, spleen, kidneys, heart, lungs, and brain. Compared with the other organs, these substances are still required to cross the BBB or blood–cerebrospinal fluid (CSF)

barriers to reach the brain. In addition to the systemic pathways, nanomaterials can directly translocate to the brain via nerves.[45] The possible pathways of dental nanomaterials entering the CNS are described below.

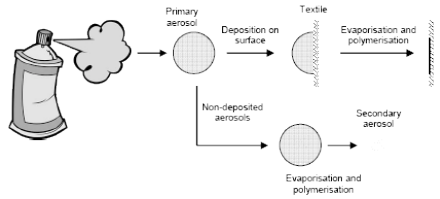
BBB pathway

The BBB is mainly composed of the cerebrovascular endothelium, which is sealed with tight junctions (TJs). Additional structures, such as pericytes, astrocyte end-feet, and a discontinuous basal membrane, are supportive cells to the BBB. All of these structures associated with the surrounding neurons constitute a complex and functional "neurovascular unit"[46] (Figure 1). The unique structural characteristics of the BBB are the intracellular TJs and the absence of Weibel–Palade bodies, which are significantly different from other vascular endothelial cells and can prevent most of the substances from entering into the CNS. In addition to these physical barriers, the BBB also possesses some metabolic barriers to the delivery of therapeutic agents.[47] First, the endothelia of the BBB are deficient in pinocytic vesicles, and thus, they can only allow for the low pinocytosis of certain substrates. Second, a series of intra- and extracellular enzymes that are expressed by the cellular components will limit the transport of a substance through the BBB. The complex interactions between the drugs and these enzyme systems often lead to therapeutic failure. Finally, efflux systems (such as P-glycoprotein) of the endothelial cells also play an important role in the elimination of harmful endogenous and exogenous molecules. Another associated structure that serves to prevent potentially harmful substances from entering the brain is known as the blood–CSF barrier.[48] This barrier is formed by choroid plexus epithelial cells, which possess similar TJs but a smaller surface area compared with the BBB endothelia. The blood–CSF barrier helps to prevent macromolecules from penetrating into the CSF, and this function is further reinforced by the active transport systems, which actively remove therapeutic organic acids from the CSF.[49]

****

Atmospheric Aerosols- The Pariculates –Nano-and there effects

Aerosols are minute particles suspended in the atmosphere. When these particles are sufficiently large, we notice their presence as they scatter and absorb sunlight. Their scattering of sunlight can reduce visibility (haze) and redden sunrises and sunsets.



The dispersal of volcanic aerosols has a drastic effect on Earth's atmosphere. Follow an eruption, large amounts of sulphur dioxide (SO_2), hydrochloric acid

Owner 4/17/2017 10:45 AM

Comment [183]: In this report I added the actual effect of the nanoparticles that would descend from above as well these are also in the food and water table but with the aerosoling the upper atmosphere these would spread in the use of carbon nano and silicates they would have in the chemtrails making this not only an atmospheric issue but a planetary one as well

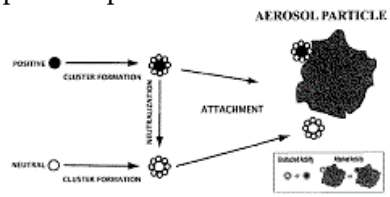
Owner 4/17/2017 10:45 AM

Comment [184]: Today they would be termed nano~ years past they were called ultrafine particles

(HCL) and ash are spewed into Earth's stratosphere. HCL, in most cases, condenses with water vapor and is rained out of the volcanic cloud formation. SO_2 from the cloud is transformed into sulphuric acid, H_2SO_4. The sulphuric acid quickly condenses, producing aersol particles which linger in the atmosphere for long periods of time. **The interaction of chemicals on the surface of aerosols, known as heterogeneous chemistry**, and the tendency of aerosols to increase levels of chlorine gas react with nitrogen in the stratopshere, is a prime contributor to stratospheric ozone destruction.

Aerosols interact both directly and indirectly with the Earth's radiation budget and climate. As a direct effect, the aerosols scatter sunlight directly back into space. As an indirect effect, aerosols in the lower atmosphere can modify the size of cloud particles, changing how the clouds reflect and absorb sunlight, thereby affecting the Earth's energy budget.

Aerosols also can act as sites for chemical reactions to take place (heterogeneous chemistry). The most significant of these reactions are those that lead to the destruction of stratospheric ozone. During winter in the polar regions, aerosols grow to form polar stratospheric clouds. The large surface areas of these cloud particles provide sites for chemical reactions to take place.



These reactions lead to the formation of large amounts of reactive chlorine and, ultimately, to the destruction of ozone in the stratosphere. Evidence now exists that shows similar changes in stratospheric ozone concentrations occur after major volcanic eruptions, like Mt. Pinatubo in 1991, where tons of volcanic aerosols are blown into the atmosphere (Fig. 1).

Volcanic Aerosol

Three types of aerosols significantly affect the Earth's climate. The first is the volcanic aerosol layer which forms in the stratosphere after major volcanic eruptions like Mt. Pinatubo. The dominant aerosol layer is actually formed by sulfur dioxide gas which is converted to droplets of sulfuric acid in the stratosphere over the course of a week to several months after the eruption (Fig. 1). **Winds in the stratosphere spread the aerosols until they practically cover the globe. Once formed, these aerosols stay in the stratosphere**

Owner 4/17/2017 10:45 AM

Comment [185]: Oxidative stress as a common mechanism for cell damage induced by nano- and ultrafine particles is well documented; fullerenes are model compounds for producing superoxide. A wide range of nanomaterial species have been shown to create reactive oxygen species both *in vivo* and *in vitro*. Species which have been shown to induce free radical damage include the C_{60} fullerenes, quantum dots, and carbon nanotubes [30,60-66]. Nanoparticles of various sizes and chemical compositions are able to preferentially localize in mitochondria where they induce major structural damage and can contribute to oxidative stress

Owner 4/17/2017 10:45 AM

Comment [186]: Some recent studies have shown the potential of nanomaterials to elicit a phytotoxic response in the ecosystem. In the case of alumina nanoparticles, one of the US market leaders for nano-sized materials, 99.6% pure nanoparticles with an average particle size of 13 nm were shown to cause root growth inhibition in five plant species [4

for about two years. They reflect sunlight, reducing the amount of energy reaching the lower atmosphere and the Earth's surface, cooling them. The relative coolness of 1993 is thought to have been a response to the stratospheric aerosol layer that was produced by the Mt. Pinatubo eruption. In 1995, though several years had passed since the Mt. Pinatubo eruption, remnants of the layer remained in the atmosphere. Data from satellites such as the NASA Langley Stratospheric Aerosol and Gas Experiment II (SAGE II) have enabled scientists to better understand the effects of volcanic aerosols on our atmosphere.

Desert Dust

The second type of aerosol that may have a significant effect on climate is desert dust. Pictures from weather satellites often reveal dust veils streaming out over the Atlantic Ocean from the deserts of North Africa. Fallout from these layers has been observed at various locations on the American continent. Similar veils of dust stream off deserts on the Asian continent. The September 1994 Lidar In-space Technology Experiment (LITE), aboard the space shuttle Discovery (STS-64), measured large quantities of desert dust in the lower atmosphere over Africa (Fig. 2). The particles in these dust plumes are minute grains of dirt blown from the desert surface. They are relatively large for atmospheric aerosols and would normally fall out of the atmosphere after a short flight if they were not blown to relatively high altitudes (15,000 ft. and higher) by intense dust storms.

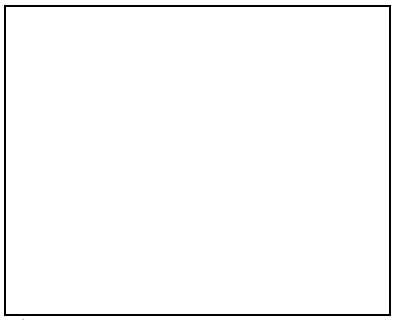


Fig. 1 - LITE Measurements Over Northwestern Africa, Atlas Mountains

Because the dust is composed of minerals, the particles absorb sunlight as well as scatter it. Through absorption of sunlight, the dust particles warm the layer of the atmosphere where they reside. This warmer air is believed to inhibit the

formation of storm clouds. Through the suppression of storm clouds and their consequent rain, the dust veil is believed to further desert expansion.

Recent observations of some clouds indicate that they may be absorbing more sunlight than was thought possible. Because of their ability to absorb sunlight, and their transport over large distances, desert aerosols may be the culprit for this additional absorption of sunlight by some clouds.

Human-Made Aerosol

The third type of aerosol comes from human activities. While a large fraction of human-made aerosols come in the form of smoke from burning tropical forests, the major component comes in the form of sulfate aerosols created by the burning of coal and oil. The concentration of human-made sulfate aerosols in the atmosphere has grown rapidly since the start of the industrial revolution. At current production levels, human-made sulfate aerosols are thought to outweigh the naturally produced sulfate aerosols. **The concentration of aerosols is highest in the northern hemisphere where industrial activity is centered**. The sulfate aerosols absorb no sunlight but they reflect it, thereby reducing the amount of sunlight reaching the Earth's surface. **Sulfate aerosols are believed to survive in the atmosphere for about 3-5 days.**

The sulfate aerosols also enter clouds where they cause the number of cloud droplets to increase but make the droplet sizes smaller. The net effect is to make the clouds reflect more sunlight than they would without the presence of the sulfate aerosols. Pollution from the stacks of ships at sea has been seen to modify the low-lying clouds above them. These changes in the cloud droplets, due to the sulfate aerosols from the ships, have been seen in pictures from weather satellites as a track through a layer of clouds. In addition to making the clouds more reflective, it is also believed that the additional aerosols cause polluted clouds to last longer and reflect more sunlight than non-polluted clouds.

Climatic Effects of Aerosols

Sept. 16, 1994 - Astronaut Carl J. Meade tests the new Simplified Aid for EVA Rescue (SAFER) system 130 nautical miles above Earth. The hardware supporting the LIDAR-in-Space Technology Experiment (LITE) is in the lower right. A TV camera on the Remote Manipulator System arm records the Extravehicular Activity.-- The additional reflection caused by pollution aerosols is expected to have an effect on the climate comparable in magnitude to that of increasing concentrations of atmospheric gases. **The effect of the aerosols**, however, will be opposite to the effect of the increasing atmospheric trace gases - cooling instead of warming the atmosphere.

Owner 4/17/2017 10:45 AM

Comment [187]: Toxicological studies of fibrous and tubular nanostructures have shown that at extremely high doses these materials are associated with fibrotic lung responses and result in inflammation and an increased risk of carcinogenesis. Single-walled carbon nanotubes (SWCNT) have been shown to inhibit the proliferation of kidney cells in cell culture by inducing cell apoptosis and decreasing cellular adhesive ability. In addition, they cause inflammation in the lung upon instillation [26,33,47-49]. Multi-walled carbon nanotubes (MWCNT) are persistent in the deep lung after inhalation and, once there, are able to induce both inflammatory and fibrotic reactions [47].

Owner 4/17/2017 10:45 AM

Comment [188]: Charge properties and the ability of carbon nanoparticles to affect the integrity of the blood-brain barrier as well as exhibit chemical effects within the brain have also been studied. Nanoparticles can overcome this physical and electrostatic barrier to the brain.

Owner 4/17/2017 10:45 AM

Comment [189]: In addition, high concentrations of anionic nanoparticles and cationic nanoparticles are capable of disrupting the integrity of the blood-brain barrier. The brain uptake rates of anionic nanoparticles at lower concentrations were greater than those of neutral or cationic formulations at the same concentrations. This work suggests that neutral nanoparticles and low concentration anionic nanoparticles can serve as carrier molecules providing chemicals direct access to the brain and that cationic nanoparticles have an immediate toxic effect at the blood-brain barrier [55,56].

The warming effect of the **greenhouse gases(nano aerosols) is expected to take place everywhere**, but the cooling effect of the pollution aerosols will be somewhat regionally dependent, near and downwind of industrial areas. No one knows what the outcome will be of atmospheric warming in some regions and cooling in others. Climate models are still too primitive to provide reliable insight into the possible outcome. Current observations of the buildup are available only for a few locations around the globe and these observations are fragmentary.

Understanding how much sulfur-based pollution is present in the atmosphere is important for understanding the effectiveness of current sulfur dioxide pollution control strategies.

The Removal of Aerosols

It is believed that much of the removal of atmospheric aerosols occurs in the vicinity of large weather systems and high altitude jet streams, where the stratosphere and the lower atmosphere become intertwined and exchange air with each other. In such regions, many pollutant gases in the troposphere can be injected in the stratosphere, affecting the chemistry of the stratosphere. Likewise, in such regions, the ozone in the stratosphere is brought down to the lower atmosphere where it reacts with the pollutant rich air, possibly forming new types of pollution aerosols.

Aerosols As Atmospheric Tracers

Aerosol measurements can also be used as tracers to study how the Earth's atmosphere moves. Because aerosols change their characteristics very slowly, they make much better tracers for atmospheric motions than a chemical species that may vary its concentration through chemical reactions. Aerosols have been used to study the dynamics of the polar regions, stratospheric transport from low to high latitudes, and the exchange of air between the troposphere and stratosphere.

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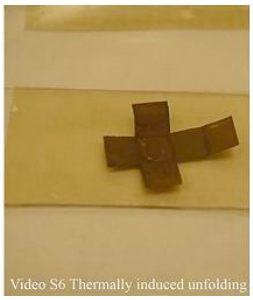
WSU researchers develop shape-changing 'smart' material

by Staff Writers



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Comment [190]: In addition, gene expression profiling was conducted on human epidermal keratinocytes exposed to SWCNT that showed a similar profile to alpha-quartz or silica. Also, genes not previously associated with these particulates before from structural protein and cytokine families were significantly expressed [52]. Dosing keratinocytes and bronchial epithelial cells *in vitro* with SWCNT has been shown to result in increases in markers of oxidative stress [50,53,54]

Owner 4/17/2017 10:45 AM

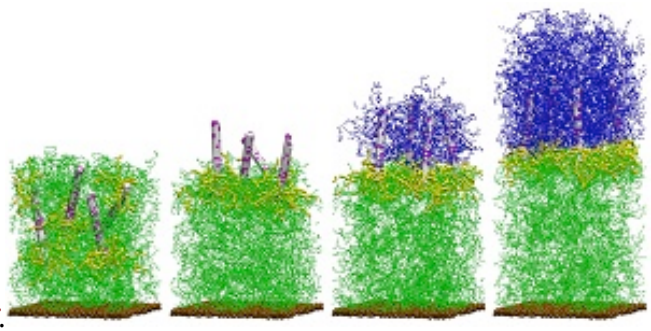
Comment [191]: Recently published research indicates that there is a range of concentrations where quantum dots used in bioimaging have the potential to decrease cell viability, or even cause cell death, thus suggesting that further toxicological evaluation is urgently needed

Owner 4/17/2017 10:45 AM

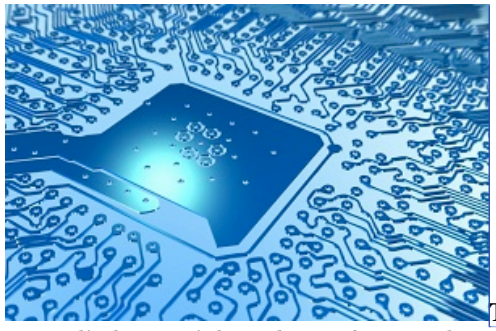
Comment [192]: Dermal exposure to MWCNT has been modeled through cell culture and points to the nanoparticles' ability to localize within and initiate an irritation response in target epithelial cells [50]. Proteomic analysis conducted in human epidermal keratinocytes exposed to MWCNT showed both increased and decreased expression of many proteins relative to controls. These protein alterations suggested dysregulation of intermediate filament expression, cell cycle inhibition, altered vesicular trafficking/exocytosis and membrane scaffold protein down-regulation

Pullman WA (SPX) Jul 03, 2016

Washington State University researchers have developed a unique, **multifunctional smart material that can change shape from heat or light and assemble and disassemble itself**.



They have filed a provisional patent on the work.--**This is the first time researchers have been able to combine several smart abilities, including shape memory behavior, light-activated movement and self-healing behavior, into one material**.

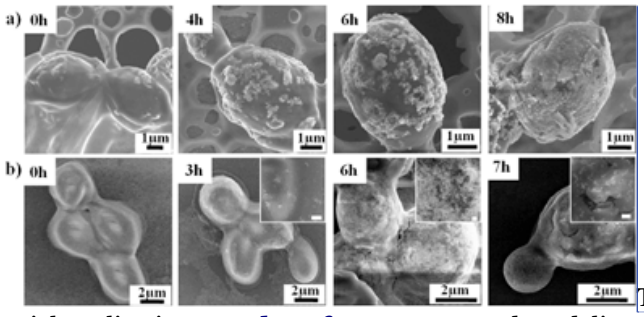


They have published their work in ACS Applied Materials and Interfaces.--The work is led by Michael Kessler, professor and Berry Family director and in the WSU School of Mechanical and Materials Engineering (MME), and Yuzhan Li, MME staff scientist, in collaboration with Orlando Rios, a researcher at Oak Ridge National Laboratory.

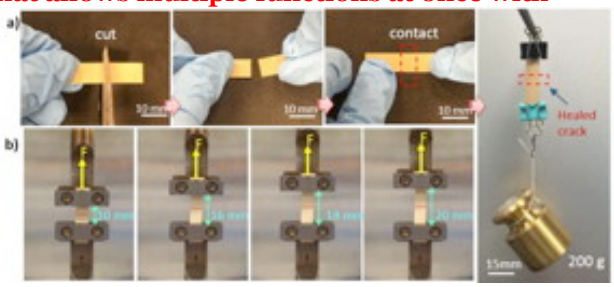
Adding functional versatility--Smart materials that can react to external stimuli, like light or heat, have been an interesting novelty and look almost magical as they mysteriously fold and unfold

Owner 4/17/2017 10:45 AM
Comment [193]: As you can see this polymer is altering from the original lattice (see the bars of code or fullerene) and then is changing it's construct from it's original template to the new design or program~ starts with 3 and then is expanded as the materials are being incorporated in the process

Owner 4/17/2017 10:45 AM
Comment [194]: Self repair CPU chip or AI chip

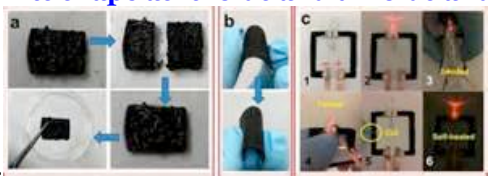


themselves. They have a variety of potential applications, **such as for actuators**, drug delivery systems **and self-assembling devices**. For instance, **smart materials could change shape** to unfold a solar panel on a space satellite without need of a battery-powered mechanical device.--But smart materials haven't come into widespread use because they are difficult to make and often can only perform one function at a time. Researchers also have struggled to reprocess the material so its special properties can continually repeat themselves.-The WSU research team **developed a material that allows multiple functions at once with potential to add more**.



Fold and unfold, remember and heal

The team worked with a class of long-chain molecules, called liquid crystalline networks (LCNs), which **provide order in one direction and give material unique properties**. The researchers took advantage of the way **the material changes in response to heat to induce a unique three-way shape shifting behavior**. **They added groups of atoms that react to polarized light and used dynamic chemical bonds to improve the material's reprocessing abilities.** - -"We knew these different technologies worked independently and tried to combine them in a way that would be compatible," said Kessler.-**The resulting material reacts to light, can remember its shape as it folds and unfolds and can heal itself when damaged**.

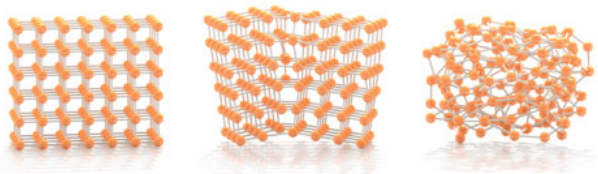


For instance, a razor blade scratch in the material **can be fixed by applying ultraviolet light**. The

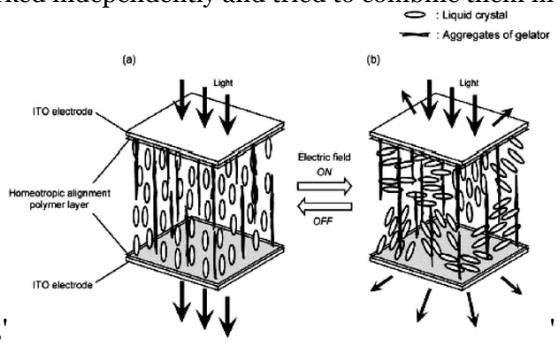
Owner 4/17/2017 10:45 AM

Comment [195]: **This is a cage that delivers nano quatum dots to lattices or it can deliver origami which is a protein that acts as a circuit board to assemble a consortium of constructs that can as well alter there shape and function and as you can see if attacked the protein polymer will repair itself and this is also applicable to carbon nano particulates**

material's movements can be preprogrammed and its properties tailored.--The Oak Ridge National Laboratory researchers used facilities at their Center for Nanophase Materials Sciences to study the mechanisms responsible for the material's unique abilities. -The research is in keeping with WSU's Grand Challenges, a suite of research initiatives aimed at large societal issues. **It is particularly relevant to the challenge of smart systems and its theme of foundational and emergent material -networks**



(LCNs) , which provide order in one direction and give material unique properties. The researchers took advantage of the way the material changes in **response to heat to induce a unique three-way shape shifting behavior. They added groups of atoms that react to polarized light and used dynamic chemical bonds to improve the material's reprocessing abilities**. -"We knew these different technologies worked independently and tried to combine them in a way



that would be compatible,' ' said Kessler.-The **resulting material reacts to light, can remember its shape as it folds and unfolds and can heal itself when damaged**. For instance, a **razor blade scratch in the material can be fixed by applying ultraviolet light**. **The material's movements can be preprogrammed and its properties tailored.**-The Oak Ridge National Laboratory researchers used facilities at their Center for Nanophase Materials Sciences to study the mechanisms responsible for the material's unique abilities.-The research is in keeping with WSU's Grand Challenges, a suite of research initiatives aimed at large societal issues. It is particularly relevant to the challenge of smart systems and its theme of foundational and **emergent material**

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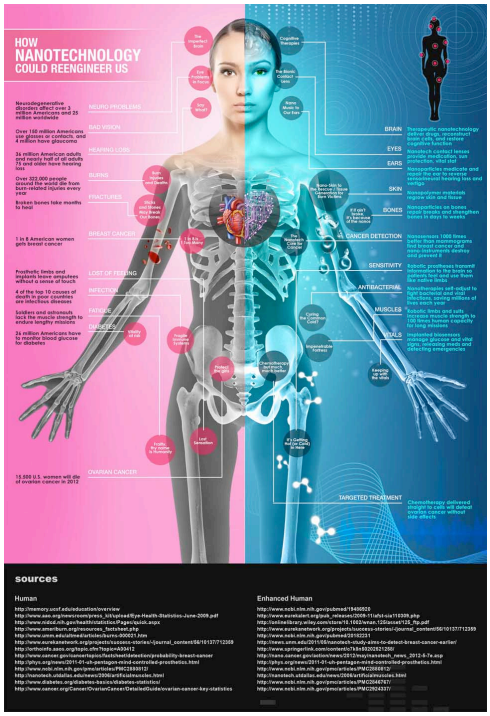
Comment [196]: Pay strict attention to this ~ this can have a program already implemented and run a specific design or protocol



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Silicon Dioxide (SiO2) Nanopowder

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Nanoparticles (SiO2) SSA: 180-600m2/g
Nanoparticles (SiO2) Color: white
Nanoparticles (SiO2) Bulk Density: <0.10 g/cm3

Nanoparticles (SiO2) True Density: 2.4 g/cm3

Silicon Oxide Nanoparticles (SiO2) Certificate of Analysis				
SiO2	Ti	Ca	Na	Fe
99.5%	120ppm	70ppm	30ppm	20ppm

Silicon Oxide Nanoparticles Applications:
Paint, plastic, color rubber, magnetic materials, in addition, nano-silica can be widely used in ceramics (sugar) porcelain, gypsum, batteries, paints, adhesives, cosmetics, glass, steel, fiber, glass, and many other fields of environmental protection products the upgrading.

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There is a wide range of product application in different fields with a large different amount of dosage, from 0.5 to 5.5%. The end user shall determine the quantity to be added through testing and make the best dosage choice for the best use.

Silicon Oxide Nanoparticles SDS

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SiO2, 98+%, 400 nm
SiO2, 99.5+%, S-type, Spherical, 15-20 nm
SiO2, 99.5+%, P-type, Porous, 15-20 nm
SiO2, 98+%, 60-70 nm
SiO2, 99+%, 20-30 nm
SiO2, 99%, 8nm
SiO2, 95.9+%, 20-30 nm, coated with KH570
SiO2, 96.3+%, 20-30 nm, coated with KH550
SiO2, 97.3+wt%, 15nm, coated with 2wt% Silane
SiO2 water dispersion, 25 wt%, 5-35 nm
SiO2 water dispersion 25wt%, 30 nm
SiO2 in 2-Propanol, Amorphous,15wt%, 25nm
SiO2 in 1, 2-Propanediol, 25wt%, 25nm
SiO2 in Ethylene Glycol, 25wt%, 25nm

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The nanosilica hazard- another variable entity

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- Johan A Martens and
- Peter H HoetEmail author

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Particle and Fibre Toxicology20107:39

DOI: 10.1186/1743-8977-7-39

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Received: 24 June 2010

Accepted: 3 December 2010

Published: 3 December 2010

Abstract

Silica nanoparticles (SNPs) are produced on an industrial scale and are an addition to a growing number of commercial products. SNPs also have great potential for a variety of diagnostic and therapeutic applications in medicine. Contrary to the well-studied crystalline micron-sized silica, relatively little information exists on the toxicity of its amorphous and nano-size forms. Because nanoparticles possess novel properties, kinetics and unusual bioactivity, their potential biological effects may differ greatly from those of micron-size bulk materials. In this review, we summarize the physico-chemical properties of the different nano-sized silica materials that can affect their interaction with biological systems, with a specific emphasis on inhalation exposure. We discuss recent *in vitro* and *in vivo* investigations into the toxicity of nanosilica, both crystalline and amorphous. **Most of the *in vitro* studies of SNPs report results of cellular uptake, size- and dose-dependent cytotoxicity, increased reactive oxygen species levels and pro-inflammatory stimulation**. Evidence from a limited number of *in vivo* studies demonstrates largely **reversible lung inflammation, granuloma formation and focal emphysema, with no progressive lung fibrosis.** Clearly, more research with standardized materials is needed to enable comparison of experimental data for the different forms of nanosilicas and to establish which physico-chemical properties are responsible for the observed toxicity of SNPs.

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Comment [197]: Most of the in vitro studies of SNPs report results of cellular uptake, size- and dose-dependent cytotoxicity, increased reactive oxygen species levels and pro-inflammatory stimulation. Evidence from a limited number of in vivo studies demonstrates largely reversible lung inflammation, granuloma formation and focal emphysema, with no progressive lung fibrosis

Introduction

Over the past decade, the definition of nanoparticles has been controversial. **Nanoparticles** are commonly defined as objects with a diameter less than 100 nm, but no clear size cut-off exists, and this usual boundary does not appear to have a solid scientific basis. Other definitions of nanoparticles have been proposed, and the most recent proposal [1] **is based on surface area rather than size** (a nanoparticle should have specific surface area > 60 m^2/cm^3), thus reflecting the **critical**

importance of this parameter in governing the reactivity and toxicity of nanomaterials. Physico-chemical properties that may be important in understanding the **toxic effects of nanomaterials include primary particle size, agglomeration/aggregation state, size distribution, shape, crystal structure, chemical composition, surface chemistry, surface charge, and porosity**. Aspects of these properties have been discussed in several reviews of nanotoxicology [2, 3, 4].

Silica is the common name for materials composed of silicon dioxide (SiO_2) and occurs in crystalline and amorphous forms. Crystalline silica exists in multiple forms. Quartz, and more specifically □-quartz is a widespread and well-known material. Upon heating, □-quartz is transformed into □-quartz, trydimite and cristobalite. **Porosil is the family name for porous crystalline silica**. Quartz exists in natural and synthetic forms, whereas all **porosils are synthetic. Amorphous silica can be divided into natural specimens (e.g., diatomaceous earth, opal and silica glass) and human-made products.**--The application of synthetic amorphous silica, especially silica nanoparticles (SNPs), has received wide attention in a variety of industries. **SNPs are produced on an industrial scale as additives to cosmetics, drugs, printer toners, varnishes, and food**. In addition, nanosilica is being developed for a host of biomedical and biotechnological applications such as cancer therapy, DNA transfection, drug delivery, and enzyme immobilization [5, 6, 7, 8, 9]. Barik et al. [10] recently reviewed the impact of nanosilica on basic biology, medicine, and agro-nanoproducts. With the growing commercialization of nanotechnology products, **human exposure to SNPs is increasing, and many aspects related to the size of these nanomaterials have raised concerns about safety** [11]. Until recently, most research has focused on silica particles 0.5 to 10 □m, mainly in crystalline forms**, but nanosilica may have different toxicological properties as compared with larger particles.** The unique physico-chemical properties of nano-sized silica that make them attractive for industry may present potential hazards to human health, **including an enhanced ability to penetrate intracellular targets in the lung and systemic circulation**. -Biocompatibility is a critical issue for the industrial development of nanoparticles [12, 13]. Even though no acute cytotoxicity has been observed or reported, **the uptake of the nanoparticles by cells may eventually lead to perturbation of intracellular mechanisms**. The ability of silica-coated nanomaterials **to penetrate the blood-brain barrier** also strongly suggests that extensive studies are required to clarify the potential chronic toxicity of these materials [14].

A number of SNPs have recently been shown to cause adverse health effects *in vitro* and *in vivo* (discussed later in this review). However, most of the studies have used poorly characterized particles in terms of their composition and physico-chemical properties. **The distinct physico-chemical properties of nanoparticles indeed determine their interaction with the cell/within the cell, and even subtle differences in such properties can modulate the toxicity and modes of action**. The results of toxicity studies then become difficult to interpret and compare, and, as a result, drawing appropriate conclusions is nearly impossible. Although SNPs could certainly provide benefits to society, their interaction with biological systems and potential toxic effects must be carefully addressed.----In this review, we discuss silica materials with a special attention to the physico-chemical properties that can affect their potential interaction with biological systems. We aim to provide an overview of the recent *in vitro* and *in vivo* investigations **of the toxicity of nanosilica, both in**

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Comment [198]: toxic effects of nanomaterials include primary particle size, agglomeration/aggregation state, size distribution, shape, crystal structure, chemical composition, surface chemistry, surface charge, and porosity-- this basically tells you that there is no form of nano then which is safe and with so many variables that can lead to high levels of toxic side effects toxicity is going to be inevitable

crystalline and amorphous forms, rather than review the toxicity of micron-sized silica and quartz. A summary of the present knowledge on the potential toxic effects of nano-sized silica particles is needed**, because their toxicological pattern appears distinct from that of micron-sized silica particles.**

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Synthesis & Characterization of Silica Materials

Classification of natural and synthetic silica materials

"Silica" is the name given to materials with the chemical formula of silicon dioxide, SiO_2. Silicas can be amorphous or crystalline, porous or non-porous (dense), anhydrous or hydroxylated [15], regardless of their natural or synthetic nature. In a silica material, the silicon atom is in **tetrahedral coordination with 4 oxygen atoms**. Theoretically, an infinite variety of 3-D-ordered structures can be built from oxygen-sharing silicate tetrahedra. The number of known crystalline silica materials is limited, which leaves much room for research and development. In amorphous silica, the **tetrahedra are randomly connected. ---**In nature, amorphous silica can have different origins. Silica can be condensed from vapors emitted in volcanic eruptions. **Natural silica can also be deposited from supersaturated natural water or polymerized in living organisms (biogenic silica**). These amorphous biogenic silicas **can be found as isolated particles, skeletal structures or surface elements in different living organisms**. Many microcrystalline silica minerals such as flint, chert and chalcedony are derived from biogenic silica after crystallization by compaction**. Kieselguhr (diatomaceous earth) occurs at various stages of transformation [15] and therefore often exhibits both crystalline and amorphous silica constituents.**

Physico-chemical characteristics of synthetic silica materials related to toxicity

The silica materials presenting a toxicological hazard to human health are mainly synthetic materials and natural quartz. The physico-chemical properties of silica materials largely **depend on the synthetic procedures used for their preparation**. Therefore, we will briefly discuss silica synthesis processes.

Silica synthesis

Silica is mainly synthesized from an aqueous solution, with dissociated monomeric silicic acid, $Si(OH)_4$, or from a vapor of a silicon compound such as silicon tetrachloride.--Waterglass is a concentrated alkaline sodium silicate solution with anhydrous composition corresponding to Na_2SiO_3**. It is the most common reagent for silica production in aqueous solution**. Waterglass is a sodium salt of silicic acid that forms silicic acid upon acidification. When the concentration of $Si(OH)_4$ exceeds about 2.10^{-3} M, condensation to polysilicic acids (Figure 1) occurs, thus leading to the formation of colloidal silica particles [15]. -

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Comment [200]: this is what breaks down silica

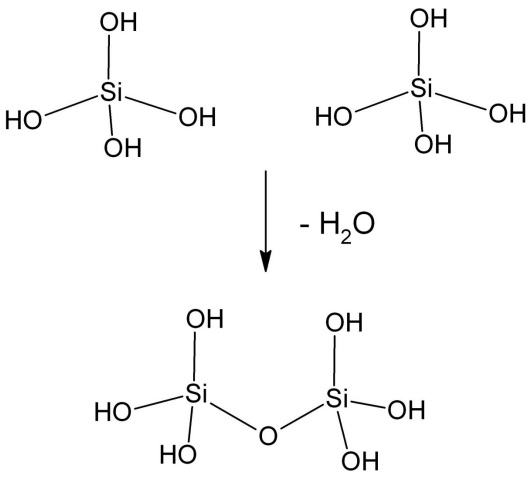

Figure 1

Polymerization of silicic acid molecules through formation of siloxane bond and water.

The polymerization and the formation of silica can be represented as follows:

$[Si_nO_{2n-nx/2}(OH)_{nx}] + m\ Si(OH)_4 \rightarrow$

$[Si_{n+m}O_{2n-nx/2+2m(2-p)}(OH)_{nx+4(m-p)}] + 2\ pm\ H_2O$

Where:

n = number of silicon atoms in a polysilicic acid molecule or particle,

x = number of OH groups per silicon atom in the polymer ($0 \leq x \leq 3$),

m = number of monomeric silicic acid molecules added to the polymer, and

p = fraction of the hydroxyl groups per monomeric silicic acid molecule that are converted to water during the **polymerization reaction** [15].

Amorphous silica particles are formed by polymerization of **monomers** in the aqueous solution **supersaturated with silicic acid**. Various silica materials are produced in liquid phase processes (Figure 2).

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Comment [201]: polymerization reaction -- **Monomers form polymers by forming chemical bonds or binding supramolecularly through a process called polymerization. Sometimes polymers are made from bound groups of monomer subunits (up to a few dozen monomers) called oligomers**

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Comment [202]: Monomers
The word monomer comes from *mono-* (one) and *-mer* (part). Monomers are small molecules which may be joined together in a repeating fashion to form more complex molecules called polymers

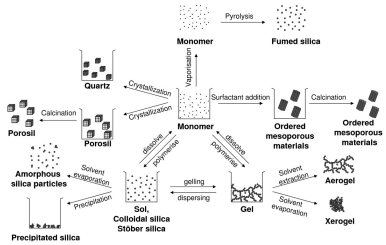

Figure 2

General scheme of silica synthesis processes. Adapted and complemented from [177].

Colloidal silica or silica sol is most often produced in a multi-step process in which the **alkaline silicate solution is partially neutralized with a mineral acid**. Alternatively, this pH neutralization can be achieved by electrodialysis. The resulting silica suspension is stabilized by pH adjustment. Finally a solid concentration up to 50 wt% is reached by water evaporation. **Silica sol nanoparticles show a perfect spherical shape and identical size** as a result of extensive Ostwald ripening [15]. **Stöber silica** sol is prepared by controlled hydrolysis and condensation of tetraethylorthosilicate (TEOS) in ethanol to which **catalytic amounts of water and ammonia are added**. The Stöber procedure can be used to obtain **monodisperse spherical amorphous** silica particles with **tunable size and porosity** [16].

Silica gel is obtained by destabilizing silica sol. Silica gel is an open 3-D network of aggregated sol particles. The pore size is related to the size of the original silica sol particles composing the gel.

Precipitated silica is formed when a sol is destabilized and precipitated.

Ordered mesoporous silica is obtained by a **supramolecular assembly of silica around surfactant micelles**. Typical surfactant molecules are amphiphilic polymers such as tribloc copolymers or quaternary alkylammonium compounds. These organic supramolecular templates are evacuated from the mesopores, typically via a calcination step. Calcination is a controlled combustion process leading to oxidation and decomposition of the template molecules into small volatile products such as NO_x, CO_2 and H_2O, which can leave the pores. **The diameter of the mesopores (2-50 nm)** is determined by the type of surfactant applied [17, 18].

A completely different synthesis route of amorphous silica starts from $SiCl_4$ in the vapor phase. Silicon tetrachloride is oxidized in a hydrogen flame at temperatures exceeding 1000°C and polymerized into amorphous non-porous SNPs. **This nanopowder has very low bulk density and high specific surface area, typically 200 to 300 m²/g**. This material is called **pyrogenic or fumed silica**, referring to the special synthesis conditions [15].---**The synthesis of dense crystalline silica such as** quartz **from aqueous solution is a slow process requiring heating the solution to accelerate the formation process in a so-called hydrothermal synthesis** [15]. Alternatively, under high pressure, amorphous silica can be transformed to

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Comment [204]: Micelle: Particle of colloidal dimensions that exists in equilibrium with the molecules or ions in solution from which it is formed.[1][2]
Micelle (polymers): Organized auto-assembly formed in a liquid and composed of amphiphilic *macromolecules*, in general amphiphilic di-
or tri-block copolymers made of solvophilic and solvophobic blocks.
***Note 1*: An amphiphilic behavior can be observed for water and an organic solvent or between two organic solvents.**
***Note 2*: Polymeric micelles have a much lower critical micellar concentration**
(CMC) than soap or surfactant micelles, but are nevertheless at equilibrium
with isolated macromolecules called unimers. Therefore, micelle formation and stability are concentration-dependent.[3]

crystalline material by microcrystallization. **The appearance of quartz ranges from macroscopic crystals to microcrystalline powder**s. Large crystals are grown at high temperature and pressure in industry. Smaller quartz crystals are conveniently obtained by grinding large crystals. Alpha-quartz is formed under moderate temperature and pressure conditions and is the most abundant form of quartz. At temperatures exceeding 573°C, □-quartz can transform into □-quartz [19]. At atmospheric pressure and temperatures higher than 870°C, quartz is transformed into tridymite and at temperatures more than 1470°C into cristobalite [15, 20]. **These high-temperature polymorphs of quartz have the same elemental composition but a different crystal structure and can persist metastably at lower temperatures**.-

Dense and porous crystalline materials can be distinguished by framework density. **The framework density is conveniently defined as the number of tetrahedrally coordinated atoms (T-atoms) per nm³**. For dense structures, such as quartz, tridymite and cristobalite, values of 22 to 29 T-atoms/nm³ are common, whereas for **porosils belonging to the zeolite material family**, as few as 12.1 T-atoms/nm³ are present [21]. The framework structure of a porosil is denoted with a 3-letter code. Descriptions are available in the *Atlas of Zeolite Framework Types*[22].

Porosils are crystallized in aqueous media in the presence of organic molecules that act as porogens or template molecules defining the size and shape of the pores. Their evacuation is typically achieved through calcination. Among the porosils are clathrasils and zeosils [23, 24]. **Zeosils have cages with windows or channels of a sufficiently free dimension to allow molecules to diffuse in and out, a property known as molecular sieving** [25]. **Clathrasils have cages with windows that are delineated with a 6-membered ring of SiO units, thus presenting a free aperture of barely 0.28 nm. Even a molecule as small as oxygen has no access to the cavities of a clathrasil**. The organic template molecules engaged in the crystallization of a clathrasil cannot be removed easily from the pores [23, 24].

When heated above 1700°C, any type of silica (amorphous or crystalline) melts. During cooling, the disordered structure is solidified, and a dense amorphous silica glass or vitreous silica is formed [15].

Physico-chemical properties

The properties of silica materials considered essential for their potential **toxicity are crystallinity, particle size and morphology, porosity, chemical purity, surface chemistry and solubility** [26]. An overview of the properties of silica materials involved in silica toxicity is provided in Table 1.

Table 1

Overview of silica materials and relevant properties

Material	Nature of product	Crystallinity	Particle size	Porosity	Polarity	Purity	Applications	Ref
Colloidal silica	Sol	Amorphous	1-1000 nm	Dense	Hydrophillic	Very high	Binders, ink	[15]

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Comment [205]: the designation metastable state usually is reserved for states whose lifetimes are unusually long.

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Comment [206]: Porosils are crystallized in aqueous media in the presence of organic molecules that act as porogens or template molecules defining the size and shape of the pores

Material	Nature of product	Crystallinity	Particle size	Porosity	Polarity	Purity	Applications	Ref
Stober silica	Sol	Amorphous	10-1000 nm	Tunable porosity	Hydrophillic	Very high	Research	[16]
Precipitated silica	Powder	Amorphous	5-6 nm primary particles precipitated to 500 nm - 50 □m aggregates	Tunable porosity	Hydrophillic	Very high	Filler and performance additive	[15]
Silica gel	Powder	Amorphous	0.5 - 5 nm primary particles gelled to networks and milled to 500 □m - 6 mm aggregates	Tunable, void spaces between primary particles	Hydrophillic	Very high	Dessicant, filler and performance additive	[15]
Mesoporous silica	Powder	Amorphous	50 - 1000 nm, aggregated because of calcinations	Mesoporous	Hydrophobic	Very high	Drug delivery, catalysis, imaging	[8]
Pyrogenic silica (fumed silica)	Powder	Amorphous	2-50 nm primary particles fused to 1-250 □m aggregates	Void spaces between primary particles	Hydrophobic	Very high	Tickner, performance additive	[15]
Vitreous silica (fused silica glass)	Powder	Amorphous	50-2000 □m	Dense	Hydrophobic (grinded: hydrophilic)	Variable	Glass	[15, 19]
Quartz	Powder	Crystalline	50 nm-several □m	Dense	Hydrophobic/(grinded: hydrophilic)	Variable	Geologic mineral, Piezoelectricity	[19, 20]
Cristobalite	Powder	Crystalline	1 □m - several cm	Dense	Hydrophobic	Variable	Geologic mineral	[20]
Zeosils (porosil)	Powder	Crystalline	0.05-5000 □m	Porous Pore diameter: 0.4-1.2 nm	Hydrophillic/hydrophobic	Very high	Adsorbent	[25]
Clathrasils (porosil)	Powder	Crystalline	0.5-5000 □m	Porous	Hydrophillic/hydrophobic	Very high	Gas separation	[24]

Material	Nature of product	Crystallinity	Particle size	Porosity	Polarity	Purity	Applications	Ref
				Pore diameter: 0.2-0.3 nm				
Diatomeus earth, kieselguhr	Powder	Amorphous, partially crystalline	5-120 □m	Dense	Hydrophillic/hydrophobic	Low (90%)	Filter, filling material	[15]

Crystallinity

In crystalline structures such as quartz and porosils, **the arrangement of atoms is ordered in all dimensions**. According to the International Union of Pure and Applied Chemistry (IUPAC), the atoms must **be arranged periodically with long-range order (at least 10 repeats in all directions)** and produce sharp maxima in a diffraction experiment to observe x-ray diffraction (XRD) crystallinity [27]. **The threshold for observing crystallinity depends on the unit cell size (size of the repeated unit in a crystal**). For materials with **large unit cells, such as porosils, the minimum particle size required is about 10 nanometers to observe a distinct, sharp XRD pattern.** Amorphous silica may present some short-range order but lacks long-range order in 3 dimensions and does not exhibit a sharp XRD pattern. Of note, the surface of a crystal represents a discontinuity that can be seen as a defect. With the presence of a less-structured or even partially amorphous rim, crystals may behave like amorphous particles. Thus, particles with an ordering at limited-length scales or with amorphous regions may be classified as amorphous.

Particle size and morphology

Nanoparticles are obtained by direct synthesis of silica sol [15] or by crystallization of nano-sized crystals of quartz or porosils [25]. The particle size is determined by the synthesis parameters. Amorphous silica sol particles tend to adopt the spherical shape so as to reach a minimum of interfacial surface area. **The particle size of commercial silica sols prepared from sodium silicate is from 10 to 25 nm** (Figure 3 left). Sols with larger primary particles can be prepared from TEOS by Stöber synthesis, for example (Figure 3 middle). **Grinding and milling processes reduce particle size**. These techniques **are most often applied to quartz, silica gel and vitreous silica**. The obtained products generally have a broad size distribution.

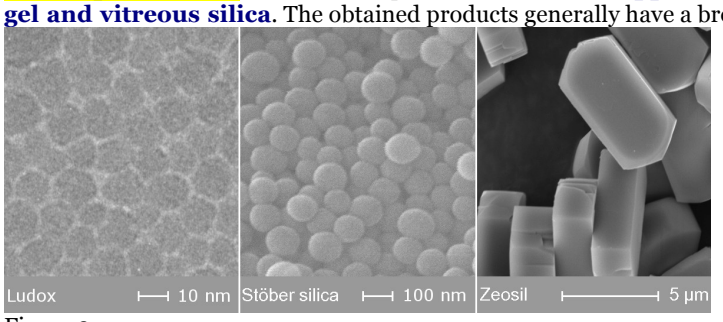

Figure 3

Electron microscopy images of Stöber silica sol particles (left) and MFI type zeosil (right).

Crystalline particles exhibit crystal planes at the surface, and the morphology of the crystalline nanoparticles depends on the crystal class such as cubic, hexagonal, tetragonal, and orthorhombic (Figure 3 right). For all nanomaterials, in aqueous environment, **the primary nano-sized silica particles tend to form aggregates.**

Porosity

According to IUPAC [28], pores are classified according to **their diameter into micropores (< 2 nm), mesopores (2-50 nm) and macropores (> 50 nm**). **Amorphous sol particles can be microporous or non-porous (dense).** The porosity of Stöber silica can be tuned by adapting the synthesis parameters: decreasing the ratio of water to TEOS **promotes particle growth by aggregating smaller sub-particles, thus leading to rough particle surfaces with micropores**. In contrast, smooth particle surfaces are obtained with conditions of high ratio of water to TEOS [29]. **Silica gel is a powder with particle size in the micrometer range or larger and is, typically, mesoporous. -Zeosils and clathrasils have characteristic pores and cages in the micropore size range, depending on framework topology**. Examples of porosil frameworks are shown in Figure 4[22].

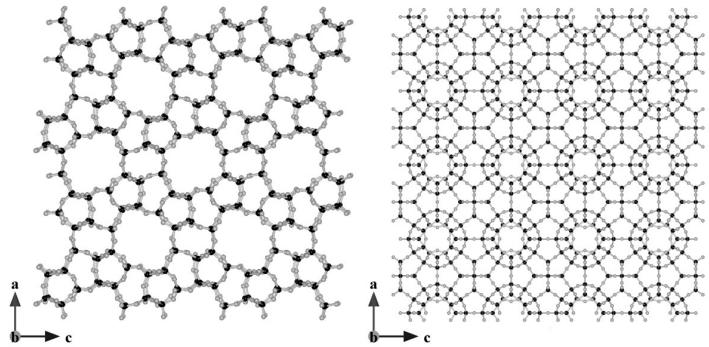

Figure 4

Atomic representation of (left) a zeosil with microporous channels (MFI type) and (right) clathrasil with a denser framework (SOD type). Black and gray circles represent silicon and oxygen atoms, respectively. Figure made with Vesta 2.0.3 [178] with unit cell coordinates from [22].

When the silica is presented as a nanopowder, porosity can be an intrinsic and extrinsic characteristic: stapling of the elementary nanoparticles gives rise to an interparticle porosity, which often is difficult to distinguish from the intrinsic intraparticle porosity, especially when dealing with mesoporosity.

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Comment [207]: anything in these ranges will penetrate skin or translocate throughout the body making this problematic due to size and shape

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Comment [208]: in these ranges the system can flush or remove -due to the particle size is large enough for the body to recognize and to function

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Comment [209]: intrinsic: belonging to the essential nature of a thing : occurring as a natural part of something

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Comment [210]: extrinsic 1. not contained or included within; extraneous
2. originating or acting from outside; external

Hydrophilic-hydrophobic properties

The hydrophilicity of a silica material increases with the number of silanols, or silicon-bonded hydroxyl groups, capable of forming hydrogen bonds with physical water molecules. The chemical formula of silica is represented as $SiO_2.xH_2O$, in which water represents chemical water contained in silanol groups present on the surface of the silica material. These water molecules are not to be confused with crystal water, such as that present in many inorganic salt crystals. The surface chemistry of silica is depicted in Figure 5. Vicinal hydroxyl groups (one hydroxyl group per tetrahedron) located at **mutual distances smaller than 3 nm are engaged in hydrogen bonding**. Geminal hydroxyls (2 hydroxyl groups per tetrahedron) are considered to occur in minor concentrations. Isolated silanols are positioned too far apart to be engaged in hydrogen bonding. Because of the differing chemistry of these 3 types of silanol groups**, they are not all equivalent in their adsorption behavior or chemical reactivity.** Vicinal hydroxyls interact strongly with water molecules and are responsible for the excellent water adsorption properties of silica, which are exploited in industrial gas drying operations, for example.

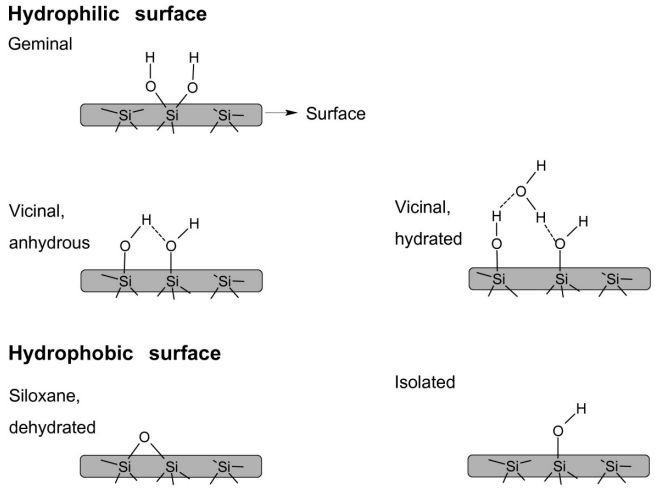

Figure 5

Hydroxyl groups on the surface of silica. Covalent bonds and hydrogen bonds are presented as full and dotted lines, respectively.

The reported concentration of hydroxyl groups per square nanometer on the surface of amorphous silica ranges from 4 to 5 OH/nm^2[12]. As compared with amorphous silica, the crystalline forms of silica generally contain a lower concentration of surface hydroxyl groups [15]. Hydrogen-bonded water molecules are removed when silica is heated at 170°C under atmospheric pressure or at room temperature under vacuum.

Colloidal silica, precipitated silica and ordered mesoporous silica and silica gel are hydrophilic because of their high concentration of silanols. Silicagel, for example, can adsorb water in quantities up to 100% of its proper weight.

Porosils typically are hydrophobic because they lack silanols in the pores of their framework. Silica produced at high temperature, such as pyrogenic and vitreous silica, or calcined at temperatures exceeding 800°C, is almost entirely dehydroxylated. In a dehydroxylation reaction, neighboring silanol groups are condensed into siloxane bonds (Figure 5 bottom) and water molecules. Some isolated silanol groups may persist on the surface [15]. Because hydrogen bonding on siloxanes is unfavorable, dehydroxylated silica is hydrophobic. Grinding of hydrophobic bulk materials such as quartz and vitreous silica induces silicon and oxygen radicals and surface charges. These charges increase the hydrophilic surface [19, 30].

Solubility

The dissolution and precipitation of silica in water chemically involves hydrolysis and condensation reactions, respectively, catalyzed by OH^- ions (Figure 1).

For micrometer-sized nonporous amorphous silica, the equilibrium concentrations of $Si(OH)_4$ at 25°C in water corresponds to 70 ppm at pH 7. The silica solubility depends on the surface curvature of the (nano)particles. SNPs and nanoporous silica show enhanced equilibrium solubility, of 100-130 ppm [12]. According to Vogelsberger et al. [31], the solubilization of amorphous SNPs in physiological buffer at 25°C is accelerated because of the large surface area exposed. The solubility equilibrium is reached only after 24 to 48 h. Crystalline silica such as quartz has a much lower equilibrium solubility, of 6 ppm [15].

In summary, when dealing with silica, the physico-chemical properties such as amorphous versus crystalline nature, porosity, particle size and degree of hydroxylation must be specified. An overview of silica materials described in the scientific literature and in the research and development environment is provided in Table 1.

Toxicity Of Silica

Background

Health effects of silica and epidemiological studies

Until recently, toxicological research into silica particles focused mainly on "natural" crystalline silica particles of 0.5 to 10 □m (coarse or fine particles). This research was/is fed by the clear association of occupational inhalation exposure and severe health effects, mainly on the respiratory system. The typical lung reaction induced by chronic inhalation of crystalline silica is silicosis, a generally progressive fibrotic lung disease (pneumoconiosis), exemplified by the development of silicotic nodules composed of silica particles surrounded by whorled collagen in concentric layers, with macrophages, lymphocytes, and fibroblasts in the periphery. Epidemiologic studies have found that silicosis may develop or progress even after occupational exposure has ended; therefore, above a given lung burden of particles, silicosis was suggested to progress without further exposure [32, 33, 34]. Calvert et al. [35] recently reported an association of crystalline silica (mainly quartz) exposure and silicosis, as well as lung cancer, chronic obstructive pulmonary disease (COPD), and pulmonary tuberculosis. The carcinogenicity of quartz and cristobalite has been shown in several epidemiological studies [36, 37, 38]. In 1997, the International Agency for Research on Cancer (IARC) classified some

crystalline silica polymorphs (quartz and cristobalite) in group 1 (sufficient evidence for the carcinogenicity to experimental animals and to humans), whereas amorphous silica (silicon dioxide without crystalline structure) was classified in group 3 (inadequate evidence for carcinogenicity) [39]. This classification has recently been confirmed [40]. Checkoway and Franzblau [41] reviewed occupational epidemiologic literature on the interrelations among silica exposure, silicosis and lung cancer and concluded that the appearance of silicosis is not necessarily required for the development of silica-associated lung cancer. Hnizdo and Vallyathan [42] suggested that chronic exposure to levels of crystalline silica dust, which does not cause disabling silicosis, may cause chronic bronchitis, emphysema, and/or small airway disease leading to airflow obstruction, even in the absence of radiological evidence of silicosis. Evidence has linked silica exposure to various autoimmune diseases (systemic sclerosis, rheumatoid arthritis, lupus, chronic renal disease), as reviewed by Steenland and Goldsmith [43]. A study by Haustein et al. [44] reported on silica-induced (silica dust) scleroderma.

Amorphous silica has been far less studied than has the crystalline form [39]. Warheit [45] briefly described the inhalation toxicity data related to amorphous silica particulates and concluded that some forms of amorphous silica are more potent in producing pulmonary effects as compared to others. He also emphasized the great need for adequate toxicological testing of many of these amorphous silicates given their importance in commerce and widespread potential for exposure. Workers exposed to precipitated or fumed silica did not exhibit pneumoconiosis [46, 47], but evidence of pulmonary fibrosis was reported in workers exposed to amorphous silica dust produced as a byproduct of silicon metal production [48]. Merget et al. [49] reviewed the current knowledge of the health effects of a wide range of amorphous forms of silica in humans. The major problem in the assessment of health effects of biogenic amorphous silica is its contamination with crystalline silica. This problem applies particularly to the well-documented pneumoconiosis among diatomaceous-earth workers. Although the data are limited, a risk of chronic obstructive bronchitis disease, COPD or emphysema cannot be excluded [49]. Animal inhalation studies involving synthetic amorphous silica (colloidal silica, fumed silica and precipitated silica) showed at least partially reversible inflammation [50, 51], granuloma formation and emphysema, but no progressive fibrosis of the lungs [52, 53]. However, high doses of amorphous silica may result in acute pulmonary inflammatory responses, which could conceivably trigger long-term effects, despite a low biopersistence of the particles [54]. The debate on the health effects of micron-sized crystalline or amorphous silica is beyond the scope of this article. Readers are referred to other publications [35, 36, 37, 38, 41, 55, 56, 57].

Mechanisms of toxic action

As mentioned, most of the toxicological research into silica has focused on crystalline silica particles of 0.5 to 10 □m (coarse or fine particles). Despite the relatively large amount of available studies, the mechanisms of crystalline silica toxicity at the cellular and molecular levels are still unclear, and whether any single mechanism underlies all the above-mentioned diseases induced by these particles is uncertain [43]. However, severe inflammation following exposure to silica particles appears to be a common initiating step [58, 59].

The crucial role of reactive oxygen species (ROS) in the inflammatory, fibrogenic and carcinogenic activity of quartz is well established [60, 61]. Oxidative membrane and DNA damage are considered the most important mechanisms involved in the health effects of

micron-sized crystalline silica. A few of the numerous reports clearly demonstrate these findings: ROS generated by the silica surface can induce cell membrane damage via lipid peroxidation that may subsequently lead to increased cellular permeability [62], perturbation of intracellular calcium homeostasis [63] and alterations in signaling pathways. Schins et al. and Fanizza et al. [64, 65] demonstrated that respirable quartz particles induce oxidative DNA damage in human lung epithelial cells. Li et al. [66, 67] demonstrated that micron-sized quartz particles induce ·OH generation through an iron-dependent mechanism. A close association of ·OH and iron ion concentration has been reported for amorphous silica particles [66, 67]. The study of Ghiazza et al. [30] indicates that crystallinity might not be a necessary prerequisite to make a silica particle toxic; both quartz and vitreous silica showed stable surface radicals and sustained release of HO· radicals. When tested on macrophages, vitreous silica and pure quartz showed a remarkable potency in cytotoxicity, release of nitrite and tumor necrosis factor □ (TNF-□) production, suggesting a common behavior in inducing of oxidative stress [30]. Ding et al. [68] discuss the molecular mechanisms of silica-induced lung injuries with a focus on NF-kB activation, generation of cyclooxygenase II and tumor necrosis factor □ (TNF-□). The review of Castranova [69] summarizes evidence that *in vitro* and *in vivo* exposure to crystalline silica results in activation of NF-kB and AP-1 signaling pathways. *In vitro* and *in vivo* animal studies, as well as investigations in humans, strongly support the role of macrophage products in the development and progression of silicosis [70]. Such products include a large panel of cytokines [71], with TNF-□ seeming to determine the development of silica-induced pulmonary fibrosis [72]. In addition, recent evidence implicates interleukin 1□ (IL-1□) and its activation by the NALP-3 inflammasome [73].

A large body of experimental work in the past 20 years has shown that 2 main factors seem to govern the hazardous nature of crystalline silica: particle surface reactivity and the form of silica [74]. Fenoglio et al. [75] evaluated these factors systematically, studying synthetic quartz samples differing only in size and shape. Cytotoxicity appeared to be primarily governed by the form of the particles and the extent of the exposed surface. Several studies indicate that the surface silanol groups are directly involved both in membranolysis [76, 77, 78] and in toxicity to alveolar cells [79, 80]. Therefore, the distribution and abundance of silanols determines the degree of hydrophilicity (see "Physico-chemical properties of synthetic silica materials related to toxicity" described above) and seems to modulate cell toxicity [80, 81]. Experimental work with respirable silica particles and the survey of published data by Bagchi [82] suggest that the toxicity of these particles is caused by the large amount of positive charges they carry. Ghiazza et al. [83] reported that formation of a vitreous phase at the surface of some commercial diatomaceous earth prevents the onset of oxidative stress effects. Donaldson and Borm [84] emphasized that the ability of quartz to generate ROS could be modified by a range of substances that affect the quartz surface, such as substances originating from other minerals. The authors concluded that the toxicity of quartz is not a constant entity and may vary greatly depending on the origin/constitution of the sample.

The origin/synthesis of SNPs plays a crucial role in determining the physico-chemical properties of these particles and, consequently, their potential interactions with biological systems. Surface area, surface morphology, surface energy, dissolution layer properties, adsorption and aggregation properties are relevant parameters. Depending on the manufacturing process, amorphous silica has a wide range of physico-chemical properties that determine its industrial application. Bye et al. [85] showed that the cytotoxic activity of different forms of amorphous silica does not depend on a crystalline silica component but, rather, is caused by surface charges and the morphologic features

of particles. Synthetic amorphous silica has been the subject of dissolution testing with a simulated biological medium, and the silica dissolution rate was reported as being more rapid than the reverse precipitation rate [86]. Solubility has been defined as a key driver in the clearance mechanisms involved in amorphous silica removal from lung [87]. Warheit [45] reviewed pulmonary responses to different forms of silica and reported that cristobalite produced the greatest lung injury, quartz produced intermediate effects, and amorphous silica produced minimal effects. In terms of analytical technique, small differences in dissolution exist among these different forms of silica, and dissolution, in turn, influences pulmonary effects through the concept of persistence. In addition, components from the biological system may react with the surface of the particle. A systematic investigation of iron-containing SNPs as used in industrial fine-chemical synthesis demonstrated the presence of catalytic activity that could strongly alter the toxic action of nanoparticles [88].

On the whole, considering the great variety of silica forms, degree of crystallinity, surface state and the presence of contaminants, there is a critical need for carefully characterized standard silica samples to unravel the relationships between physico-chemical factors and toxicity, both micron- and nano-sized. The main goal of this review is to focus on the toxicity of nanosilica, which has never been properly reviewed. Moreover, nanosilica occurs mainly in amorphous forms, and the potential hazard posed by these nanomaterials cannot be simply related to, as has already been reviewed many times, studies of micron-sized crystalline materials.

Silica nanoparticles

The growing abundance and industrial applications of nanotechnology has resulted in a recent shift of toxicological research towards nanoparticles [89, 90, 91, 92, 93, 94]. Ultrafine particles (< 0.1 □m) have been demonstrated to cause greater inflammatory responses and particle-mediated lung diseases than have fine particles (< 2.5 □m) per given mass [95, 96, 97]. Also, experiments involving silica have shown that nanoparticles, both ultrafine colloidal silica [98, 99] and crystalline silica [99], have a greater ability to cause lung injury as compared with fine particles. Thus, the unique properties (i.e., small size and corresponding large specific surface area; cell penetrating ability) of nano-sized SiO_2 are likely to impose biological effects that differ greatly from micron-scale counterparts.

In vitro studies of nanosilica toxicity

A structured summary of *in vitro* studies of the toxicity of SNPs can be found in Table 2.
Table 2

In vitro studies on nanosilica particles (SNPs) toxicity

Silica form	Size (primary)	Material characterization	Cells used	Test	Biological endpoints and findings	Ref
Amorphous	40 nm- 5 □m	Not specified	A549	• Replication and transcription assays	• Uptake of all particles into the cytoplasm and nuclear	[100]

Silica form	Size (primary)	Material characterization	Cells used	Test	Biological endpoints and findings	Ref
			HEp-2 RPMI 2650 RLE-6TN N2a	• Cell proliferation and cell viability assay • Proteasome activity assay • Immunofluorescence and microscopy	localization of nanoparticles between 40 and 70 nm • The uptake of NSPs in the nucleus induced aberrant clusters of topoisomerase I and protein aggregates in the nucleoplasm	
Amorphous (luminescent)	50 nm	• Synthesis (ref. to literature)	A549 rat alveolar macrophages	• laser scanning confocal microscope • Comet Assay • Pulse Field Gel Electrophoresis (PFGE) • Western Blot Analysis of DNA Adducts/DNA Agarose Gel • DNA Repair Enzyme Activity Assay • Cell Proliferation Assay • Vybrant Apoptosis Assay	• Uptake not detected in the nuclear region • As compared to the A549 cells, the nanoparticle penetration rate was much faster in the rat alveolar macrophages • No significant toxic effects observed at the molecular and cellular levels below a concentration of 0.1 mg/ml	[101]
Amorphous (colloidal)	15 and 46 nm	• Particle sizes and distribution • Surface area (268 and 52.5 m^2/g for 15 and 46 nm particle, respectively), crystalline structure, major trace metal	A549	• SRB (sulforhodamine B) and LDH assays • Reduced glutathione (GSH) level • DCFH assay (ROS	• Cytotoxicity was dose- and time-dependent • Reduced glutathione (GSH) levels and elevated MDA production after	[102]

Silica form	Size (primary)	Material characterization	Cells used	Test	Biological endpoints and findings	Ref
		impurities • Hydrodynamic particle size in water suspension		generation) • Malondialdehyde (MDA) assay	exposure to 15 nm SNPs	
Amorphous	60 and 100 nm	• Size distribution analysis • Endotoxin concentration	A549 THP-1 Mono Mac 6; co-cultures	• LDH assay • Cytokine expression (TNF-**α**, IL-6, IL-8) • Light and transmission electron microscopy (TEM)	• Cytotoxicity differed among the cell lines and was dose- and size-dependent (smaller particles were more toxic) • co-cultures showed an increased sensitivity to particles concerning the cytokine release in comparison to the mono-cultures of each cell type	[103]
Amorphous	~14 nm	• Size distribution	A549 L-132 HeLa MNNG/ HOS	• MTT and WST-1 assays • Trypan blue exclusion and LDH assay • Annexin V-PI assay (fluorescence microscopy) • DCFH assay • IL-8 expression (ELISA)	• Little cytotoxic effects in 4 cell lines tested at the concentration below 250 ☐g/ml within 48 h • Exposing cancer cells to high concentrations (250-500 ☐g/ml) for 72 h resulted in an inflammatory response with oxidative stress and membrane damage, which	[104]

Silica form	Size (primary)	Material characterization	Cells used	Test	Biological endpoints and findings	Ref
					varied with cell type (A549>HOS > HeLa) • SNPs triggered an inflammation response without causing considerable cell death for both cancer cells and normal cells	
Amorphous	10 and 80 nm	o Provided by producer for the primary particles (surface area: 640 and 440 m^2/g for 10 and 80 nm particle, respectively) o Hydrodynamic particle size (in cell culture medium)	A549	• MTT and LDH assays • DCFH assay • Intracellular glutathione (GSH) concentration • Membrane lipid peroxidation (LPO) • Assay of glutathione reductase and glutathione peoxidase	• Cytotoxicity was dose-dependent • SNPs induced reactive oxygen species and membrane lipid peroxidation in dose-dependent manner • Both sizes of SNPs had little effect on GSH level and the activities of glutathione metabolizing enzymes	[105]
Amorphous	7 and 5-15 nm	o Surface area (350 and 644 m^2/g for 7 and 5-15 nm particle, respectively) o Size distribution (in the test medium)	Beas-2B	• Incorporation of SNPs into the cells (confocal LSM) • MTT assay • PI staining (flow cytometry) • Apoptosis • DCFH assay	• SNPs were incorporated into the cells and distributed around the nucleus area • SNPs induced oxidative stress via ROS formation and induction of of antioxidant	[106]

Silica form	Size (primary)	Material characterization	Cells used	Test	Biological endpoints and findings	Ref
				• Oxidative stress responding transcription factors (Western blotting)	enzymes (SOD and HO-1) • Induction of Nrf-2-ERK MAP kinase signaling pathway was observed • Overall, cells exposed to 5-15 nm SNPs (porous) showed a more sensitive response than those exposed to 7 nm SNPs (fumed)	
Amorphous	10-20 nm	o Provided by manufacturer (surface area: 140-180 m^2/g) o Primary particle size o Endotoxin content (LPS)	A549	• MTT and LDH assays • DCFH assay • SOD activity determination • Nitrate/nitrite determination • DNA oxidative damage assay	• Cytotoxicity was dose- and time-dependent • SNPs stimulated the ROS generation, GSH depletion and lower expression of SOD activity in a dose-dependent manner • No NO production and significant DNA oxidative damage was observed after treatment of cells with SNPs • Co-treatment of LPS with SNPs enhanced observed cytoxicity and	[107]

Silica form	Size (primary)	Material characterization	Cells used	Test	Biological endpoints and findings	Ref
					generation of oxidative stress	
Amorphous	30, 48, 118 and 535 nm	• Synthesis method • Hydrodynamic particle size (in water and cell culture medium)	HEL-30	• MTT and LDH assays • Reduced glutathione (GSH) and DCFH assay • Transmission electron microscopy (TEM)	• Cytotoxicity was dose- and size-dependent (smaller particles were more toxic) • Uptake of all particles into the cytoplasm (nuclear uptake not studied) • GSH level reduced significantly of after exposure to 30 nm nanoparticles • No significant Reactive Oxygen Species (ROS) formation	[108]
Amorphous	70, 300 and 1000 nm	Not specified	XS52	• TEM analysis of cells • LDH assay • Proliferation ([^{3}H]-Thymidine incorporation assay)	• SNPs of 300 and 1000 nm were incorporated into the cells and located in cytoplasm only; nanoparticles of 70 nm were located in nucleus as well as cytoplasm • Cell proliferation was inhibited by treatment with SNPs of all sizes in dose-dependent	[109]

Silica form	Size (primary)	Material characterization	Cells used	Test	Biological endpoints and findings	Ref
					manner • The growth of the cells was more strongly inhibited by smaller-sized SNPs	
Amorphous	15, 30 and 365 nm	• Size distribution • Zeta potential • Amorphous structure	HaCaT	• CCK assay • Cell cycle assay • Annexin V-PI assay (Flow cytometry) • 2D-DIGE and, IEF and SDS_PAGE (protein expression) • Western blot	• Cytotoxicity was dose- and size-dependent (smaller particles were more toxic) • Apoptosis was dose- and size-dependent (smaller particles induced higher apoptosis frequency) • Up-regulated proteins were classified as oxidative stress-associated proteins; cytoskeleton-associated proteins; molecular chaperones; energy metabolism-associated proteins; apoptosis and tumor-associated proteins	[110]
Amorphous	15 nm	• Size distribution	HaCaT	• Flow cytometric analysis of	• Treatment with SNPs induced Global DNA	[111]

Silica form	Size (primary)	Material characterization	Cells used	Test	Biological endpoints and findings	Ref
		• Zeta potential • Amorphous structure		methylated DNA • Real-time PCR • Western blot	hypomethylation	
Amorphous	21 and 80 nm	• Particle preparation and dispersion • Size, morphology and chemical states of elements • Hydrodynamic particle size (dispersed in water)	WS1 CCD-966sk MRC-5 A549 MKN-28 HT-29	• MTT and LDH assays	• Toxicity was seen at concentrations exceeding 138 □g/ml • Susceptibility to NSPs differed among tested cell lines	[113]
Amorphous	20 nm	Only provided by producer (surface area: 640 ± 50 m^2/g)	RAW264.7	• Membrane fluidity measurements (FRAP technique by LSCM) • DCFH assay • Intracellular free calcium content	• Exposure to SNPs increased ROS generation and decrease of the membrane fluidity • Perturbation of Intracellular free calcium homeostasis was responsible for observed cytotoxicity	[114]
Amorphous	14 nm	Only provided by producer (surface area: 200 m^2/g)	Caco-2	• LDH and WST-1 assay • Fpg-modified comet assay • Total GSH content	• Cytotoxicity observed • Oxidative DNA damage • Significant depletion of intracellular GSH	[115]
Amorphous	21, 48 and 86 nm	• Size distribution analysis • Surface area (225, 106 and 39 m^2/g for	L-02	• MTT and LDH assays • TEM assay	• Cytotoxicity was dose- time - and size-dependent (smaller	[116]

Silica form	Size (primary)	Material characterization	Cells used	Test	Biological endpoints and findings	Ref
		21, 48 and 86 nm particle, respectively) • structure		• DCFH, MDA and GSH assay • Annexin V-PI assay (flow cytometry) • DNA ladder assay • Western blot	particles were more toxic) • 21 nm SNPs induced ROS generation, lipid peroxidation and GSH depletion in a dose-dependent manner • 21 nm SNPs induced apoptosis in a dose-dependent manner	
Amorphous	4-40 nm (mean size: 14)	Not specified	HDMEC	• MTS assay • transmission electron microscopy (TEM) • Ki67 expression and IL-8 release	• The particles were internalized but they did not exert cytotoxic effects • Reduction of the proliferative activity and a pro-inflammatory stimulation were observed	[117]
Amorphous (monodisperse)	14, 15, 16, 19, 60, 104, 335 nm	• Particle preparation and stability • shape and size distribution • surface area (196, 179, 183, 145, 33, 28 and 7.7 m^2/g for 14, 15, 16, 19, 60, 104 and 335 nm particle, respectively)	EAHY926	• MTT and LDH assays • Annexin V-PI assay	• Cytotoxicity was dose- and size-dependent (smaller particles were more toxic and affected the exposed cells faster) • Cell death predominantly caused by necrosis	[118]

Silica form	Size (primary)	Material characterization	Cells used	Test	Biological endpoints and findings	Ref
		• micropore volume • Hydrodynamic particle size (in water and cell culture medium)				
Amorphous	21 and 48 nm	• Size distribution analysis • Surface area (225 and 106 m^2/g for 21 and 48 nm particle, respectively) • structure	H9c2(2-1)	• MTT and LDH assays • Hematoxylin and eosin staining • DCFH, intracellular MDA and GSH assays • Flow cytometry (cell cycle) • Western blot	• Cytotoxicity was dose- time - and size-dependent (smaller particles were more toxic) • ROS generation in a dose-dependent manner; increased level of MDA and decreased concentration of GSH indicated oxidative stress • Cell cycle arrest in G1 phase • Dose-dependent expression of p53 and p21 for 21 nm SNP	[119]
Amorphous	From 20 nm to below 400 nm	• the dispersion characteristics (size, size distribution, size evolution) • zeta potential	3T3-L1	• comet assay	• No detectable genotoxicity (the results were independently validated in two separate laboratories)	[120]
Amorphous (monodisperse)	16, 60 and 104 nm	• Particle preparation and stability • shape and size	A549	• MTT assay • cytochalasin-B micronucleus assay (CBMN) alone or in	• Results suggest that non-cytotoxic doses of SNPs may be capable of	[122]

Silica form	Size (primary)	Material characterization	Cells used	Test	Biological endpoints and findings	Ref
		distribution • surface area (183, 33 and 28 m^2/g for 16, 60 and 104 nm particle, respectively) • micropore volume • Hydrodynamic particle size (in water and cell culture medium)		combination with FISH-centromeric staining • Alkaline Comet assay • Measurements of cell-associated silica (ICP-MS)	inducing slight chromosome breakage, loss and mitotic slippage, and at higher concentration possibly mitotic arrest.	
Amorphous (monodisperse)	from 2 up to 335 nm	• Particle preparation and stability • shape and size distribution • surface area (from 232 to 7.7 m^2/g) • micropore volume • Hydrodynamic particle size (in water and cell culture medium) • Zeta potential	J774 EAHY926 3T3 Human erythrocytes	• MTT and WST-1 assays • RBC hemolysis	• in murine macrophages, the cytotoxic response, after treatment with SNPs of 17 different sizes, increased with external surface area and decreased with micopore volume • in human endothelial cells and mouse embryo fibroblast the cytotoxicity increased with surface roughness and decrease in diameter • the hemolytic activity of SNPs in human erythrocytes increased with the diameter of	[141]

Silica form	Size (primary)	Material characterization	Cells used	Test	Biological endpoints and findings	Ref
					SNPs	
Amorphous	30 nm	• Provided by producer for primary partilcles (surface area: 165 m^2/g) • Hydrodynamic particle size (in PBS and cell culture medium) • Adsorption of proteins from the test media in the absence of cells	3T3 hT RAW264.7	• MTS assay • Uptake (flow cytometry) • DCFH assay • Lysosomal membrane integrity • Mitochodrial membrane potential • Apoptosis (caspase-3, and caspase-7 activation; Annexin V-PI assay)	• SNPs depleted serum proteins from cell culture media • SNPs cytotoxicity was dose-, time- and cell line dependent-dependent • SNPs induced significant ROS generation in all cell lines • No detectable destabilization of lysosomal membranes was observed • Incubation with SNPs decreased mitochodrial membrane potential in hT and RAW cells • SNPs triggered different extent of cell apoptosis depending on the cell line tested	[140]
Amorphous (mesoporous)	110 nm (pore diameter of ~2.5 nm)	• Structure • surface area (910 m^2/g) • pore volume • stability in	3T3-L1 MCF-7 K562	• Confocal microscope • TEM • Flow cytometry	• Particles were internalized into cells and accumulated in cytoplasm • No apparent cytotoxicity	[123]

Silica form	Size (primary)	Material characterization	Cells used	Test	Biological endpoints and findings	Ref
		aqueous solution				
Amorphous (mesoporous)	Not specified (MCM-41 particle type)	• Synthesis and functionalization of particles • Zeta potential • Cylindrical pores with a diameter around 5 nm	HeLa	• MTT, WST-1 and LDH assays • Flow cytometry for PI • TEM observations	• No cytotoxicity was observed up to 50 □g/ml • Particles interfered with MTT assay	[126]
Amorphous (mesoporous)	108, 110, 111 and 115 nm	• Synthesis (ref to the previous study) and surface modification • Zeta potential • Surface area (780, 980, 930 and 1050 m^2/g for 108, 110, 111 and 115 nm particle, respectively) • pore volume and pore size distribution (2.6-2.0 nm)	hMSCs 3T3-L1	• MTT assay • Flow cytometry for the uptake • Cellular differentiation and cytochemical assay	• The modulation of surface charge and its threshold affects the uptake and is specific to cell type • Positive correlation of positive surface charge and the uptake by the cells • Uptake was through clathrin and actin-dependent endocytosis • Uptake did not affect cells viability, proliferation and differentiation	[125]
Amorphous (mesoporous silica nanorods capped with iron oxide NPs)	200 × 80 nm (pore diameter of ~3 nm)	• Preparation and functionalization	HeLa	• Confocal fluorescence microscopy	• Particles were endocytosed by the cells and biocompatible (concentration used: 0.2 mg/mL)	[127]

Silica form	Size (primary)	Material characterization	Cells used	Test	Biological endpoints and findings	Ref
Amorphous (mesoporous)	30, 50, 110, 170 and 280 nm	• Synthesis, suspension stability (no interparticle aggregation), hydrodynamic diamaters, zeta potential	HeLa	• MTT • onfocal laser scanning microscopy • ICP-MS	• Cellular uptake is highly particle size-dependent (with the optimum size of 50 nm); little cytotoxicity up to 100 mg/ml	[128]
Amorphous (mesoporous) loaded with anticancer drugs)	<130 nm (pore diameter of ~2 nm)	• Preparation, shape, aggregation/stability in aqueous solution	PANC-1 AsPC-1 Capan-1 MKN45 SW480	• Fluorescence and confocal microscopy	• The particles offer the possibility of controlled release of anticancer drugs (non-loaded particles did not caused cytotoxicity)	[129]
Amorphous (mesoporous)	150 nm (pore diameter of ~2.4 nm)	• Synthesis, functionalization, surface area (850 m^2/g), zeta potential	HeLa	• Flow cytometry • Fluorescence microscopy	• Uptake of particles can be regulated by different surface functionalization • More negatively charged particles were able to escape from endosomes	[130]
Amorphous (mesoporous) Commercially available amorphous silica material	100 - 300 nm (pore diameter of ~3 nm) -	• Synthesis (ref. to the previous study), funcionalization, surface area (1138 m^2/g), pore volumes, number of silanol group • Funcionalization	Rabbit RBCs	• Hemolysis assay • UV/Vis spectroscopy • Flow cytometry	• The hemolytic activity of silica nanoparticles depends only on the concentration of negatively charged silanol groups • Mesoporous particles exhibit a high compatibility towards RBCs as most of the	[131]

Silica form	Size (primary)	Material characterization	Cells used	Test	Biological endpoints and findings	Ref
					silanols are located in the interior of the particles that are not accessible by the RBCs membranes	
Amorphous (mesoporous)	300-650 nm (pore diameter of 31Å) and SBA-15 type (>hundreds of nm, pore diameter of 55 Å)	• Synthesis, • Order of mesostructures, surface area (821 and 506 m^2/g), wall thickness, composition	HL-60 Jurkat	• Oxygen consumption assay • ATP formation assay • Cellular GSH assay	• Particles with larger size and larger pores caused concentration- and time dependent inhibition of cellular respiration • Both nanoparticles were toxic to the isolated mitochondria • No significant changes in cellular glutathione level was observed	[132]
Amorphous (mesoporous and silica nanospheres)	250 nm; 166x320 nm (pore diameter = 3.5 nm)	• Synthesis and functionalization • Number of particles per gram, surface area (4.1 and 0.2 m^2/particle for mesoporous and spherical particle, respectively)	SK-N-SH	• Staining with trypan blue and determination of viable cells using a hemacytometer	• The cytotoxicity of particles was related to the adsorptive surface area of the particle (the most toxic malodorous silica are those with the largest BET surface areas) • Dependency of cytotoxicity on the nature of the	[133]

Silica form	Size (primary)	Material characterization	Cells used	Test	Biological endpoints and findings	Ref
					attached functional groups cannot be ruled out	
Amorphous (mesoporous)	270 ± 50 nm (pore diameter of 3.9 nm) and 2.5 □m ± 500 nm (pore diameter of 2.8 nm)	• Synthesis • The structural and textural characterizations • Surface area(520 and 547 m^2/g for 270 nm and 2.5 □m particle, respectively) • LPS concentration analysis	Human monocyte-derived dendritic cells	• Apoptosis/necrosis (Annexin V/PI assay) • production of cytokinesIL-10 and IL-12p70,IL-12, IL-10 • confocal microscopy, TEM	• Viability, uptake and immune regulatory markers were affected with increasing size and dose	[134]
Amorphous (mesoporous)	190, 420 and 1220 nm	• Synthesis and functionalization • Size distribution • Dispersity and porosity • Surface area (220-650 m^2/g) • Zeta potential	MDA-MB-468 COS-7	• MTT assay • The biodegradation experiments • Intracellular localization of particles	• The cytotoxicity of particles was highly correlated with particle sizes ((smaller particles were more toxic) • The biodegradation products of spherical E-MS particles showed no toxicity • The residual surfactant bound to the particles has a much smaller contribution to the cytotoxicity than the free one • The smaller particles were more easily	[135]

Silica form	Size (primary)	Material characterization	Cells used	Test	Biological endpoints and findings	Ref
					endocytosed and consequently located within lysosomes	
Amorphous	100 and 200 nm	• rod-shaped and spherical particles (Stöber), not-coated and coated with fibronectin or polyethylene glycol (PEG), • Primary and aggregate size, surface area (9.2 and 4.6 m^2/g for silica rods and 27.3 and 14.2 m^2/g for silica spheres), crystallinity, impurities, zeta potential	MET-5A	• LDH assay • Expression of IL-8 • Simulated stretch imposed on the cells	• Dosimetric comparison of acicular and isotropic particulate materials is not straightforward • In the absence of simulated lung function (stretch), cells showed no significant enhancement of cytotoxicity or inflammation release • PEG surface treatment tended to reduce the cytotoxicity and IL-8 release from particle exposures suggesting the significance of adhesive interactions e.g. for membrane binding/signal transduction	[136]
Amorphous	130 nm and 155 nm; iron oxide particle with silica shell (80 nm)	• Size distribution • Reference given for the description in detail	Hmy2 Jurkat U937 PC3;	• MTT assay and Trypan Blue exclusion • Scanning electron microscopy • DCFH assay	• The cytotoxicity of particles depended on the cell type tested • No direct correlation	[137]

Silica form	Size (primary)	Material characterization	Cells used	Test	Biological endpoints and findings	Ref
			human peripheral blood cells		between ROS production and cell toxicity. • PEG-ylation of SNP protected the particles from protein adsorption on the external surface of the NPs and consequently no agglomeration in culture medium was observed. • The availability of the particles to be internalized by the cells depended on the size and morphology of the aggregates.	
Crystalline	Particle sizes not uniform (7.21, 9.08 and 123.21 nm)	• Size and concentration	WIL2-NS	• MTT assay • Population Growth Assay • Apoptosis Assay by Flow Cytometry • Cytokinesis Block Micronucleus Assay • Comet Assay • HPRT Mutation Assay	• Significant dose-dependent decrease in viability • with increasing dose of particles • Fourfold increase in micronucleated binucleated cells frequency was detected, while no significant difference was measured by the Comet assay	[99]

Chen and von Mikecz [100] investigated the effects of nanoparticles on structure, function, and proteasomal proteolysis in the cell nucleus by incubating different cell lines with unlabeled and fluorescently labeled **amorphous silica** particles of different sizes [100]. SiO_2 particles between 40 nm and 5 □m were applied to epithelial cells in culture and observed on confocal laser scanning microscopy with differential interference contrast. Particles of all tested sizes penetrated the cytoplasm; however, nuclear localization was observed exclusively in cells treated with SiO_2 nanoparticles between 40 and 70 nm. Fine and coarse SiO_2 particles (0.2-5 □m) were exclusively located in the cytoplasm and accumulated around the nucleus, forming nuclear indentations. The uptake of SNPs in the nucleus induced aberrant clusters of topoisomerase I and protein aggregates in the nucleoplasm -- the former inhibiting replication, transcription, and cell proliferation -- without altering cell viability. Cells treated with fine (0.5 □m) or coarse (5 □m) SiO_2 particles had the same replication and transcription activity as that of untreated control cells [100].

Jin et al. [101] investigated the potential toxicity of luminescent **amorphous** SNPs (50 nm) in freshly isolated rat alveolar macrophage cells and human lung epithelial cells (A549 cells). The SNPs penetrated the cells but were not detected in the nuclear region and did not cause significant toxic effects at the molecular and cellular levels below a concentration of 0.1 mg/ml.

Lin et al. [102] investigated the cytotoxicity of **amorphous (colloidal)** SNPs (15 and 46 nm) in cultured human alveolar epithelial cells (A549 cells). Cell viability decreased in a time- and dose-dependent manner (down to 100 □g/ml), and nanoparticles of both sizes were more cytotoxic than were fine quartz particles (Min-U-Sil 5). Exposure to 15-nm SNPs generated oxidative stress in A549 cells as reflected by reduced glutathione (GSH) levels, elevated production of malondialdehyde (MDA) and lactate dehydrogenase (LDH) leakage, which is indicative of lipid peroxidation and membrane damage, respectively [102]. In the study by Wottrich et al. [103], A549 cells and macrophages (THP-1, Mono Mac 6) exposed to 60 nm **amorphous** SNPs showed distinctly higher mortality than did larger silica particles (diameter 100 nm). Another study by Choi et al. [104], involving A549 cells and **amorphous** SNPs (14 nm), showed a pro-inflammatory response triggered by nanoparticles without blocking cell proliferation or causing cell death to any great extent. A recent work by Akhtar et al. [105] examined cytotoxicity (by MTT and LDH assay) and oxidative stress (ROS levels, membrane lipid peroxidation, GSH level and activity of GSH metabolizing enzymes) in A549 cells exposed for 48 h to **amorphous** SNPs of 10 and 80 nm. The SNPs were cytotoxic to studied cells through oxidant generation (ROS and membrane lipid peroxidation) rather than depletion of GSH. Eom and Choi [106] studied oxidative stress caused by **amorphous** SNPs (7 and 5-15 nm) in human bronchial epithelial cells (BEAS-2B) and observed the formation of ROS and induction of antioxidant enzymes.

Shi et al. [107] exposed A549 cells to **amorphous** SNPs (10-20 nm) at concentrations up to 200 □g/ml and observed low cytotoxicity as measured by MTT and LDH assays. However, co-treatment with the same nanoparticles and lipopolysaccharide, a bacterial product that may contaminate (nano)materials, significantly enhanced the cytotoxicity.

Yu et al. [108] examined the cytotoxic activity (by MTT and LDH assay) of well-dispersed **amorphous silica** particles (30-535 nm) in mouse keratinocytes. All sizes of particles were taken up into the cell cytoplasm; nuclear uptake was not studied. The toxicity was dose and size dependent, with 30- and 48-nm particles being more cytotoxic than 118-

and 535-nm particles. The reduced GSH level significantly decreased only after exposure to 30-nm nanoparticles [108]. Nabeshi et al. [109] showed the size-dependent cytotoxic effects of **amorphous silica** particles (70, 300 and 1000 nm) on mouse epidermal Langerhans cells. The smallest particles induced greater cytotoxicity (by LDH assay) and inhibited cellular proliferation (by [^{3}H]-thymidine incorporation). The observed effects were associated with the quantity of particle uptake into the cells.

Yang et al. [110] evaluated the effects of **amorphous** SNPs (15 and 30 nm) and micron-sized silica particles on cellular viability, cell cycle, apoptosis and protein expression in the human epidermal keratinocyte cell line HaCaT. Microscopy examination revealed morphological changes after 24-h exposure; cell growth also appeared to be significantly inhibited. The cellular viability of HaCaT cells was significantly decreased, and the amount of apoptotic cells was increased in a dose-dependent manner after treatment with nano- and micron-sized SiO_2 particles. Furthermore, smaller silica particles were more cytotoxic and induced a higher apoptotic rate. Proteomic analysis revealed differential induction of expression of 16 proteins by SiO_2 exposure; proteins were classified into 5 categories according to their functions: oxidative stress-associated proteins, cytoskeleton-associated proteins, molecular chaperones, energy metabolism-associated proteins, and apoptosis and tumor-associated proteins. The expression levels of the differentially expressed proteins were associated with particle size [110]. In a recently published study [111], the same research group used these SNPs to study the global DNA methylation profiles in HaCaT cells; the authors reported that nanosilica treatment can induce epigenetic changes.

Cousins et al. [112] exposed murine fibroblasts to small **amorphous (colloidal) silica** particles (7, 14 and 21 nm) over a long incubation period (1, 3 and 7 days and up to 7 weeks) and observed a distinctive cellular response affecting the morphologic features, adhesion and proliferation of the fibroblasts but not cell viability. Chang et al. [113] exposed selected human fibroblast and cancer cell lines for 48 h to **amorphous** SNPs and assessed cellular viability by MTT and LDH assays. Cytotoxicity was seen at concentrations > 138 □g/ml and depended on the metabolic activity of the cell line. However, the average primary size of tested silica particles was 21 and 80 nm, but their average hydrodynamic particle size was 188 and 236 nm, respectively, so in media, aggregates/agglomerates were formed.

In the study of Yang et al. [114], cell membrane injury induced by 20-nm **amorphous silica** nanoparticles in mouse macrophages was closely associated with increased intracellular oxidative stress, decreased membrane fluidity, and perturbation of intracellular calcium homeostasis.

Besides inhalation, ingestion is considered a major uptake route of nanoparticles into the human body [3]; however, the possible harmful effects of engineered nanoparticles in the gastrointestinal tract are still largely unknown. Recently, Gerloff et al. [115] investigated the cytotoxic and DNA damaging properties of **amorphous** fumed SiO_2 nanoparticles (14 nm) in the human colon epithelial cell-line Caco-2. Exposure to SNPs for up to 24 h caused cell mortality, significant DNA damage and total glutathione depletion. The results of an *in vivo* study of mice fed nanosized silica are discussed in section 3.2.2.

Ye et al. [116] reported on induced apoptosis in a human hepatic cell line after exposure to **amorphous (colloidal)** SNPs (21, 48 and 86 nm). The viability of cells was assessed

by LDH and MTT assay; oxidative stress was studied by measurement of ROS, lipid peroxidation and GSH concentration; and apoptosis was quantified by annexin V/propidium iodide staining and DNA ladder assays. Nano-SiO_2 caused cytotoxicity in a size-, dose- and time-dependent manner.

Because nanoparticles are probably distributed by the blood stream (e.g., with medical applications), endothelial cells would also come in direct contact with these particles, for pathogenic particle-endothelial interactions. Peters et al. [117] evaluated the effects of 4- to 40-nm **amorphous SiO_2** particles *in vitro* on human dermal microvascular endothelial cell function and viability. The particles were internalized but did not exert cytotoxic effects (MTS assay). However, cells showed impaired proliferative activity and pro-inflammatory stimulation. Napierska et al. [118] reported a dose-dependent cytotoxicity (by MTT and LDH assay) of **monodisperse amorphous** SNPs (16-335 nm) in a human endothelial cell line. The toxicity of the particles was strongly related to particle size; smaller particles showed significantly higher toxicity and also affected the exposed cells faster. Ye et al. [119] evaluated the toxicity of **amorphous** SNPs (21 and 48 nm) towards rat myocardial cells. Exposure to the SNPs for up to 48 h resulted in size-, dose- and time-dependent cytotoxicity, smaller particles again showing higher toxicity.

Barnes et al. [120] reported no detectable genotoxic activity (by Comet assay) of **amorphous** SNPs (20 nm to < 400 nm) in 3T3-L1 fibroblasts at 4 or 40 □g/ml silica for 24 h. The particle dispersions were carefully characterized and the results were independently validated in 2 separate laboratories. In a recent review, Gonzalez et al. [121], in a literature review, compared 2 genotoxicity tests -- the alkaline Comet assay and the micronucleus test - in terms of chemical composition and size of engineered SNPs: engineered SNPs did not seem to induce DNA strand breakage. However, when **monodisperse amorphous** SNPs of 3 different sizes (16, 60 and 104 nm) were selected to assess the genotoxic potential of these particles in A549 lung carcinoma cells with a well-validated assay (the *in vitro* cytochalasin-B micronucleus assay), at non-cytotoxic doses, the smallest particles showed an apparently higher-fold induction of micronucleated binucleated (MNBN) cells [122]. When considering the 3 SNPs together, particle number and total surface area accounted for MNBN induction because they were significantly associated with the amplitude of the effect.

Crystalline nanosilica

Wang et al. [99] investigated cytotoxicity (by MTT assay) and genotoxicity of ultrafine **crystalline SiO_2 particulates** (UF-SiO_2) in cultured human lymphoblastoid cells. A 24-h treatment with 120 □g/ml UF-SiO_2 produced a fourfold increase in MNBN cells, with no significant difference as measured by the Comet assay. However, the ultrafine crystalline silica used was extracted from commercially available crystalline silica and the particle sizes were not uniform [99].

Mesoporous silica

The cytoxicity of **amorphous mesoporous SNPs** (**MSNs**) was recently studied intensively because they are promising materials for drug delivery systems and cell markers [8, 123, 124]. Several studies have demonstrated that efficient cellular uptake of MSNs could be achieved at concentrations < 50 □g/ml, with no cytotoxic effects observed up to 100 □g/ml in different mammalian cells [125, 126, 127, 128, 129, 130]. Lu

et al. [128] reported on the optimal size of ~50 nm MSNs for cell uptake. Slowing et al. [131] reported that, contrary to the known cytotoxicity of amorphous SNPs toward red blood cells, **mesoporous** SNPs exhibit high biocompatibility at concentrations adequate for potential pharmacological applications.

However, studies have reported cytotoxicity of mesoporous silica nanomaterials. Tao et al. [132] investigated the effects of two types of **MSNs** (pore diameters of 31 and 55 Å) on cellular bioenergetics (cellular respiration and ATP content) in myeloid and lymphoid cells and isolated mitochondria. Only cells exposed to MSNs with larger size and larger pores showed concentration- and time-dependent inhibition of cellular respiration, and both nanoparticles were toxic to the isolated mitochondria. Di Pasqua et al. [133] reported that the toxicity of **MSNs** towards human neuroblastoma cells was related to the adsorptive surface area of the particle. However, the nature of the functional groups playing a role could not be excluded. Vallhov et al. [134] investigated the effects of **mesoporous** SNPs of different sizes (270 nm and 2.5 □m) on human dendritic cells and found viability, uptake and immune regulatory markers affected by increasing size and dose. He et al. [135] evaluated the influence of size and concentration of **mesoporous** SNPs (190, 420 and 1220 nm) on cytotoxicity in human breast cancer cells and monkey kidney cells. The cytotoxicity of the particles was associated with particle size: silica of 190 and 420 nm in diameter showed significant cytotoxicity at concentrations > 25 □g/ml; whereas particles of 1220 nm in diameter showed slight cytotoxicity at 480 □g/ml. The smaller particles were suggested to be more easily endocytosed and consequently located within lysosomes [135].

Surface-modified/functionalized silica

Brown et al. [136] attempted to evaluate the role of shape in particle toxicity in the lung; the authors compared the response of rod-shaped and spherical **amorphous silica particles** (Stöber), not coated or coated with fibronectin or polyethylene glycol (PEG), under stretched and static conditions. The dosimetric comparison of materials with different shapes (e.g., needle-shaped or acicular and isotropic) was not straightforward. Non-coated particles induced an increase in IL-8 and LDH release, whereas a surface modification with PEG mitigated this effect, which suggested the significance of adhesive interactions for membrane binding/signal transduction, for example [136].

Diaz et al. [137] described the interactions of two **amorphous** silica particles - a pristine particle, without any coating, and PEGylated silica particles (average size 130 and 155 nm), as well as an iron oxide particle with a silica shell (80 nm) -- with different human peripheral blood cells, several human tumor cell lines and mouse peritoneal macrophages. The effects depended on the cell analyzed: although all particles were phagocytosed and were able to induce ROS expression in mouse macrophages, they differentially affected the human cell lines and peripheral blood cells, both in terms of internalization and ROS induction. The availability of the particles to be internalized by the cells seemed to strongly depend on aggregation, especially on the size and morphology of the aggregates [137].

Almost all of the existing cytotoxicity studies of SNPs involved monocultures of cells that are organ specific. The exception is the study by Wottrich et al. [103], in which co-cultures of epithelial cells (A549) and macrophages (THP-1, Mono Mac 6) exposed to 60- and 100-nm amorphous SNPs showed an increased sensitivity to the cytokine release as compared with monocultures of each cell type. The enhanced responses to nanoparticles

in different contact and non-contact co-cultures were reported in studies by Herseth et al. [138, 139] with micron-sized crystalline silica, showing that more realistic models should be applied to study interactions between nanoparticles and cells or organs of interest.

Few recently published studies have systematically investigated nanomaterial properties in terms of the degree and pathways of cytotoxicity. Sohaebuddin et al. [140] selected nanomaterials of different composition, including silica, to analyze the effects of size and composition on 3 model cell lines: fibroblasts, macrophages and bronchiolar epithelial cells. The authors concluded that the physico-chemical properties of size and composition both determined the cellular responses and induced cell-specific responses. In another recent study, Rabolli et al. [141] studied the influence of size, surface area and microporosity on the *in vitro* cytotoxic activity of a set of 17 stable suspensions of **monodisperse amorphous** SNPs of different sizes (2-335 nm) in 4 different cell types (macrophages, fibroblasts, endothelial cells and erythrocytes). The response to these nanoparticles was governed by different physico-chemical parameters that varied by cell type: in murine macrophages, the cytotoxic response increased with external surface area and decreased with micopore volume; in human endothelial cells and mouse embryo fibroblasts, the cytotoxicity increased with surface roughness and decrease in diameter; and in human erythrocytes, the hemolytic activity increased with the diameter of the SNP [141].

Overall, most of these *in vitro* studies involving different SNPs documented the cytotoxic effects of these nanomaterials. The determinants of the observed cytotoxicity seem to be complex and vary with the particles used and cell type tested. Unfortunately, for many published studies, adequate material characterization is still missing. The mere cytotoxicity reported with some particles does not strictly imply hazard. However, this observation indicates that a proactive development of nanomaterials should consider physical, chemical and catalytic properties of nanoparticles.

In vivo studies of nanosilica toxicity

Along with particle size, surface area and particle number appear to be integral components contributing to the mechanisms of lung toxicity induced by nano-sized particles. The high deposition rate of ultrafine particulates is a result of a small aerodynamic diameter and is assumed to be important in the lung inflammatory process. Some evidence suggests that inhaled nanoparticles, after deposition in the lung, largely escape from alveolar macrophage clearance and gain greater access to the pulmonary interstitium via translocation from alveolar spaces through epithelium [3, 142]. A summary of the *in vivo* responses to SNPs can be found in Table 3.

Table 3

In vivo studies on nanosilica particles (SNPs) toxicity

Silica form	Size (primary)	Material characterization	Exposure model	Test	Biological endpoints and findings	Ref
Quartz	10-20 nm (average size: 12), 30-65	• Synthesis	Rats instilled intratracheally with various particle types (1	• Bronchoalveolar lavage (BAL) fluid analysis: cell counts, differentials, and pulmonary biomarkers	Exposures to the various quartz particles produced differential degrees of	[148]

Silica form	Size (primary)	Material characterization	Exposure model	Test	Biological endpoints and findings	Ref
	(average size: 50), 300 nm - 2 □m	• Surface area • Crystallinity • Metal impurities	or 5 mg/kg), sacrificed at 24 h, 1 week, 1 month, and 3 months post-exposure	(Lactate dehydrogenase (LDH), alkaline phosphatase (ALP), and lavage fluid protein) • Cell proliferation • Morphological/Histopathology examination • Hemolytic Potential of particles	pulmonary inflammation and cytotoxicity, which were not consistent with particle size but correlated with surface activity, particularly hemolytic potential.	
Silica dust	10 ± 5 nm; and 0.5-10 □m (80% of the particles 1-5 □m)	• Composition uknown • Surface area	Rats instilled intratracheally (20 mg), sacrificed 1 and 2 months after dosing	• The changes of lung/body coefficient and hydroxyproline content • Pathologic examination • Immunohistochemical staining for IL-4 and TGF-beta1	One month after instillation cellular nodules (Stage I silicosis) were found in the nanosized SiO_2 group, while in microsized SiO_2 group Stage II, II+ of silicotic nodules were observed. Two months after instillation, still only Stage I silicotic nodules in nanosilica group were found, while in the micro-silica group the disease progressed and Stage II+, and III silicotic nodules were found. The experiment revealed that in rats the effect of fibrogenesis of nano-SiO_2 might be milder than that of micro-SiO_2.	[147]
Ludox colloidal silica	-	• Mass median aerodynamic diameter (2.9, 3.3	Rats Inhalation (nose-only) for 2 or 4 weeks at	• Lung silica analysis • BAL analysis: cell differential	The inflammatory responses, mainly seen as increased	[143]

Silica form	Size (primary)	Material characterization	Exposure model	Test	Biological endpoints and findings	Ref
		and 3.7 □m) • Chamber Ludox concentration	concentrations 10, 50 and 150 mg/m³. Additional groups of rats exposed for 4 weeks were given a 3-month recovery period	counts and biochemical assay (LDH, ALP, lavage fluid protein) • Pulmonary macrophage cell culture and phagocytosis assay • SEM ananlysis • Additional groups of animals were processed for cell labeling studies or lung deposition studies.	numbers of neutrophils in BALF, following the 2 and/or 4 weeks of exposure was evident at 50 mg/m³ (or higher) group. Three months after exposure most biochemical parameters returned to control values. Results showed that exposures to 150 mg/m3 Ludox for 2 or 4 weeks produced pulmonary inflammation along with increases in BAL protein, LDH, and alkaline phosphatase values (p less than 0.05) and reduced macrophage phagocytosis. Autoradiographic studies demonstrated that the labeling indices of terminal bronchiolar and lung parenchymal cells were generally increased in the 50 and 150 mg/m3 groups after 2 and 4 weeks of exposure but, with one exception, returned to normal levels following a 3-month postexposure period.	
Aerosol containing colloidal silica	Average size: 22 nm	• Mass median aerodynamic diameter (2.9, 3.3 and 3.7 □m)	Rats inhalation (from 10 to 150 mg/m³), 6 h/day, 5 days/week for 4	• Lung silica determination • Body weights and clinical	No effects after exposure to the lowest concentration Lung weights were	[52]

Silica form	Size (primary)	Material characterization	Exposure model	Test	Biological endpoints and findings	Ref
		• Chamber Ludox concentration	weeks; 3 months postexposure	observations • Clinical pathology (urine and blood samples) • Histopathology	increased significantly after 4 exposure to 50 and 150 mg/m^3. A dose dependent alveolar macrophage response, polymorphonuclear leukocytic infiltration, and Type II pneumocyte hyperplasia in alveolar duct regions was reported. Lung-deposited nanosilica cleared rapidly from the lungs with half-times of approximately 40 and 50 days for the 50 and 150 mg/m^3 groups, respectively. The lungs did not show fibrotic scar tissue formation or alveolar bronchiolarization.	
Colloidal silica	(UFCSs, average size of 14 nm) fine colloidal silica particles (FCSs; average size of 213 nm)	• Size distribution • Surface area • Metal composition	Mice instilled intratracheally (3 mg) and sacrificed 0.5, 2, 6,12 and 24 h after dosing	• Histopathology • Immunohistochemistry • Electron microscopy	Histopathological examination revealed for both sizes bronchiolar degeneration, necrosis, neutrophilic inflammation, alveolar type II cell swelling and alveolar macrophage accumulation. UFCs induced extensive alveolar hemorrhage, a more severe bronchiolar epithelial cell necrosis and neutrophil influx in alveoli compared to	[98]

Silica form	Size (primary)	Material characterization	Exposure model	Test	Biological endpoints and findings	Ref
					FCSs. Electron microscopy demonstrated UFCSs and FCSs on bronchiolar and alveolar wall surface as well as in the cytoplasm of alveolar epithelial cells, alveolar macrophages and neutrophils. The findings suggest that UFCSs (possibly linked to larger surface area) have greater ability to induce lung inflammation and tissue damages than FCSs.	
Colloidal silica	average size: 14 nm	• Size distribution • Surface area • Metal composition	Mice instilled intratracheally (0.3,3,10,30 or 100 □g) and sacrificed 3 days after dosing; 1 to 30 days postexposure	• BAL analysis: cells quantification, viability and differentiation, total protein concentration • Histopathology • Immunohistochemistry • Apoptosis (TUNEL assay)	Exposure up to 100 □g of UFCSs produced moderate to severe pulmonary inflammation and tissue injury 3 days post exposure. Mice instilled with 30 □g of UFCSs and sacrificed at intervals from 1 to 30 days post-exposure showed moderate pulmonary inflammation and injury on BALF indices at acute period; however, these changes gradually regressed with time. Histopathological and immunohistochemical examination correlated to BALF	[146]

Silica form	Size (primary)	Material characterization	Exposure model	Test	Biological endpoints and findings	Ref
					data. A significant increase of the apoptotic index (TUNEL) in lung parenchyma at all observation times was reported. The findings suggest that instillation of a small dose of UFCSs caused an acute, but transient, lung inflammation and tissue damage in which oxidative stress and apoptosis may be involved.	
Amorphous silica	14 nm	• Endotoxins content	Mice instilled intratracheally (2,10 and 50 mg/kg) and sacrificed 24 h, 1,4 and 14 weeks after dosing	• BAL analysis: total protein and endotoxin concentration, cell differential counts • Histopathology • Real-time PCR • Immunohistochemistry	Significantly increased lung weights, total BAL cells and proteins were observed until 1 week after treatment. Particles induced acute inflammation (with neutrophils) at an early stage and chronic granulomatous inflammation at the later stage. The significant up-regulation of cytokines (IL-1□, IL-6, IL-8, and TNF-□) and chemokines (MCP-1 and MIP-2) was observed during the early stages, but there were no changes after week 1.	[144]

Silica form	Size (primary)	Material characterization	Exposure model	Test	Biological endpoints and findings	Ref
					In conclusion, Instillation of nanoparticles induced transient but very severe lung inflammation.	
Amorphous silica	37.9 ± 3.3 nm	• Size distribution • Surface area • Particle number	Rats inhalation (24.1 mg/m^3, 40 min/day, 4 weeks The age factor involved 3 levels (young/ adult/old)	• Electrocardiography • BAL analysis • Hemorheological analysis • Serum biomarker assay • Pathology	Inhalation of SNP under identical conditions caused the strongest pulmonary and cardi ovascular alterations in old rats, yet less change in young and adult rats. Observed changes included pulmonary inflammation, myocardial ischemic damage, atrio-ventricular blockage, and increase in fibrinogen concentration and blood viscosity. Old individuals were more sensitive to nanoparticle exposure than the young and adult rats. The risk of causing pulmonary damages was: old > young > adult. The risk of cardiovascular disorder was observed only in old age.	[145]
Amorphous silica	37 nm and 83 nm	• The generation of nanosilica aerosol • Size distribution	Rats inhalation (3.7 × 10^7 or 1.8 × 10^8 particles/cm^3), 6h/day, for 1- or	• Bal analysis: cell counts, differentials, enzymatic activity of LDH,and ALP • Genotoxicity endpoints (micronuclei induction)	One- or three-day aerosol exposure produced no significant pulmonary inflammatory, genotoxic, or adverse lung histopathological	[149]

Silica form	Size (primary)	Material characterization	Exposure model	Test	Biological endpoints and findings	Ref
			3-days several post-exposure time points (up to 2 months)		effects in rats exposed to very high particle numbers corresponding to a range of mass concentrations (1.8 or 86 mg/m^3).	
Amorphous silica	14 nm	o Daily mean mass median aerodynamic diameter (2.1 ± 0.1 □m)	Rats inhalation (head/nose only; 26.9 ± 3 mg/m^3), 6h/day during 6 days); Challenging the animals by inhalation to a minimally irritating concentration of allergen trimellitic anhydride (TMA)	• Breathing parameters • Cellular and biochemical changes in BAL • Histopathological airway changes	Exposure to SNPs alone resulted in transient changes in breathing parameters during exposure, and in nasal and alveolar inflammation with neutrophils and macrophages. Exposure to particles before a single TMA challenge resulted in only a slightly irregular breathing pattern during TMA challenge. Pre-exposure to particles also diminished the effect of TMA on tidal volume, laryngeal ulceration, laryngeal inflammation, and the number of BAL eosinophils in most animals. When the additional group of animals was exposed to nanosilica before a second challenge to TMA, the pulmonary eosinophilic infiltrate and edema induced by a second TMA challenge in control animals was diminished by the preceding silica	[153]

Silica form	Size (primary)	Material characterization	Exposure model	Test	Biological endpoints and findings	Ref
					exposure, but the number of lymphocytes in BAL was increased.	
Amorphous silica	~30 nm and ~30 □m	• Size distribution	Feeding of mice for 10 weeks (total fed amount of 140 g/kg mice)	• Blood analysis • Cytological analysis of lungs and liver tissue sections • Analysis of silicon in organs	The nano-sized silica particle dieted group showed higher value of ALT (alanine aminotransferase) than normal and micron-sized silica dieted groups. H&E staining of the liver of the nano-sized particle dieted group indicated some fatty liver pattern. The contents of Si in the livers of the groups were almost the same.	[154]
Amorphous silica (organically modified)	20-25 nm	• Synthesis • Conjugation with fluorophore • Radiolabelling	Mice injected intravenously with SPN (2.0 mg/kg body weight)	• Fluorescence imaging (CRi) • MicroPET imaging • Histological Analysis	Greater acummulation of nanoparticles in liver, spleen and stomach that in kidney, heart and lungs. Almost 100% of the injected nanoparticles were effectively cleared out of the animals over a period of 15 days via the hepatobiliary excretion. No signs of organs toxicity were observed.	[155]
Amorphous (mesoporous) silica	150 nm, 800 nm and 4 □m (pore sizes of 3 nm, 7	• Synthesis • Size • Endotoxins content	Rats injected subcutaneously (30 mg per rat), Mice injected intraperitoneally and	• Hematoxylin and eosin staining and histological examination	When the particles were injected subcutaneously, the amount of residual material decreased progressively over 3	[156]

Silica form	Size (primary)	Material characterization	Exposure model	Test	Biological endpoints and findings	Ref
	nm and 16 nm)		intravenously		months, with good biocompatibility on histology at all time points. Intra-peritoneal and intra-venous injections in mice resulted in death or euthanasia. No toxicity was seen with subcutaneous injection of the same particles in mice. Microscopic analysis of the lung tissue of the mice indicated that death may be due to thrombosis.	
Amorphous silica	75, 311 and 830 nm	Not specified	Mice injected intravenously (10-100 mg/kg)	• H&E staining; histological analysis of the liver, kidney, spleen and lung • Biochemical assays • Gadolinium chloride, cyclophosphamide and hepatic hydroxyproline assay	70 nm SNP induced liver injury at 30 mg/kg body weight, while SP300 or 1000 had no effect even at 100 mg/kg. Administration of 70 nm SNP dose-dependently increased serum markers of liver injury, serum aminotransferase and inflammatory cytokines. Repeated administration of 70 nm SNP twice a week for 4 weeks, even at 10 mg/kg, caused hepatic fibrosis.	[157]
Amorphous silica	50, 100 and 200 nm	• Synthesis • Fluorescence	Mice injected intravenously (50 mg/kg)	• Confocal laser scanning microscopy	Significant increase of inflammation in the liver at 12 h for the	[158]

Silica form	Size (primary)	Material characterization	Exposure model	Test	Biological endpoints and findings	Ref
		labeling		• Immunofluorescence staining • Fluorescence microplate readings	100 and 200 nm silica nanoparticles treatment groups. The tissue distribution and excretion of the injected particles were different depending on particle size. As particle sizes increased, more particles were trapped by macrophages in the liver and spleen. All particles were cleared via urine and bile; however, the 50 nm silica nanoparticles excreted faster than the other two particles.	

In 1991, Warheit et al. [143] performed a rat inhalation study (nose-only) with an **aerosol of colloidal silica** (mass median aerodynamic diameter 2.9, 3.3 and 3.7 □m) for 2 or 4 weeks at concentrations up to 150 mg/m^3, and some groups of rats were allowed to recover for 3 months. The inflammatory responses, mainly seen as increased numbers of neutrophils in bronchoalveolar lavage fluid (BALF), with the 2 and/or 4 weeks of exposure were evident at ≥ 50 mg/m^3 concentration. Three months after exposure, most biochemical parameters returned to control values [143].

Lee and Kelly [52] studied the effects of repeated inhalation (6 h/day, 5 days/week for 4 weeks) of an **aerosol of colloidal silica** (mass median aerodynamic diameter 2.9, 3.3 and 3.7 □m; concentration up to 150 mg/m^3) in rats. The authors reported a dose-dependent alveolar macrophage response, polymorphonuclear leukocytic infiltration, and type II pneumocyte hyperplasia in alveolar duct regions. Lung-deposited nanosilica were cleared rapidly from the lungs, with half-times of approximately 40 and 50 days for the 50 and 150 mg/m^3 treatment groups, respectively. The lungs did not show formation of fibrotic scar tissue or alveolar bronchiolarization [52].

Cho et al. [144] investigated inflammatory mediators (24 h, and 1, 4 or 14 weeks after exposure) induced by intratracheal instillation in mice of up to 50 mg/kg of ultrafine **amorphous silica** with a primary particle diameter of 14 nm. The authors observed significantly increased lung weights, total cell numbers and levels of total protein in BALF up to 1 week after treatment. The histopathological examination revealed acute inflammation, with neutrophils and chronic granulomatous inflammation. The

expression of cytokines (IL-1□, IL-6, IL-8, and TNF-□) and chemokines (monocyte chemoattractant protein 1 and macrophage inflammatory protein 2) was significantly increased during the early stages, with no changes after week 1 [144].

Chen et al. [145] studied age-related differences in response to **amorphous** SNPs (average size 38 nm). Changes in serum biomarkers, pulmonary inflammation, heart injury and pathology were compared in young (3 weeks), adult (8 weeks) and old (20 months) rats that inhaled tested nanoparticles for 4 weeks (40 min/day). Old animals appeared to be more sensitive to nanoparticle exposure than were young and adult rats. The risk of pulmonary damage was old > young > adult, but the risk of cardiovascular disorder was observed only in old animals [145].

Kaewamatawong et al. [98] compared acute pulmonary toxicity induced in mice by ultrafine **colloidal silica** particles (UFCSs; average size 14 nm) or fine colloidal silica particles (FCSs; average size 213 nm) after intratracheal instillation of 3-mg particles. Histopathological examination with both sizes revealed bronchiolar degeneration, necrosis, neutrophilic inflammation, alveolar type II cell swelling and alveolar macrophage accumulation. However, UFCSs induced extensive alveolar hemorrhage, more severe bronchiolar epithelial cell necrosis and neutrophil influx in alveoli as compared with FCSs. Electron microscopy showed UFCSs and FCSs on the bronchiolar and alveolar wall surface and in the cytoplasm of alveolar epithelial cells, alveolar macrophages and neutrophils. The findings suggest that UFCSs (possibly linked to size and/or larger surface area) have a greater ability to induce lung inflammation and tissue damage than do FCSs [98]. The same research group reported acute and subacute pulmonary toxicity of low-dose UFCS particles in mice after intratracheal instillation [146]. Exposure of up to 100 □g UFCSs produced moderate to severe pulmonary inflammation and tissue injury 3 days after exposure. Mice instilled with 30 □g UFCSs and sacrificed at intervals from 1 to 30 days after exposure showed moderate pulmonary inflammation and injury on BALF indices at the acute period; however, these changes gradually regressed with time. Concomitant histopathological and laminin immunohistochemical results were similar to BALF data. The authors reported a significant increase in the apoptotic index (TUNEL) in lung parenchyma at all observation times. The findings suggest that instillation of a small dose of UFCSs causes acute but transient lung inflammation and tissue damage in which oxidative stress and apoptosis may be involved [146].

In a study of fibrogenesis, Wistar rats were intratracheally instilled with silica (of unknown composition) nano- (10 ± 5 nm) and microparticles (0.5-10 □m), and were sacrificed 1 and 2 months after dosing [147]. One month after instillation, cellular nodules (Stage I silicosis) were found in the nano-sized SiO_2 group, whereas more severe lesions were found in the micron-sized SiO_2 treatment group (Stage II and Stage II+ of silicotic nodules). One month later, the nano-sized SiO_2 group still showed only Stage I silicotic nodules, whereas the micron-silica group showed disease progression and Stage II+ and III silicotic nodules. Therefore, in rats, the effect of nano-SiO_2 on fibrogenesis might be milder than that of micron-SiO_2. Nanoparticles, because of their size, probably diffuse more easily to other pulmonary compartments than do microparticles [147].

Warheit et al. [148] compared the toxicity of synthetic **nanoquartz** particles (12 and 50 nm) to mined Min-U-Sil quartz (500 nm) and synthetic fine-quartz particles (300 nm) and (2) evaluated the surface activity (hemolytic potential) of the different samples in terms of toxicity. Rats were instilled with the different particle types (1 or 5 mg/kg), and

pulmonary toxicity was assessed with BALF biomarkers, cell proliferation, and histopathological evaluation of lung tissue at 24 h, 1 week, 1 month, and 3 months after exposure. Exposure to the quartz particles of different sizes produced pulmonary inflammation and cytotoxicity, with nanoscale quartz of 12 nm and Min-U-Sil quartz being more toxic than fine quartz and nanoscale quartz of 50 nm. The pulmonary effects were not consistent with particle size but were associated with surface activity, particularly hemolytic potential [148].

In a recent work by Sayes et al. [149], rats inhaled freshly generated aerosolized **amorphous** SNPs of 37 and 83 nm for a short-term period. In contrast to previous studies' measurements, particle number rather than particle mass was chosen as dose metrics (3.7×10^7 or 1.8×10^8 particles/cm^3) for 1- or 3-day exposure. Pulmonary toxicity (cell counts, differentials, enzymatic activity of LDH and alkaline phosphatase (ALP) in BALF) and genotoxicity endpoints (micronuclei induction) were assessed from 24 h up to 2 months after exposure. One- or 3-day aerosol exposure produced no significant pulmonary inflammatory, genotoxic or adverse lung histopathological effects in rats exposed to very high particle numbers in a range of mass concentrations (1.8 or 86 mg/m^3).

Recently, airway irritants were suggested to facilitate allergic sensitization [150, 151, 152]. Arts et al. [153] examined the effect of pre-exposure to synthetic (fumed) **amorphous** SNPs (14 nm) on elicitation of airway hypersensitivity reactions by the low-molecular-weight allergen trimellitic anhydride (TMA). Brown Norway rats were topically sensitized with TMA, exposed (head or nose only) to SNPs for 6 h/day for 6 days and then challenged by inhalation with a minimally irritating concentration of TMA. One day later, breathing parameters, cellular and biochemical changes in BALF, and histopathological airway changes were studied. Exposure to SNPs alone resulted in transient changes in breathing parameters during exposure and in nasal and alveolar inflammation with neutrophils and macrophages. Exposure to particles before a single TMA challenge resulted in only a slightly irregular breathing pattern during TMA challenge. Interestingly, pre-exposure to particles diminished the effect of TMA on tidal volume, laryngeal ulceration, laryngeal inflammation, and the number of BALF eosinophils in most animals. When an additional group of animals was exposed to nanosilica before a second challenge to TMA, the pulmonary eosinophilic infiltrate and edema induced by a second TMA challenge in control animals was diminished by the preceding silica exposure, but the number of lymphocytes in the BALF was increased. The authors concluded that SNPs could reduce as well as aggravate certain aspects of TMA-induced respiratory allergy [153].

As mentioned, next to inhalation, ingestion is considered a major route for the uptake of nanoparticles in the human body. So et al. [154] studied the effects on mice fed nano- and micron-sized **amorphous** silica particles (30 nm and approximately 30 □m, respectively). After feeding the animals for 10 weeks (total amount of 140 g silica/kg mouse), blood was tested biochemically and hematologically. The group fed SNPs showed higher serum values of alanine aminotransferase as compared with the other groups (both control and micron-silica treated). Although the contents of Si in the livers of the groups were almost the same, hematoxylin and eosin staining revealed a fatty liver pattern in the group treated with SNPs [154].

The successful use of nanoparticles in the clinic requires exhaustive studies on the behavior of these particles *in vivo*. Unfortunately, biocompatibility, biodistribution and

clearance studies of silica-based nanoparticles are sparse. Kumar et al. [155] used nanoparticles of organically modified **amorphous** silica (ORMOSIL; amino-terminated; 20-25 nm) to study biodistribution, clearance and toxicity in a mouse model. Particles conjugated with fluorophore and radiolabeled were injected systemically in mice. Biodistribution studies showed a greater accumulation of nanoparticles in liver, spleen and stomach than in kidney, heart and lungs. Over 15 days, almost 100% of the injected nanoparticles were effectively cleared out of the animals via hepatobiliary excretion, without any sign of organ toxicity. Hudson et al. [156] examined the biocompatibility of **mesoporous** silica particles (150 nm, 800 nm and 4 □m) after injection in rats and mice. When the particles were injected subcutaneously in rats, the amount of residual material decreased progressively over 3 months, with no significant injury to surrounding tissues. Subcutaneous injection of the same particles in mice produced no toxic effects. In contrast, intra-peritoneal and intra-venous injection in mice resulted in death; microscopic analysis of the lung tissue of the mice indicated that death might have been due to pulmonary thrombosis. Nishimori et al. [157] evaluated the acute toxicity of **amorphous** silica particles (70, 300 and 1000 nm) after a single intravenous injection in mice and reported that 70-nm silica injured the liver but not the spleen, lung or kidney. Moreover, chronic administration of 70-nm nanoparticles (injections every 3 days for 4 weeks) caused liver fibrosis. Cho et al. [158] examined the impact of the size of **amorphous** SNPs on toxicity, tissue distribution and excretion. Fluorescence dye-labeled 50-, 100- and 200-nm silica particles were intravenously injected in mice. The incidence and severity of inflammation with the 100- and 200-nm SNPs was significantly increased in the liver at 12 h; the 50-nm particles induced a slight but nonsignificant inflammatory response. The tissue distribution and excretion of the injected particles differed depending on particle size. With increasing particle size, more particles were trapped by macrophages in the liver and spleen. All particles were cleared via urine and bile; however, the 50-nm SNPs were excreted faster than were the other 2 particle sizes [158].

In *vivo* versus *in vitro*; amorphous versus crystalline

Park and Park [159] performed *in vitro* and *in vivo* studies to investigate oxidative stress and pro-inflammatory responses induced by **amorphous** SNPs (average primary size 12 nm). RAW 264.7 cells derived from mouse peritoneal macrophages were exposed to SNPs (5-40 ppm) *in vitro* and showed ROS generation and decreased intracellular GSH levels, as well as increased levels of nitric oxide released from the cultured macrophage cell line. *In vivo*, mice were treated with a single intraperitoneal dose of 50 mg/kg of nanosilica. The treatment produced activated peritoneal macrophages, increased blood level of IL-1□ and TNF-□, and increased level of nitric oxide released from peritoneal macrophages. *Ex vivo*, cultured peritoneal macrophages harvested from the treated mice showed the expression of inflammation-related genes (IL-1, IL-6, TNF- □, inducible nitric oxide synthase, cyclooxygenase 2). In the spleen, the relative distribution of natural killer cells and T cells was increased 184.8% and 115.1%, respectively, as compared with control animals, and that of B cells was decreased to 87.7% [159].

Kim et al. [160] addressed the toxicity of nano- and micron-sized silica particles (14 nm and 1-5 □m, respectively) *in vitro* and *in vivo*. *In vitro*, RAW 264.7 cells were exposed to both particle sizes for 24 h, and the cell viability was decreased in dose-dependent manner; however, apoptosis was observed only after treatment with nanoparticles. *In vivo*, mice received up to 5 mg/kg silica particles via oropharyngeal aspiration. Again, size-dependent toxicity of silica was observed; pulmonary injury and neutrophilic

infiltration were greater after treatment with nano-sized SiO_2 particles than with micron-sized silica [160].

Sayes et al. [161] assessed the capacity of *in vitro* screening studies to predict *in vivo* pulmonary toxicity of several fine or nanoscale particle types in rats. For the *in vitro* component of the study, rat lung epithelial cells, primary alveolar macrophages and alveolar macrophages-lung epithelial cell co-cultures were incubated with **quartz** particles and precipitated **amorphous** silica. In the *in vivo* component of the study, rats were exposed by intratracheal instillation to the same particles. *In vivo*, pulmonary toxicity studies demonstrated that crystalline silica particles produced sustained inflammation and cytotoxicity, whereas amorphous silica particles produced reversible and transient inflammatory responses. *Ex vivo*, pulmonary inflammation studies showed that crystalline and amorphous silica-exposed rat lung epithelial cells did not produce MIP-2 cytokines, but alveolar macrophages and, to a lesser degree, co-cultures secreted this chemotactic factor into the culture media. *In vitro* cytotoxicity studies demonstrated a variety of responses to the different particle types, primarily at high doses. When considering the range of toxicological endpoints, comparisons of *in vivo* and *in vitro* measurements revealed little correlation, particularly when considering the many variables assessed in this study such as cell types used, culture conditions and time course of exposure, as well as measured endpoints.

To summarize, extrapolating (or comparing) the results obtained *in vitro* to the *in vivo* situation is difficult and applies not only to toxicity studies with nanoparticles -- any existing *in vitro* test system lacks the complexity of animal models or the human body. However, considering the number of particles and the number of possible properties of these particles that may vary (size, shape, coating, etc.), clearly, not all can be evaluated in *in vivo* studies, and scientists have been striving to determine the correlation between the results obtained from *in vitro* and *in vivo* toxicity assessments. Although little correlation has been found in these studies with nanosilica [159, 160, 161], Lu et al. [162] tested a panel of metal oxide nanoparticles and could predict the inflammogenicity of tested nanomaterials with a battery of simple *in vitro* tests. Similar conclusions were drawn in a recent study by Rushton et al. [163]; the authors could predict the acute *in vivo* inflammatory potential of nanoparticles with cell-free and cellular assays by using NP surface area-based dose and response metrics. The authors also found that a cellular component was required to achieve a higher degree of predictive power.

Established and validated co-culture systems may provide a tool to better mimic the *in vivo* system. Using recently developed 3-D cell cultures and improving the exposure system (likewise exposure at the air-liquid interface of a human epithelial airway model reported by Brandenberger et al. [164]), could substantially improve the outcome from *in vitro* studies with nanomaterials.

Conclusions

Silica or silicon dioxide (SiO_2) is, in many forms, abundantly present in our natural environment. The adverse health effects, including lung cancer, of naturally occurring crystalline silica such as quartz and cristobalite have been thoroughly documented in occupational settings. Naturally occurring amorphous silica such as diatomaceous earth is considered less harmful. Most of the synthetic (manufactured) silicas used in a large variety of applications are amorphous. For silica in general, the property most significantly linked to the toxicological potential is the crystallinity. For micron-sized

crystalline silica, oxidative stress and, linked to it, oxidative DNA and membrane damage, are probably the most important mechanisms involved in the inflammogenic and fibrogenic activities (reviewed by [60]) and/or carcinogenic activity [39, 165], for example. These mechanisms do not apply to amorphous silica, which has therefore been far less studied. Moreover, the adverse health effects of biogenic (natural) amorphous silica is often attributed to a certain degree of contamination with crystalline silica [49]. Synthetic amorphous silica (colloidal silica, fumed silica and precipitated silica) is not involved in progressive fibrosis of the lung [52, 53]; however, high doses of amorphous silica may result in acute pulmonary inflammatory responses [54].

Interest in using SNPs is growing worldwide, especially for biomedical and biotechnological applications such as cancer therapy, DNA transfection, drug delivery, and enzyme immobilization [5, 6, 7, 8, 9]. In general, SNPs are synthetic, which has an advantage over natural silica in that they contain fewer or no impurities than do natural silica, and the physico-chemical properties are known and well controlled during production. Exposure to SNPs during the production process and their downstream use is probably minimal for sols and gels because the nanoparticles are trapped/immobilized within their matrix. However, the inhalation potential of low-density fumed silica powders or freeze-dried nanoparticles may be high without adequate precautions.

Results of a growing number of *in vitro* studies indicate that the particle surface area may play a crucial role in the toxicity of silica [75, 166]. The cytotoxic activity of silica particles can be related to their surface interfacing with the biological milieu rather than to particle size or shape [75]. Surface silanol groups are directly involved (as shown *in vitro*) in hemolysis [76, 77, 78] and in alveolar epithelial cell toxicity [79, 80]. This observation indirectly links the hydrophilicity to cellular toxicity [80, 81]. The size and surface physico-chemical features of SNPs contribute decisively to the biological effects of SiO_2 nanoparticles. The complexity of protein-SNP interactions should not be underestimated; these interactions appear to be affected by the size of SNPs as well [167, 168, 169, 170, 171]. The effect of other physico-chemical properties of SNPs on health, such as porosity, chemical purity, surface chemistry and solubility, are less well studied, and therefore no definite conclusions can be formulated (summary of the data can be found in Table 2). Comparison of published studies leads to the conclusion that even a small modification of the surface can result in a more or less marked change of a biological effect [2, 3, 172]. Few *in vitro* studies have emphasized that the response to SNPs varies by cell type [137, 140, 141].

Considering the use of SNPs for medical applications, biocompatibility and toxicokinetics need to be documented in great detail because, despite no observation of acute (cyto)toxicity, the uptake of the particles by cells may eventually lead to perturbation of intracellular mechanisms. For instance, the ability of silica-coated nanomaterials to penetrate the blood-brain barrier supports the urgent need for extensive studies to clarify the potential chronic toxicity of these materials [14]. The successful use of nanoparticles in the clinic requires exhaustive and elaborate *in vivo* studies [155]. Of note, the toxicity of SNPs can depend on not only the material itself but also the administration route to the living body, as was shown by Hudson et al. [156]: subcutaneous injection presented good biocompatibility, whereas intraperitoneal and intravenous injection led to fatal outcomes.

Unfortunately, only limited short-term and no chronic *in vivo* studies of SNPs are available (summary of the data is found in Table 3), and the current data do not clarify

whether amorphous SNPs - showing augmented cytotoxicity and presumably processing oxidative DNA damaging potential -- are less or more harmful as compared with micron-sized silica.

Determining the association of results from *in vitro* and *in vivo* toxicity assessments is difficult; however, the common feature seems to be cytotoxicity and inflammatory response after exposure to SNPs.

To conclude, the available studies of the toxicity of SNPs are relatively few, especially as compared to the vast number of studies of titanium dioxide or carbon nanotubes. Besides the relative lack of information on the safety or hazards of SNPs, often conflicting evidence is emerging in the literature as a result of a general lack of standard procedures, as well as insufficient characterization of nanomaterials in biological systems. For all studies, a crucial issue remains the careful, accurate characterization of particle size and morphologic features (especially in the biological media used for experimental set-up), composition, particle surface area and surface chemistry [173]. Moreover, equally important to the physico-chemical characterization of the material is the control of assays and assay conditions [174, 175]. Only with the complete description of the NP and assay can the results of reported studies be comparable with those of other studies conducted with similar nanomaterials [159, 176].

Until now, the health effects of SNPs have mainly been studied in terms of exposure via the respiratory tract, after acute or sub-acute exposure; other exposure routes should also be checked (e.g. blood, skin, gastrointestinal tract). Studies of chronicity are needed to supplement and verify the existing data. Information is insufficient to clearly identify and characterize the health hazards SNPs pose, and defining the appropriate conditions for safe use of these materials is currently not possible.

Notes

Dorota Napierska, Leen CJ Thomassen contributed equally to this work.

Declarations

Acknowledgements

This work was supported by the Belgian Science Policy program "Science for a Sustainable Development" (SD/HE/02A). JAM acknowledges the Flemish government for long-term structural funding (Methusalem).

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Competing interests

The authors declare that they have no competing interests.

Authors' contributions

DN, LCJT and PHH drafted the manuscript. DN provided key input in the literature search. LCJT and JAM wrote the section on synthesis and characterization of silica materials. LCJT prepared all figures. DL contributed to drafting the paper. All authors read and approved the final manuscript.

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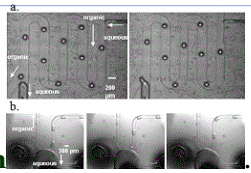
The key to mass-producing nanomaterials

Researchers create a system that can scale-up production of the smallest -- but among the most useful -- materials of this century

Nanoparticles -- tiny particles 100,000 times smaller than the width of a strand of hair -- can be found in everything from drug delivery formulations to pollution controls on cars to HD TV sets. With special properties derived from their tiny size and subsequently increased surface area, they're critical to industry and scientific research.-- They're also expensive and tricky to make. **Now, researchers at USC have created a new way to manufacture nanoparticles that will transform the process from a painstaking, batch-by-batch drudgery into a large-scale, automated assembly line**. The method, developed by a team led by Noah Malmstadt of the USC Viterbi School of Engineering and Richard Brutchey of the USC Dornsife College of Letters, Arts and Sciences, was published in *Nature Communications* on Feb. 23**. Consider, for example, gold nanoparticles. They have been shown to be able to easily penetrate cell membranes -- an unusual feat, given that most penetrations of cell membranes by foreign objects can damage or kill the cell**. Their ability to slip through the cell's membrane

makes gold nanoparticles ideal delivery devices for medications to healthy cells, or fatal doses of radiation to cancer cells. However, a single milligram of gold nanoparticles currently costs about $80 (depending on the size of the nanoparticles). That places the price of gold nanoparticles at $80,000 per gram -- while a gram of pure, raw gold goes for about $50. "It's not the gold that's making it expensive," Malmstadt said. "We can make them, but it's not like we can cheaply make a 50 gallon drum full of them." Right now, the process of manufacturing a nanoparticle typically involves a technician in a chemistry lab mixing up a batch of chemicals by hand in traditional lab flasks and beakers. Brutchey and Malmstadt's new technique instead relies on microfluidics -- technology that manipulates tiny droplets of fluid in narrow channels. "In order to go large scale, we have to go small," Brutchey said. Really small. **The team 3D printed tubes about 250 micrometers in diameter -- which they believe to be the smallest, fully enclosed 3D printed tubes anywhere.** For reference, your average-sized speck of dust is 50 micrometers wide. They then built a parallel network of four of these tubes, side-by-side, and ran a combination of two non-mixing fluids (like oil and water) through them. **As the two fluids fought to get out through the openings, they squeezed off tiny droplets. Each of these droplets acted as a micro-scale chemical reactor in which materials were mixed and nanoparticles were generated. Each microfluidic tube can create millions of identical droplets that perform the same reaction**



. This sort of system has been envisioned in the past, but its hasn't been able to be scaled up because the parallel structure meant that if one tube got jammed, it would cause a ripple effect of changing pressures along its neighbors, knocking out the entire system. Think of it like losing a single Christmas light in one of the old-style strands -- lose one, and you lose them all. Brutchey and Malmstadt bypassed this problem **by altering the geometry of the tubes themselves, shaping the junction between the tubes such that the particles come out a uniform size and the system is immune to pressure changes**. Malmstadt and Brutchy collaborated with Malancha Gupta of USC Viterbi and USC graduate students Carson Riche and Emily Roberts.-**Story Source-**The above post is reprinted from materials provided by **University of Southern California**. -**Journal Reference**-Carson T. Riche, Emily J. Roberts, Malancha Gupta, Richard L. Brutchey, Noah Malmstadt. **Flow invariant droplet formation for stable parallel microreactors**. *Nature Communications*, 2016; 7: 10780 DOI: 10.1038/ncomms10780 -University of Southern California. "The key to mass-producing nanomaterials: Researchers create a system that can scale-up production of the smallest -- but among the most useful -- materials of this century." ScienceDaily. ScienceDaily, 24 February 2016. <www.sciencedaily.com/releases/2016/02/160224100343.htm>.

Owner 4/17/2017 10:45 AM

Comment [211]: The majority of reactions relevant to the preparation of fine chemicals, however, involve the use of solid reagents, catalysts, products, and by-products. The manipulation of cells and biomolecules in microfluidics also demands control strategies for heterogeneous matter

Owner 4/17/2017 10:45 AM

Comment [212]: This is how the nano spreads throughout the body by forcing the fluid levels out and increasing voltage or electrical flow and heat destroying the myelin and cells and even the mitochondria and as a result hijacking the dna and spreading and as a result you have more self replication and self assembly in the nano materials to assimilate into the biology

Owner 4/17/2017 10:45 AM

Comment [213]: Called a morphology and with this tech this would allow the changes to take place within the nano structure itself including the dna

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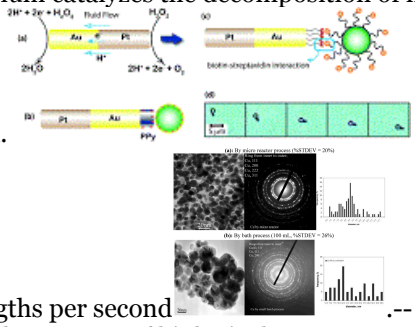
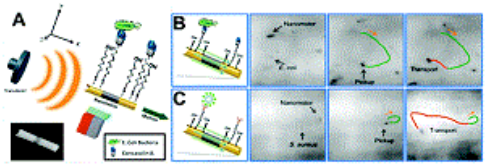
Micro-Scale Chemical Reactor

The microfluidic synthesis of inorganic crystals, another example of the flow and reaction of solids in micro-scales, can generate advanced nanomaterials for applications in catalysis, material science. The **nanomotors** are rod-shaped particles, 370 nm in diameter, consisting of 1-µm-long platinum and gold segments. The platinum catalyzes the decomposition of hydrogen peroxide and formation of oxygen.  The rods move at speeds of up to 10 body lengths per second .-- Nanowire motor for selective capture and transport of biological targets. 

Owner 4/17/2017 10:45 AM

Comment [214]: The majority of reactions relevant to the preparation of fine chemicals, however, involve the use of solid reagents, catalysts, products, and by-products. The manipulation of cells and biomolecules in microfluidics also demands control strategies for heterogeneous matter

Owner 4/17/2017 10:45 AM

Comment [215]: The new synthetic systems consist of suspensions of nanorods, composed of two or more metallic segments, in aqueous hydrogen peroxide. One of the metals catalyzes the decomposition of the hydrogen peroxide, liberating oxygen, which provides thrust to propel the nanorods through the solution.

Owner 4/17/2017 10:45 AM

Comment [216]: "Catalytically driven nanomotors are autonomous in that they do not require external electric, magnetic, or optical fields as energy sources," he adds. "Instead, the input energy is supplied locally and chemically."

Efficient method of producing metallic nanoparticles

VTT Technical Research Centre of Finland Ltd has developed a new, cost-efficient method of producing various types of metallic nanoparticles. Nanoparticles can be used in applications such as conductive and magnetic inks, medical diagnostics and drug dosing, **tailoring the electrical and magnetic properties of polymers and energy technology**. VTT is seeking a party interested in commercializing the technique.-- **VTT's aerosol technology reactor for nanoparticle production can generate a variety of pure metal particles, particles of various alloys and carbon-coated particles**. The reactor can efficiently produce hundreds of grammes or even kilogrammes of nanoparticles per day.-"Demand has outstripped supply in the nanoparticle markets. This has been an obstacle to the development of product applications; nano-metal composites are scarce and often available in small quantities only. We wanted to demonstrate that it was possible to produce nanomaterials in considerable quantities cost-effectively," comments Ari Auvinen of VTT, head of the research team.-When developing the reactor, the aim was to achieve a production figure of 200-3,000 grammes per day. This has already been clearly exceeded. Due to the extremely small material wastage incurred when using this

equipment, remote-control production can be maintained for several days**. In most cases, industrial production of metallic nanoparticles involves chemical reduction in liquid solutions, which requires the design of product-specific solutions.** Plasma synthesis, which consumes large amounts of energy and involves significant material wastage, is another generally used method.-In the design of the reactor developed by VTT, the scalability and cost-effectiveness of the synthesis process were key criteria. For this reason, **synthesis is performed under air pressure at a comparatively low temperature.** This means that the equipment can be built from materials commonly used in industry and energy consumption is low. The process generates an extremely high particle concentration, enabling a high production speed but with low gas consumption. **In addition, even impure metallic salts can be used as a raw material, which keeps the price low.-VTT has demonstrated the practical functionality of its reactor by testing the production of various nanometals, metallic compounds and carbon-coated materials**. Materials such as carbon-coated magnets, which can be used as catalysts in biorefineries -- say, in the production of biofuels -- have been produced in the reactor. Following synthesis, magnets used as catalysts can be efficiently gathered in and recycled back into the process.-**Nanoparticles have also been tested in the manufacture of magnetic inks and inks that conduct electricity in printed electronics**. **For example, VTT succeeded in using a permalloy ink** to print a magnetically anisotropic material, which can be used in the manufacture of magnetic field sensors.-VTT's third application trial involved the prevention of microwave reflection. The tests showed that reflection can be reduced by even 10,000 times in polymers, by adding particles which increase radar wave attenuation.-VTT's researchers believe that the reactor has many applications in addition to those already mentioned. The silicon nanoparticles it produces may even enable lithium battery capacity to be boosted by a factor of 10. Other possible applications, all of which require further investigation, include high permeability polymers, nanomagnets for medical diagnostics applications, materials for the 3D printing of metal articles, and silicon-based materials for thermoelectric and solar power components.-VTT is currently seeking a party interested in commercializing the technique.-**Story Source-**The above post is reprinted from materials provided by **Technical Research Centre of Finland (VTT)**.-Technical Research Centre of Finland (VTT). "Efficient method of producing metallic nanoparticles." ScienceDaily. ScienceDaily, 20 April 2015. <www.sciencedaily.com/releases/2015/04/150420084741.htm>.

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New artificial cells mimic nature's tiny reactors

Artificial cells that mimic their natural counterparts help scientists learn the secrets of complex processes, such as how plant cells turn sunlight, water, and carbon dioxide into fuel. Today's artificial cells often become unstable when materials transit the membrane**. Scientists have developed a new artificial**

Owner 4/17/2017 10:45 AM

Comment [217]: Nanoparticles in tattoo ink could cause cancer Researchers from the UK's University of Bradford have raised concerns about the dangers of some tattoo inks and their potential to cause illnesses, including cancers. -Evidence has been found that nanoparticles from the ink can leave the skin (most likely via its dense network of blood and lymphatic vessels) and be transported to other organs of the body. Professor Tobin, Director of the University's Centre for Skin Sciences (CSS) believes that toxins in the dyes may accumulate in the spleen or the kidneys – the organs which filter impurities from the blood.-Given the enormous increase in tattooing in the last 10 years, Professor Tobin believes this is a potentially significant public health concern.-Millions of people around the world are tattooed. A 2006 American study found that up to 24% of people aged between 18 and 50 had tattoos. Despite a 2010 study by German scientists suggesting that millions of people in the Western world may have transient or persisting health problems after tattooing.-To date, much of the debate around nanomaterials in consumer products has focused on the use of nanomaterials in sunscreen. One of the significant toxicity concerns associated with nanomaterials is their ability to produce free radicals that can damage DNA and proteins. Tattoos involve the injection of chemicals such as titanium dioxide (anatase and rutile forms), aluminium oxide and carbon black directly into the dermis. This has scientists worried. A recent study, looking at a range of tattoo inks, found that the vast majority contained significant **amounts of nanoparticles. In particular, black pigments – those most used by tattooists – are usually made of carbon nanoparticles.**
-Carbon black is classified by the International Agency for Research on Cancer as possibly carcinogenic to human beings, based partly on inhalation studies on rats. Studies have shown that carbon black nanoparticles can cause inflammation and damage DNA. This damage is believed to be due to free radical production. **Furthermore, studies confirm that nanoparticles of titanium dioxide and carbon black are more toxic and generate free radicals to a greater extent than larger** ... [12]

cell where lipid vesicles (small pools of fatty molecules) self-assemble around treated water droplets. The result is an artificial cell or microscopic bioreactor.

The Impact

This new type of cell-like bioreactor could offer substantial advantages for **carrying out complex synthesis processes that mimic natural processes**. It could also offer benefits in conducting massively parallel chemical reactions.

Summary

Scientists discovered a new process for spontaneously forming "artificial cells" that can function as bioreactors through the self-assembly of polymer-rich water droplets within lipid-rich water droplets. In essence, the artificial bioreactor is composed of a shell membrane through which reactants and products can selectively pass through, and an interior environment where the reactions occur. Lipid-, polymer-, and gel-based processes for preparing bioreactors modelled after biological cells have been previously developed; maintaining stable reaction-relevant internal environments while simultaneously allowing reactants and products to easily pass through have remained a key challenge. --Now, researchers at the Pennsylvania State University **have developed a new type of water-in-water composite emulsion, based on self-assembly of microscale aqueous droplets surrounded by nanoscale lipid capsules in a continuous aqueous phase. These lipid-stabilized water-in-water assemblies provide an exciting alternative to traditional giant lipid vesicles, or liposomes, as artificial cell mimics.** In comparison to traditional giant liposomes, which encapsulate a similar aqueous volume within a single continuous lipid membrane, the structures introduced here offer

(1) facile encapsulation of proteins in the interior phase as well as polymer agents for controlling the progress of the desired reaction,

(2) excellent uniformity in droplet size and contents, and

(3) much greater access into and out of the interior volume.

The researchers found that negatively charged lipid capsules, each on the order of 100 nanometers in diameter, self-assemble at the aqueous interface of polymer-rich droplets that are tens of microns in diameter. The repulsion between the **lipid capsules due their negative charges forced them to maintain their assembled structure, essentially gluing them together and stabilizing the overall bioreactor composite**. A particularly exciting capability of these **composite assemblies is the preferential partitioning of DNA within the interior**

FREEDOM 4/17/2017 10:45 AM
Comment [218]: Cell bio reactor would be similar to what we cal ATP in the mitochondria~

FREEDOM 4/17/2017 10:45 AM
Comment [219]: Basically the are utilizing liposome where you have a bilayer of fat with water in the middle~basically this is how some pathogens hide in the body as well to basically sabotage the host ~ so in essence what they have made is something or another thing that can hijack the body through a lipsomal delivery self assembling inside a fat

FREEDOM 4/17/2017 10:45 AM
Comment [220]: Pass through any interior environment….

FREEDOM 4/17/2017 10:45 AM
Comment [221]: Here is a concept what happens when you have this in the drinking water or in water outlets in lakes and streams and possibly oceans??

FREEDOM 4/17/2017 10:45 AM
Comment [222]: A Clustering-or a building of a polymer for assembling as a possible foundation and allowing things to go through~ working like a cellular wall

FREEDOM 4/17/2017 10:45 AM
Comment [223]: How polymers construct and adhere together

compartment based on the length of the DNA, which bodes well for designing and preparing micro-reactors in which combinations of reactants can be selectively introduced and maintained at desired levels. In addition, ribozyme-induced cleavage of RNA encapsulated within the interior is as another example of the bioreactor's unique capability.--**Story Source-**The above post is reprinted from materials provided by **Department of Energy, Office of Science**. -**Journal Reference**-Daniel C. Dewey, Christopher A. Strulson, David N. Cacace, Philip C. Bevilacqua, Christine D. Keating. **Bioreactor droplets from liposome-stabilized all-aqueous emulsions**. *Nature Communications*, 2014; 5: 4670 DOI: 10.1038/ncomms5670 --Department of Energy, Office of Science. "New artificial cells mimic nature's tiny reactors." ScienceDaily. ScienceDaily, 6 October 2015. <www.sciencedaily.com/releases/2015/10/151006124005.htm>.

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Health Effects of NANO- Aerosols
ARTICLE in INHALATION TOXICOLOGY·

Carbonaceous aerosol, a major component of particulate matter (PM), gases, and vapors in the atmosphere, has been associated with natural and anthropogenic air pollution, reduced visibility, climate modulation, material and ecosystem damage, and adverse health effects. More recently, epidemiological studies have indicated associations between **organic fractions of ambient PM and adverse respiratory and cardiovascular health outcomes**. ***The effects of the non-PM components of the organic aerosol have received less attention because their measurement in the general environment is not mandated***. This article summarizes current knowledge of the nature, prevalence, and health effects of organic aerosols encountered in the outdoor environment, identifies key information gaps, and presents a conceptual framework for research priorities for resolving those gaps. The broad, diverse class of air contaminants comprising organic aerosols may be more important to public health than the modest attention given to them. **This review focuses on hazard identification and exposure assessment for evaluating risks to public health from ambient organic aerosols**. Current knowledge is insufficient to support a quantitative characterization of the aggregate risk from organic air contaminants. Assessments should be done for individual species or mixtures. Efforts should be taken to assemble and evaluate a common set of standard reference materials for both organic speciation and health response assays. A greater standardization of approaches across studies and laboratories would be useful to achieve uniformity in assessing health effects. Multidisciplinary research efforts are needed to improve the current regulatory-driven air quality monitoring networks for epidemiological studies. The limited array of biomarkers linking organic aerosols to health effects needs to be expanded and **specific organic compounds or classes that are associated**

FREEDOM 4/17/2017 10:45 AM
Comment [224]: It can assemble it can partition DNA meaning it can become cohesive with dna and can introduce into the dna complex reactants—almpst sounds the experiment has been ongoing ~ this is how nanopoisoning attaches and grows within the human matrix as well as animal and aquatic life and the plant kingdom

Owner 4/17/2017 10:45 AM
Comment [225]: Received 22 May 2007; accepted 10 November 2007. This article and the "Health Effects of Organic Aerosols" workshop held on October 2425, 2006, at the Electric Power Research Institute (EPRI) in Palo Alto, CA (http://www.epri.com/), are jointly sponsored by EPRI and NARSTO. The invited speakers for the workshop were Dr. Flemming Cassee, Dr. Daniel Costa, Dr. Joellen Lewis, Dr. Ning Li, Dr. Mike Madden, Dr. Jake McDonald, Dr. Annette Rohr, Dr. James Schauer, Dr. Barbara Turpin, Dr. John Watson, Dr. Ron Wyzga, and Dr. Barbara Zielinska. The authors wish to thank Ron Wyzga and Annette Rohr of EPRI, Jake McDonald of the Lovelace Respiratory Research Institute, and John Watson of the Desert Research Institute for technical advice received. Bonne Cleveland of LRRI and Jo Gerrard of DRI assisted in the assembly and editing of the arti ... [13]

Owner 4/17/2017 10:45 AM
Comment [226]: Air pollution is the introduction of particulates, biological molecules, or other harmful materials into Earth's atmosphere, causing diseases, death to humans, damage to other living organisms such as animals and food crops, or the natural or built environment- anthropogenic (resulting from globalist activities)

Owner 4/17/2017 10:45 AM
Comment [227]: Epidemiology is the study of how often diseases occur in different groups of people and why

Owner 4/17/2017 10:45 AM
Comment [228]: Using SPLAT II, the researchers measured the shape, size, density, composition, evaporation rates, and many other relevant properties of SOA-containing particles in laboratory and field. The results show that SOA particles are far from the perfect liquid spheres assumed in models. The actual SOA particles are highly viscous semi-solids that have complex ... [14]

with biological effects in human cells or animal studies need to be tested for better understanding of the exposure-response relationship.

Carbonaceous aerosol is a complex, heterogeneous mixture of organic carbon (OC) and elemental carbon (EC, also called black carbon [BC] or soot) particulate matter (PM) suspended in organic gases and vapors. It is always **encountered in the environment as a mixture with inorganic PM and gases, and the organic components of mixed PM are considered here as part of the PM phase of the organic aerosol**. **It is ubiquitous in outdoor and indoor environments**. **It is impractical to separate EC from OC because they nearly always occur together and there is a continuum of composition between the two**, although carbonates (CO_2^-) are usually excluded. Aerosol, a suspension 3 of solid or liquid particles in a gas (Hinds, 1999), is often wrongly used as a synonym for PM. **Organic aerosol includes non-PM components, and there is dynamic continuum among volatile organic compounds (VOCs), semivolatile organic compounds (SVOCs), and particulate species**. A comprehensive review of carbonaceous aerosol and its effects could fill several volumes. Citations in this review are limited to prior reviews and examples supporting key points. This review was informed by the recent workshop **"Health Effects of Organic**

Aerosols," held in Palo Alto, CA, on October 2425, 2006, but it is not a summary per se of that workshop (see http://www.narsto.org/event.src?ID = 10). Both the workshop and this review were motivated by the knowledge that carbonaceous aerosol constitutes a substantive portion of ambient air pollution, as well as by growing recognition of its health importance, measurement difficulties, and the lack of integrative, tutorial reviews of the topic. In its criteria document on PM, the U.S. Environmental Protection Agency (U.S. EPA, 2004a) acknowledges that OC and EC are major fractions of ambient PM and are abundant in many source emissions. **Other than a section on "bioaerosols" (which are primarily organic), the criteria document does not review evidence for the health impacts of carbonaceous PM or carboncontaining PM as a class, nor does it portray the complex and dynamic interplay among PM and organic gaseous precursors. In contrast, the criteria document does deal with acidic and metallic components of PM**

Owner 4/17/2017 10:45 AM

Comment [229]: In other words everywhere inside or out

Owner 4/17/2017 10:45 AM

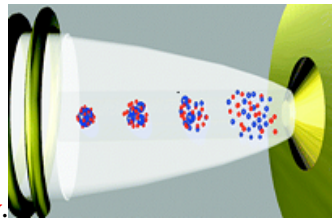
Comment [230]:
Organic aerosol includes non-PM components, and there is dynamic continuum among volatile organic compounds (VOCs), semivolatile organic compounds (SVOCs), and particulate species

Owner 4/17/2017 10:45 AM

Comment [231]:
This device measures Nanoparticles in the atmosphere

They built SPLAT II, a second generation single particle mass spectrometer. Highly precise and sensitive, this instrument allows users to study basic processes and properties of nanoparticles. Usually located in DOE's EMSL, a national scientific user facility, the instrument also travels to field sites, working on the ground and aboard research aircraft. SPLAT II has been flown to many sites in the United States, South Korea, and Germany.

Read more at:
http://phys.org/news/2015-05-years-results-secondary-aerosols-uncertainty.html#jCp

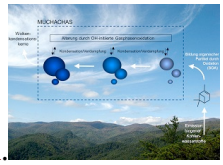


specifically. Recent reviews of air pollution and health (Donaldson et al., 2001; Green & Armstrong, 2003; Knaapen et al., 2004; Delfino, Sioutas, & Malik, 2005; Schlesinger et al., 2006; Highwood & Kinnersley, 2006; Pope & Dockery, 2006; Chow et al., 2006; Naeher et al., 2007) **deal with organic aerosol effects implicitly in terms of potential sources** (e.g., exposure to vehicle exhaust, vegetative burning) and as a portion of PM mass, but they have not explicitly tackled the subject**. Environmental concentrations of non-PM components of the organic aerosol are not regulated as "criteria pollutants," but the emissions of some are regulated as**

"hazardous air pollutants," or " (discussed later). One difficulty is our limited understanding of the extent to which carbonaceous pollutants might be grouped into coherent classes on the basis of biological activity or as targets for regulatory action. Polycyclic aromatic hydrocarbons (PAHs) constitute one of the few classes established for regulatory purposes, based largely on their hazard as carcinogens (although they have other effects as well, and not all are carcinogenic). Determination of logical or practical groupings is one of the current research needs. **The large number of chemical species, the complexity of their interrelationships across sources and physical forms**, and the high costs of their sampling and analysis make categorization challenging from both the health and regulatory perspectives. **The sources, physical/chemical/biological nature, and prevalence of environmental organic aerosols are described.** The evidence for adverse health effects is summarized. Key knowledge gaps are identified, followed by a summary of research tools and strategies necessary to reconcile those gaps. **This review intends to catalyze additional efforts to resolve the impacts of carbonaceous components of air pollution on public health, and to facilitate the evolution of research strategies that will likely be required to do** so.

NATURE AND PREVALENCE OF AMBIENT ORGANIC AEROSOL In addition to health impacts, atmospheric carbonaceous material affects the earth's climate (IPCC, 2007), tropospheric chemistry (Dentener & Crutzen, 1993), ecosystems (Cao, Zhang, & Zheng, 2006), and visibility (Watson, 2002). **Carbon accounts for 1040% of PM mass on a global scale** (e.g.,Heintzenberg, 1989), **but this fraction exceeds 50% in many urban atmospheres due to energy-related fossil-fuel and biofuel combustion**. Large-scale

wildfires consume millions of acres of forest each year, generating smoke that mostly consists of carbonaceous material. **Combustion aerosols contain EC, which is the dominant light-absorbing component in the troposphere**. The remaining carbon is in the form of organic compounds and carbonates. **There is also abundant noncombustion carbonaceous aerosol originating from plants (e.g., pollens, fungi),** and **secondary organic aerosol (SOA) formed from biogenic and anthropogenic hydrocarbon emissions that oxidize to compounds with condensable vapor pressures**. Table 1 summarizes the known major sources of atmospheric carbonaceous material. **Large uncertainties in both health effects and the earth's radiation balance are due to the complex nature of carbonaceous aerosol.**



Organic Carbon Classification Chemical Compounds Organic compounds are classified into 21 families or functional groups according to their structural features, as shown in Table 2 (Solomons, 1996). **A functional group is a cluster of atoms within a molecule that has a characteristic chemical behavior**. **Numbers and types of functional groups in the molecule determine the reactivity of each compound.** Analytical methods applicable to each functional group are specified in Table 2. Also identified are the 12 families that are often reported in aerosol studies. **Alkanes, alkenes, anenes (aromatic compounds such as PAHs), aldehydes, and carboxylic acids have been used for PM source apportionment studies to identify and quantify pollution sources and to examine their toxicity and mutagenicity in animal studies**. **Gas and Particle Phases Almost all families of organic compounds contain SVOCs, defined as organic compounds that are in equilibrium between gas and particulate phases in the atmosphere** (van Vaeck, van Cauwenberghe, & Janssens, 1984). **These are by-products of incomplete combustion that are directly emitted into the atmosphere, with vapor pressures ranging from 10-4 to 10-11 atmospheres (atm) at ambient temperature** (Pankow, 1993). **Compounds with higher vapor pressures primarily exist in the gas phase, whereas lower vapor pressure compounds are found on the surface of or inside PM**. Vapor pressure at atmospheric temperature is a major parameter to determine the extent of gas/particle partitioning. **The interaction between a compound (e.g., particle size, chemical structure, polarity) and the sorptive medium (e.g., adsorptive affinity, capacity) also affects the partition. PAHs, polychlorinated biphenyls (PCBs), polychlorinated dibenzo- p-dioxins (PCDDs), polychlorinated diphenyl ethers (PCDEs), nitroaromatics, and terpenes are SVOCs that are known to be carcinogenic** (Prince et al., 2006; Yang, Park, & Lee, 2006; Wang et al., 2006b; Ewing et al., 2006; Wigle et al., 2007; Tai et al., 2007) **and are usually present as both gases and particles**.

Owner 4/17/2017 10:45 AM

Comment [232]: Elemental Carbon or Black Carbon

Owner 4/17/2017 10:45 AM

Comment [233]: In 1993, a study of toxic emissions at Chicago's Midway Airport revealed that arriving and departing planes released more pollutants than the industrial pollution sources in the surrounding 16-square-mile area. A more recent study at London's Heathrow airport showed that aircraft contributed between 16 and 35 percent of ground level NO_x concentrations.

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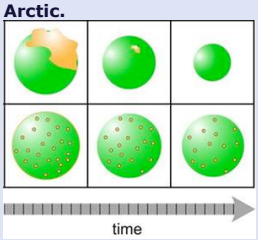
Comment [234]: The team was the first to directly measure chemical diffusivity of tracer molecules in SOA particles, determining that these particles are tar-like, and millions of times more viscous than assumed. This viscous nature allows the particles to trap toxic polycyclic aromatic hydrocarbons, PAHs, and other chemicals that would otherwise quickly evaporate. Zelenyuk and her colleagues discovered a symbiotic relationship between the PAHs and the SOA particles. The PAHs hitchhike along and, in the process, help the SOA survive longer. This hitchhiking phenomenon explains how anthropogenic pollutants from California freeways and biomass burning in Asia can be transported far away from their sources to the pristine environments, such as the Arctic.



When airborne particles (green) form before pollutants known as PAHs (yellow) glob on, the particles evaporate quickly (top row). But when the particles form in the presence of PAHs, which is what likely happens in nature, the long-lasting ...more
SOA particles, even without PAHs, are long lasting. Some scientists believed the particles evaporated nearly instantaneously at higher levels of humidity. Zelenyuk and her team found, however, that the ... [15]

TABLE 1 **Major sources of atmospheric particulate carbonaceous material with respect to organic aerosol and elemental carbon** (Mazurek et al., 1993; Watson et al., 2001; Seinfeld & Pankow, 2003; Tsigaridis & Kanakidou, 2003; Bond et al., 2004) Source origin/type **Biogenic Primary Secondary Organic aerosol (OA) Virus, bacteria, fungal spores, plant debris Oxidation of monoterpenes (-pinene, -pinene, etc.) and other reactive VOCs Incomplete biofuel/fossil fuel combustion, vegetative burning, home appliances (natural gas), vehicle exhaust, cooking Oxidation of aromatics (toluene, xylene, etc.), condensation of low vapor pressure compounds on existing particles**. Natural sources of VOCs include biogenic, volcanic, forest fires, etc. **Anthropogenic VOC sources include vehicle exhaust, liquid and evaporated gasoline, refinery fugitive, industrial coating, primers, enamel, painting, vegetative burning, cooking, etc. Elemental carbon (EC)**

Anthropogenic (including pyrogenic)

Primary-Same as primary organic aerosol

Secondary-EC from primary emitters may adsorb organic gases.

Note. VOCs: Volatile organic compounds.

In addition to SVOCs, gaseous VOCs and oxygenated hydrocarbons (OHCs) have been associated with adverse health effects. **VOCs are gases with high vapor pressures, including hydrocarbons (e.g., methane, benzene), halocarbons (e.g., chloromethane), and oxygenates (e.g., formaldehyde, acetaldehyde). Methane is a greenhouse gas. Nonmethane hydrocarbons (NMHCs) are precursors to ozone (O3) formation**. The reactivity of the NMHCs from biogenic and anthropogenic emissions vary greatly (Altshuller, 1991; Thompson & Stewart, 1991; Paulson & Seinfeld, 1992). **OHCs have oxygenated functional groups, such as alcohol, carbonyls (e.g., aldehyde, ketone), and ether, which are highly reactive and are often end-products of photochemical processes that create O3** . PM Components of Organic Aerosol PM Chemical Composition and Size Distributions In the United States, PM2.5 and PM10 mass (particles acquired with 50% inlet collection efficiency at aerodynamic diameters [dp] of 2.5 and 10 μm, respectively) are routinely measured at compliance stations and are bases for much of the exposure assessment and epidemiological studies. **Particle composition varies by source and by size**. PM2.5 mainly consists of **carbonaceous material with secondary sulfate (SO2-), ni4 trate (NO-), and ammonium (NH+), while PMcoarse (PM10-2.5) 3 4 consists mostly of geological material, with OC in the form of biological components such as detritus, fungi, pollen, and spores**. **Some VOCs condense on these particles**. An example of PM chemical composition **as a function of particle size** is illustrated in Figure 1. **Particles in the submicron PM are dominated by OC, usually associated with EC.**

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Comment [235]: The definition of submicron is something shorter or smaller than one millionth of a meter.

Organic compounds are found in cloud condensation nuclei (CCN) and water-soluble organic carbon (WSOC). The composition of the organic species that have been analyzed (using the techniques specified in Table 2) is illustrated in Figure 2. A series of water and solvent extraction and derivitization is needed to further speciate polar and nonpolar organics (Chow et al., 2007b). **Solvent extraction has been used to obtain several organic compounds (e.g., PAH, organic acids, alkanes) for source identification. Of particular interest is WSOC, which may constitute 3060% of OC** (Saxena & Hildemann, 1996; Yang, Li, & Yu, 2003). **Large OC polymers such as humic-like substances (HULIS; a group of macromolecular-size polycarboxylic acids;** Graber & Rudich, 2006) consist of a **substantial fraction of WSOC. HULIS are ubiquitious in soil and ambient PM, and can be formed as atmospheric reaction products** (Surratt et al., 2007). **HULIS can alter the hygroscopicity of PM** (Chan & Chan, 2003) **and the surface reactivity of EC** (Pignatello et al., 2006). -TABLE 2 Organic compounds families and analytical techniques applied in aerosol studies-Analytical methodsc SETDHPLC ICIC GC/MS GC/MS fluorescence PAD conductivity Comments + + Alkanes, alkenes, and alkynes are **nonpolar and soluble in organic solvents**. Both solvent extraction (SE)-GC/MS (Zhang & Anastasio, 2003; Yue & Fraser, 2004) and thermal desorption (TD)-GC/MS (Welthagen, Schnelle-Kreis, & Zimmermann, 2003; Ho & Yu, 2004b; Williams et al., 2006; Hays & Lavrich, 2007) can be applied. + + Examples of groups or Representative individual marker with compounds found IUPAC in aerosols name **an-alkanes (C14 -C42), 2,6,10,14hopanes, steranes, tetramethylpenalkyl cyclohexanes tadecane Corresponding marker with CASb registry common number name(s) pristine, norphytan, 1921-70-6 norpytane, pristane, bute hydrocarbon, robuoy norphytane nlkenes,6,10,15,19,23hexamethyl2,6,10,14,18,22tetracosahexaene squalene, skyalen, 7683-64-9 supranene, spinacene n-alkyne polycyclic aromatic hydrocarbons (PAHs) benzo[a]pyrene 3,4-benz[a]pyrene, 50-32-8 3,4-benzopyrene, 3,4-benzpyrene, 3,4-benzylpyrene, 3,4-BP, 6,7-benzopyrene, benzo[d,e,f]chrysene, Bap + + + nonadecyne -- 106073-69-2 + + fluoroalkanes, decafluorobutane chloroalkanes, bromoalkanes and iodoalkanes perfluoro-n-butane 355-25-9 + Arenes** (except for ones with polar functional groups) are nonpolar and soluble in organic solvents. In addition to SE-GC/MS (Miguel et al., 2004; Crimmins & Baker, 2006; Zhang & Anastasio, 2003), TD-GC/MS shows faster response and higher sensitivity in both online and offline detections (Welthagen, Schnelle-Kreis, & Zimmermann, 2003; Ho & Yu, 2004b; Williams et al., 2006). HPLC-fluorescence is an alternative analytical method but less sensitive compared to the GC/MS methods (Eiguren-Fernandez et al., 2004). Also, it is typically limited in detection of U.S. EPA 16 priority PAHs because of their high abundances and low separation efficiencies by HPLC. **Halides are not commonly used as source markers in aerosol studies. n-alkanols (C10 -C30), 3-cholest-5-en-3-ol cholesterol, 57-88-5 polyols 3-hydroxycholest5-ene, cholesterin + Alcohols in the atmosphere are**

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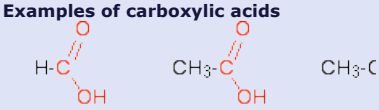
Comment [236]: What are carboxylic acids? Carboxylic acids contain a -COOH group
Carboxylic acids are compounds which contain a -COOH group. For the purposes of this page we shall just look at compounds where the -COOH group is attached either to a hydrogen atom or to an alkyl group.---
Examples of carboxylic acids



methanoic acid ethanoic acid propa

The name counts the total number of carbon atoms in the longest chain - including the one in the -COOH group. If you have side groups attached to the chain, notice that you always count from the carbon atom in the -COOH group as being number 1 ... [16]

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Comment [237]: absorbing or attracting moisture from the air.

both solvent- and water-soluble. SE followed by derivatization (i.e., reactions with silyation agents), which converts polar alcohols into less polar derivatives, has been used for GC/MS detection (Rinehart et al., 2006). Newly developed IC- pulse amperometric detection (PAD) directly qualifies (Gao et al., 2003) and quantifies (Engling et al., 2006a) water-soluble carbohydrates without derivatization and offers lower detection limits.

Family name Alkaned

Functional group structural features –Alkenec-Alkyne-Arenec **(Aromatic compounds)**-Halide—lcohold--carbohydrate oxyallyl ether 111-96-6 + Ethers **are not commonly used as source markers in aerosol studies**.

Ether Amined amino acids (e.g., alanine, glycine) 56-41-7 1,6-anhydro—D levoglucosan glucopyranose bis(2diglyme, diethylene methoxyethyl)ether glycol dimethyl ether, DEGDM(E), dimethyl carbitol, 2,5,8-trioxynonane 2-aminopropanoic alanine, ritalanine acid + Nitriled n-alkylnitriles (C14 -C32) dodecanenitrile undectl cyanide, dodecanonitrile 5399-02-0 Nitro nitro-PAHs 9-nitroanthracene ms-nitroanthracene--**Amino acids are the most representative amines in the atmosphere**. They are polar and soluble in water. Derivatization is necessary before the HPLC-fluorescence detection (Zhang & Anastasio, 2003; Matsumoto & Uematsu, 2005). Nitriles are nonpolar and soluble in organic solvents (Simoneit et al., 2003). Recent TD-GC/MS studies show faster response and higher sensitivity in both online and offline detection (Welthagen, Schnelle-Kreis, & Zimmermann, 2003; Williams et al., 2006). Nitro-PAHs are secondary organic aerosols. (Crimmins & Baker, 2006). **These often come from biogenic sources.**

Sulfoxide allyl sulfoxide dimethylsulfoxide -dimethyl sulfur oxide, 67-68-5 dimexide, dipirartril-tropico, demasorb, demavet, demeso, demsodrox dimethyl sulfone, methyl sulfone 67-71-0 + Sulfone allyl sulfone sulfonylbismethane Thiol **PAH**-thiol 2-naphthalenethiol 2-thionaphthol 91-60-1

+

Aldehyded sinapic aldehyde, 3-methoxy-4vanillin, 2-hydroxy-m- 121-33-5 coniferyl aldehyde, hydroxybenzaldehyde anisaldehyde, nonanal, vanillin, orthovenilline, syringealdehyde 6-formylguaiacol acetovanilione 1-(4-hydroxy-3methoxyphenyl)ethanone acetovanilione, 498-02-2 apocynin, apocynine, acetylgusiacol

+

Ketoned PAH-thiols are the most representative compounds for thiol in aerosols, and can be detected by either SE-GC/MS (Fine et al., 2004) or TD-GC/MS (Welthagen, Schnelle-Kreis, & Zimmermann, 2003). Aldehydes and ketones (e.g., vanillin and acetovanilione),commonly used in aerosol studies, that

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Comment [238]: Nitro-polycyclic aromatic hydrocarbons (nitro-PAHs) constitute one of the most important classes of environmental pollutants, found in air, aquatic systems, grilled food, and sediments. Nitro-PAHs are released to the environment as a result of direct emissions from incomplete combustion processes and are formed in situ in the atmosphere by gas phase oxidation and nitrite (NO3) radical reactions of PAHs. Many nitro-PAHs have been identified as mutagenic and carcinogenic agents, and continued concern about these compounds derives from the potential risk they pose to human health.

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Comment [239]: polycyclic aromatic hydrocarbons (PAHs)

are slightly polar but soluble in organic solvents. SE followed by derivatization converting polar carbonyls into less polar derivatives has been used for GC/MS detection (Zheng et al., 2002; Engling et al., 2006b). (Continued on next page) Examples of groups or Representative marker with individual IUPACa compounds found in aerosols name n-fatty acids (C5 -C28), 9-octadecenoic acid dicarboxylic acids (C6 -C28) Corresponding marker with common name(s) oleic acid, emersol 211, red oil, elaidoic acid, metaupon, pamolyn CASb registry number 112-80-1 Analytical methods b 144-62-7 monocarboxylic acids ethanedioic acid oxalic acid, aktisal, (C1 -C4), aquisal, oxaalzuur, oxiric acid dicarboxylic acids (C2 -C5) diethyl phthalate, 1,2phthalates (e.g., benzenedicarboxylic anozol, phthalol, diethylphthalate, solyanol, phthalic acid diethyl ester dimethylphthalate) acid 84-66-2 + + n-alkanoic amides (C12 -C20) decanamide capradide 2319-29-1 + + acyl chloride dodecanoyl chloride lauroyl chloride 112-16-3 + 108-31-6 cyclic acid anhydride maleic acid anhydride 2,5-dihydro-2,5dioxofuran, 2,5-furandione, 2,5-furanedione, cis-butenedioic acid anhydride, cis-butenedioic anhydride +

TABLE 2 Organic compounds families and analytical techniques applied in aerosol studies (Continued)
SETDHPLC ICIC GC/MS GC/MS fluorescence PAD conductivity Comments + Lower molecular weight (MW) mono(C1 -C4) and di-(C2 -C5) carboxylic acids are highly polar and water-soluble. They are determined by IC with conductivity detection (Topping et al., 2004; Yang et al., 2004). Fatty acids (C5 -C28) and high molecular weight (MW) di-carboxylic acids (C6 -C28) are only soluble in organic solvents. SE followed by derivatization (i.e., reaction with silyation agents), which converts the acids into less polar derivatives, is used for GC/MS detection (Kawamura + & Yasui, 2005; Rinehart et al., 2006).

Family name Carboxylic acid

Functional group structural features

Esterd

Amided

Carboxylic acid chloride

Phthalates are the most representative compounds for ester in aerosols. They are nonpolar and soluble in organic solvents (Wang et al., 2006a; Alves et al., 2007). TD-GC/MS **shows fast reaction and higher sensitivity in both online and offline detections** (Welthagen, Schnelle-Kreis, & Zimmermann, 2003; Williams et al., 2006). **Amides are nonpolar and soluble in organic solvents** (Simoneit et al., 2003)]. TD-GC/MS shows fast response and higher sensitivity in both online and offline detections (Welthagen, Schnelle-Kreis, & Zimmermann, 2003; Williams et al., 2006). **These are often components of secondary organic aerosols (SOAs).**

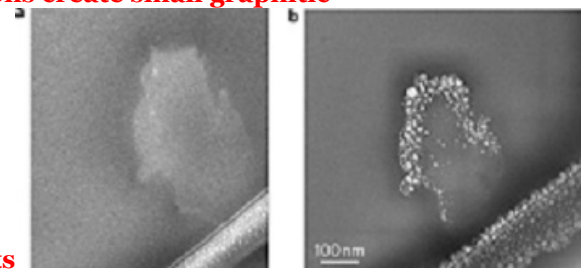
Carboxylic acid anhydride

b CAS: Chemical Abstracts Services. b The most commonly applied analytical method(s) is marked with "+." Methodas are: SE-GC/MS: solvent extraction-gas chromatography/mass spectrometry; TD-GC/MS: thermal desorption-gas chromatography/mass spectrometry; HPLC-Fluorescence: high performance liquid chromatography/ fluorescence detection; IC-PAD: ion chromatography-pulse amperometric detection; IC-Conductivity: ion chromatography-conductivity detection. d Reported in aerosol studies.Downloaded By: [Lovelace Respiratory Research Institute] At: 16:25 21 March 2008

HEALTH EFFECTS OF ORGANIC AEROSOLS
30 25 20 15 10 5 0 0. 18 0. 56 0. 32 2. 5 6 5. 62 <0 .0 5 -0 .1 0 -1 .0 0. 56 1. 00. 10 0. 18 0. 05 6 0. 32 2. 55. 62 10--Elemental Carbon Organics (OC x 1.4)
Ammonium nitrate Ammonium sulfate Geological Trace elements Salt Concentration (µg m) 3 Size Range of MOUDI Stages (µm)

FIG. 1. Chemical composition of aerosol samples in nine size fractions using Micro Orifice Uniform Deposit Impactor (MOUDI) samplers for particles sizes from <0.056 µm to 10 µm at the Fresno Supersite in urban central California, on December 28, 2000, between 1600 and 2400 Pacific standard time (PST), based on data from CARB (2007).

Epidemiological studies found correlations between adverse health outcomes and carbon measurements from British smoke (BS), BC, EC, and/or OC. More than 100,000 OC and EC measurements of PM2.5 or PM10 in ambient and source samples are taken throughout the world each year. OC and EC, as well as total carbon (TC = OC + EC) **are quantified by many different analysis methods based on different combinations of temperature, carrier gas composition, and optical properties** (Watson, Chow, & Chen, 2005). These methods usually provide equivalent results for TC, but OC and EC concentrations differ among methods. As noted earlier, atmospheric EC is never isolated **(such as crystalline graphite or diamond); it always occurs in conjunction with OC and inorganic compounds. The nature of this mixture is responsible for optical properties related to visibility, climate, and health effects. Fresh, oxygenstarved, high-temperature combustion emissions create small graphitic**



platelets that have many

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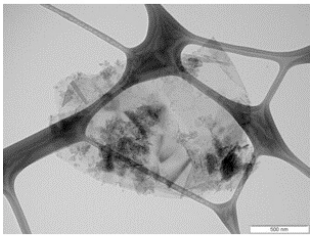
Comment [240]: Notice here the crystalline and diamond material – this is a form oof carbon and the graphine is harder then diamond and here it is in the atmosphere –one has to ask how or why is it even there and how small is it and how is it going to impact the earth and what it touches~ this is a prime source for self assembling of nanoconstructs and with this in the atmosphere and it able to construct the graphene it also has a conduit for transferring signals and energy

reactive edges. These edges may react with the human body when inhaled. As they age, they attract reactive and condensable vapors that may also be



toxic. **Their small size allows penetration into small airways of the lung**. Thermal/Optical Carbon Analyses for OC and EC In the United States, OC and EC measurements are acquired and reported for the nonurban IMPROVE (Interagency Monitoring of PROtected Visual Environments) network since 1986 (http://vista.cira.colostate.edu/views/) and the urban CSN (Chemical Speciation Network, including the Speciation Trends Network [STN]) since 2001 (http://www.epa.gov/ttn/amtic/). Several continuing multiyear data sets are available (e.g., the Fresno Supersite

[http://www.arb.ca.gov/airways/Datamaintenance/default.asp] and SEARCH [SouthEastern Aerosol Research and CHaracterization Study, http://www.atmospheric-research.com/public/ index.html]). The CSN network applies a thermal/optical transmittance (TOT) OC/EC method, often mistakenly called the "NIOSH" method (Birch & Cary, 1996), but differing from NIOSH (1999) as documented by Peterson and Richards (2002). The IMPROVE network applies the thermal/optical reflectance (TOR) method (Chow et al., 1993). TOT and TOR give different results because charring occurs on the surface deposit and on adsorbed organic vapors (Chen et al., 2004; Chow et al., 2004a). Charring takes place at >350 C in the absence of oxygen (O2). Lower molecular weight materials evaporate at <300 C, while more crystalline or graphitic EC oxides more rapidly at higher temperatures. Chow et al. (2001) reported a factor of two between EC by TOT (following a NIOSH-like protocol) and EC by TOR (following the IMPROVE protocol). Watson, Chow, and Chen (2005) show that variations in optical detection methods (i.e., TOR, TOT), analytical atmosphere, and combustion temperatures result in a difference of a factor of two to seven in reported EC concentrations.

Organic Aerosol in Ultrafine Particles (UP) Ultrafine particles (UP; <100 nm aerodynamic diameter) dominate the number distribution but constitute <10% of PM2.5 mass (Chow & Watson, 2006). Figure 1 shows that OC and EC can account for 8090% of UP mass, and is typical of other UP composition measurements (Chow & Watson, 2007). Figure 3 illustrates a diurnal variation of the size distribution at Fresno, an urban site in central California. Two early FIG. 2. Schematic of organic speciation for water-soluble organic carbon (WSOC) and solvent extractable organic carbon (SEOC) based on

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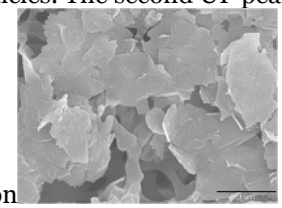
Comment [241]: Ultrafine Particles = NANO Particle –interchangeable terminology

information in Chow et al., 2007b. Pie chart fractions are for illustration; actual values vary by source. **OC = organic carbon**; HULIS = humic-like substances; **PAHs = polycyclic aromatic hydrocarbons**; and **UCM = unresolved complex materials.** Organic compound categories were compiled from references cited in the final column of Table 2. morning peaks were found. A cluster of UP (mass medium diameter [MMD] of 30 nm) was produced around 0500 h PST but was short-lived, since UP either evaporate from small particles or recondense on large particles. The second UP peak was found around 08000900 h in a smaller size fraction  (MMD of 10 nm), indicative of emissions from fresh vehicle exhaust. This peak is pronounced during **midday between 1100 and 1300 h,** representing photochemical nucleation, which starts after the surface inversion breaks up and lasts through the afternoon period. A third peak occurred during the early evening hours and is pronounced between **1900 and 2000 h, representing a combination of evening traffic and cooking with an MMD of 50 nm.** During wintertime, the evening peak is more pronounced as residential wood combustion contributes to the increase in UP concentrations during late evening hours **under stagnant atmospheric conditions**. These types of diurnal variations suggest that **UP number concentrations, and hence chemical composition, varied between day and night**. It is difficult to conduct epidemiological studies that examine health effects of only UP. Recently, studies on the spatial and temporal changes in UP near highways (Morawska et al., 1999, 2002b; Zhu et al., 2002a, 2004) have also demonstrated the importance of using UP concentrations to estimate human exposure and evaluate adverse health effects.

Sources Figure 4 shows the abundances of particulate carbon in different thermal fractions (normalized to PM2.5 mass) for different combustion emission sources. TC in PM2.5 varies from 5% in catalytic carbon to 95% in vehicle exhaust. Watson et al. (1994) **showed that higher temperature carbon (EC2 at 700 C) is more abundant in diesel vehicle exhaust, whereas low-temperature EC1 (at 550 C) is more abundant in gasoline exhaust**. Figure 4 shows that low-temperature OC1 (at 120 C) is abundant (24 ± 12% of PM2.5) in vegetative burning and high-temperature OC3 (at 450 C) is abundant (38 ± 16% of PM2.5) in cooking profiles. The abundance of these carbon fractions varied by source and can be used to relate PM2.5 to sources (Kim & Hopke, 2004; Hopke et al., 2005; Chen et al., 2007; Chow et al., 2007c; Watson et al., 2008).

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100 Diameter (nm) 10 0 5000 10000 15000 20000 25000 30000 35000

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Comment [242]: OC = organic carbon; **HULIS = humic-like substances**; **PAHs = polycyclic aromatic hydrocarbons**; and **UCM = unresolved complex materials.**

FIG. 3. Diurnal variation of the size distribution at Fresno, CA, on September 7, 2002, based on data from CARB (2007).--Figure 5 shows organic marker compounds for wood burning, cooking, gasoline, and diesel profiles (Chow et al., 2007). Benzo[ghi]perylene, indeno[123-cd]pyrene, and coronene are important source markers for gasoline exhaust (Simoneit & Mazurek, 1982; Fraser, Cass, & Simoneit, 2003). Hopanes and steranes are oil residues (Simoneit, 1999; Schauer et al., 2002; Zheng et al., 2002). Individual hopanes and steranes are highest in gasoline exhaust in Figure 5, ranging from 0.006 to 0.11% of PM2.5 mass, with only trace amounts (<0.001%) found in the cooking profile. Fluoranthene and pyrene show higher abundances in diesel exhaust (0.5%) than in gasoline exhaust (0.15%; Fraser et al., 2002, 2003). The diesel profile is also enriched with low molecular weight alkanes such as norpristane, pristane, and phytane. Levoglucosan (Rogge et al., 1998; Simoneit et al., 1999; Poore, 2002; Fine, Cass, & Simoneit, 2004) is abundant (2.5%) in biomass smoke along with guaiacol, 4-allyl-guaiacol, pimaric acid, and syringaldehyde (>0.1%). Retene is enriched by approximately threefold (0.3%) in the wood smoke profile compared to other combustion profiles. Cholesterol (Rogge et al., 1991) constitutes 0.05% of the cooking profile and <0.01% of the gasoline and diesel profiles. Other cooking emission markers include palmitoleic acid, palmitic acid, oleic acid, and stearic acid (Zheng et al., 2006). **Primary organic PM is also contributed by biological sources, and spans a size range from a few nanometers for viruses to over 10 µm for large bacteria, fungal spores, plant pollen grains, and vegetative detritus. The composition of biogenic primary PM is diverse, and there is marked spatial heterogeneity in both composition and concentration due to localized sources.** Primary biogenic PM typically comprises a very small portion of ambient PM, **but can have important local health implications**.

EVIDENCE FOR ADVERSE HEALTH EFFECTS Nature of the Information Evidence that organic aerosols adversely affect public health is convincing overall, but the information is diverse in nature, largely indirect, derived from a number of sources, and not coherently summarized. In addition to their role as PM constituents, organic aerosols have received attention mostly from concerns for either emissions from specific combustion sources or the toxicity of individual organic compounds. **The Clean Air Act deals with several of these compounds among the 188 "hazardous air pollutants" (HAPs, also commonly termed "air toxics," www.epa.gov/oar/oaq caa).** The **majority of HAPs are organic compounds** (e.g., 1,3-butadiene) or classes (e.g., PAHs), or mixtures containing substantial portions of organic matter (e.g., coke oven emissions). Of the 177 HAPs selected for the U.S. EPA 1999 National-Scale Air Toxics Assessment (U.S. EPA, 2006), 167 were organic compounds, classes, or mixtures. The subset of 33 "urban air toxics" listed in Table 3 was selected by the U.S. EPA as warranting the greatest attention (U.S. EPA, 1999b, 2006), and of these, 25 (76%) are organic compounds or classes and 21 (64%) are VOCs in ambient air.

FIG. 4. Carbon fractions in PM2.5 combustion emissions for samples collected in Texas based on information in Chow et al. (2004b). The motor vehicle profile is the composite of 34 San Antonio and Laredo, TX, roadside samples with a

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Comment [243]: Self-assembly of colloidal particles into larger structures has potential for creating materials with unprecedented properties, such as full photonic band gaps in the visible spectrum. Colloidal particles with site-specific directional interactions, so called patchy particles, are promising candidates for bottom-up assembly routes

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Comment [244]: Primary organic PM is also contributed by biological sources, and spans a size range from a few nanometers for viruses to over 10 µm for large bacteria, fungal spores, plant pollen grains, and vegetative detritus. The composition of biogenic primary
PM is diverse, and there is marked spatial heterogeneity in both composition and concentration due to localized sources

This is basically saying that all the PM –particulate matter has either integrated or already integrated and can create a whole array of different materials and effects

mixture of light and heavy-duty diesel and gasoline vehicles. The vegetative burning profile is a composite of 19 wood burning samples including pine, mesquite, tamarisk, huisache, and grass**. The coal-fired boiler profile is a composite of 26 samples using low sulfur western pulverized coal**. The cement kiln profile is a composite of 11 samples from kilns fueled with a combination of low-sulfur Wyoming and New Mexico coal, pet coke, wood chips, and oil filter fluff. The cooking profile is a composite of 12 samples from smoked, charcoal, and propane charbroiled chicken, charcoal charbroiled hamburger, and stir-fried steak. The catalytic cracker profile is a composite of five samples from a refinery heavy oil catalytic cracking unit **(silica alumina matrix catalyst with zeolite, regenerated by natural gas combustion).** Pyrolyzed carbon (OP; not shown) is part of OC (OC = OC1 + OC2 + OC3 + OC4 + OP; EC = EC1 + EC2 + EC3 OP) and varied from 0.2% in catalytic cracker to 4% in vehicle exhaust and 6% in vegetative burning. OC1, OC2, OC3, and OC4 are organic carbon evolved at 120, 250, 450, and 550 C in a 100% Helium (He) atmosphere, respectively. EC1, EC2, and EC3 are carbon fractions that evolve at 550, 700, and 800 C in a 98% He/2% oxygen (O2) atmosphere, respectively. Laser reflectance is used to monitor OP (Chow et al., 1993).

In contrast to the setting of ambient concentration-based National Ambient Air Quality Standards (NAAQS) for criteria pollutants (CPs), HAPs are managed by emission standards. These emission standards have generated less widespread public and political attention, and thus less research emphasis, than the receptor-oriented NAAQS. Few sizeable research programs focus on HAPs exposures and their effects (e.g., the National Urban Air Toxics Research Center, www.sph.uth.tmc.edu/mleland) or HAPs contributions to complex pollution mixtures (e.g., the National Environmental Respiratory Center, www.nercenter.org). In contrast to the predominant concern for the respiratory and cardiovascular effects of ambient exposure to CPs, most HAPs **have been of concern primarily for cancer, developmental effects, or neural effects from both ambient and occupational exposures**. Epidemiological studies provide the most direct evidence linking health outcomes to environmental exposures to organic aerosols. These results are attended by uncertainties about exposures, causality, and other confounding factors. Less direct evidence derives from associations between adverse health outcomes and mixtures comprised of carbonaceous components that are known to be, or can be plausibly expected to be, toxic. These studies do not typically distinguish between the effects of organic and other components of the mixture. They often address occupational exposures with concentration time products having little relevance to nonoccupational exposures. Studies of humans exposed experimentally to single organic compounds or classes, or organic-containing mixtures, fall next on the relevance scale, followed by such studies of mammals. Extrapolation of laboratory results to environmental hazards and risks is often complicated by high or unrealistic instantaneous doses or dosing methods. Experimental human subjects may not adequately model the most susceptible subpopulations**, and animal to human extrapolation is always uncertain.** Progressively further down the relevance

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Comment [245]: Again the comparisons to get the info soft pedaled

scale are results from experiments using intact **nonmammal animals, mammalian tissues,**
Percent of PM2.5 Mass 0.001 1 0.01 10 0.1

Percent of PM 2.5 Mass 0.001 0.01 0.1 10 1

0.0001
fluoranthene pyrene retene indeno[1,2,3-cd]pyrene benzo[ghi]perylene coronene 20S-13 (H),17 (H)-diacholestane 20S-13 (H),17 (H)-diasterane 20S-13 (H), 17 (H)-diasterane 20R-5 (H), 14 (H),17 (H)-ergostane 17 (H), 21 (H)-29-norhopane 17 (H), 21 (H)-29-hopane 22S-17 (H),21 (H)-30,31,32-trishomohopane 22R-17 (H),21 (H)-30,31,32-trishomohopane guaiacol 4-allyl-guaiacol levoglucosan pimaric acid syringaldehyde palmitoleic acid palmitic acid oleic acid stearic acid cholesterol phthalic acid norfarnesane farnesane norpristane pristane phytane PAHs Steranes Hopanes Polar Compounds Alkanes PAHs Steranes Hopanes

Polar Compounds Alkanes

0.0001 fluoranthene pyrene retene indeno[1,2,3-cd]pyrene benzo[ghi]perylene coronene 20S-13 (H),17 (H)-diacholestane 20S-13 (H),17 (H)-diasterane 20S-13 (H), 17 (H)-diasterane 20R-5 (H), 14 (H),17 (H)-ergostane 17 (H), 21 (H)-29-norhopane 17 (H), 21 (H)-29-hopane 22S-17 (H),21 (H)-30,31,32-trishomohopane 22R-17 (H),21 (H)-30,31,32-trishomohopane guaiacol 4-allyl-guaiacol levoglucosan pimaric acid syringaldehyde palmitoleic acid palmitic acid oleic acid stearic acid cholesterol phthalic acid norfarnesane farnesane norpristane pristane phytane Alk anes Polar Compounds Hopanes Steranes PAHs

Cooking
Cooking Uncertainty

Residential Wood Burning

Wood Burning Uncertainty

Percent of PM2.5 Mass 0.01 10 0.001 0.1 1

Percent of PM2.5 Mass 0.01 10 0.001 0.1 1

Diesel

0.0001
fluoranthene pyrene retene indeno[1,2,3-cd]pyrene benzo[ghi]perylene coronene 20S-13 (H),17 (H)-diacholestane 20S-13 (H),17 (H)-diasterane 20S-13 (H), 17 (H)-diasterane 20R-5 (H), 14 (H),17 (H)-ergostane 17 (H), 21 (H)-29-norhopane 17 (H), 21 (H)-29-hopane 22S-17 (H),21 (H)-30,31,32-trishomohopane 22R-17 (H),21 (H)-30,31,32-trishomohopane guaiacol 4-allyl-guaiacol levoglucosan

pimaric acid syringaldehyde palmitoleic acid palmitic acid oleic acid stearic acid cholesterol phthalic acid norfarnesane farnesane norpristane pristane phytane

0.0001
fluoranthene pyrene retene indeno[1,2,3-cd]pyrene benzo[ghi]perylene coronene 20S-13 (H),17 (H)-diacholestane 20S-13 (H),17 (H)-diasterane 20S-13 (H), 17 (H)-diasterane 20R-5 (H), 14 (H),17 (H)-ergostane 17 (H), 21 (H)-29-norhopane 17 (H), 21 (H)-29-hopane 22S-17 (H),21 (H)-30,31,32-trishomohopane 22R-17 (H),21 (H)-30,31,32-trishomohopane guaiacol 4-allyl-guaiacol levoglucosan pimaric acid syringaldehyde palmitoleic acid palmitic acid oleic acid stearic acid cholesterol phthalic acid norfarnesane farnesane norpristane pristane phytane Alkanes Polar Compounds Hopanes Steranes

Gasoline

PAHs

Gasoline

Diesel Uncertainty

Uncertainty

FIG. 5. Organic profiles (percent of PM2.5 mass) for residential wood burning, cooking, gasoline, and diesel, based on information in Chow et al. (2007b). Commonly used organic markers are noted with an arrow and additional markers in brackets.

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TABLE 3 Urban air toxicsa Acetaldehydeb Acroleinb Acrylonitrileb Arsenic compounds Benzeneb Beryllium compounds 1, 3-Butadieneb Cadmium compounds Carbon tetrachlorideb Chloroformb Chromium compounds a b Coke oven emissionsc 1,3,-Dichloropropeneb Diesel particulate matterc Ethylene dibromideb Ethylene dichlorideb Ethylene oxideb Formaldehydeb Hexachlorobenzeneb Hydrazineb Lead compounds Manganese compounds

Mercury compounds Methylene chloride b Nickel compounds Perchloroethyleneb Polychlorinated biphenylsc Polycyclic organic matterc Propylene dichlorideb Quinolineb 1,1,2,2-Tetrachloroethaneb Trichloroethyleneb Vinyl chlorideb

Identified for 1996 National Air Toxics Assessment, completed in 2002 (U.S. EPA, 2006). Volatile organic compounds (VOCs). c **Organic classes or organic families. cultured primary human and animal cells, cultured cell lines,**

bacteria, and finally imputation of hazard from chemical structure in the absence of data from any biological system. Multiple types of evidence exist for hazards from many organic components, and the aggregate weight of evidence from multiple research approaches or biological systems engenders greater confidence than information from any single source alone. For example, we can have reasonable confidence **that environmental exposures to the organic components of vehicle emissions have adversely affected public health, even though we have no direct confirmatory evidence**. Epidemiological studies have shown associations between adverse health outcomes and proximity to traffic. Laboratory studies have demonstrated effects of experimental exposures of humans and animals to whole gasoline and diesel emissions. Many individual compounds in engine emissions have known human toxicities at some dose, such as the carcinogenicity of benzo[a]pyrene (BaP) and the irritant potential of formaldehyde. **Extracts of organic material from PM emissions are known to be mutagenic to bacteria and mammalian cells and carcinogenic when painted on mouse skin**. SVOCs collected from **tailpipe emissions or traffic tunnels have been shown to cause inflammation and cytotoxicity when instilled into rodent lungs**. This aggregate evidence makes it implausible that the organic fractions of engine emissions do not impact public health, **There is both direct and indirect evidence that organic aerosols affect health.** In the preceding example, there is high confidence that organic engine emissions present health hazards, because of the known toxicity of some organic components; however, there has been little, if any, direct confirmation that the organic components have caused the observed effects of emissions. This indirect evidence supports little more than speculation about the portion of health impact due to organic components, the specific physicalchemical species responsible, or the doses presenting significant hazard. Because people are always exposed to organic and other components together, a statistical association with a specific organic compound might result from that species serving as an exposure index of other pollutants or combinations that actually cause some or all of the effect. Epidemiological Evidence Direct Evidence for Effects of Carbonaceous Pollutants **The most direct evidence for the health effects of environmental organic aerosols is provided by primary biogenic particles (often called "bioaerosols"),**

such as viruses, bacteria, pollens, spores, and plant and animal detritus. This evidence is commonly overlooked in discussions of relationships between air quality and health. Most of these health risks are local in nature, and many are primarily of indoor or occupational concern. Perhaps the most widespread environmental example is plant allergens. Throughout the world, particularly in developed countries, allergies to airborne plant pollens exert a substantial toll of morbidity.

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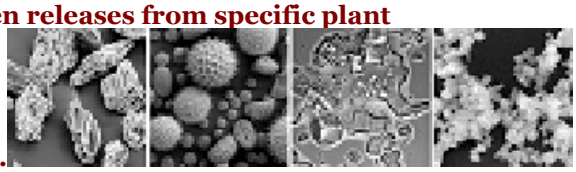
Comment [246]: Interesting with the assessing the pollutants and can determine this with little controversy and yet when it comes to the jets and there contribution just on there exhaust levels is not being properly determined and then you cannot determine the pollution contribution from there chemtrails

The causal role of specific OC in pollen proteins has been repeatedly confirmed by testing for pollen allergies and by the linkage of effects to pollen releases from specific plant species.  **Pollen allergies present one of the best-documented environmental "natural experiments" concerning organic aerosols. The widespread planting of cedar trees (Cryptomeria japonica) during post-World War II reconstruction in Japan resulted in a burgeoning "cedar pollinosis" problem that continues growing and now affects 16% of the population (Xiao et al., 2007). The frequent use of filter masks in the spring by Tokyo residents is more an attempt to reduce exposure to pollen than to traffic emiss**ions or other air contaminants (personal communication, Dr. Kazuhito Maejima, Japan Automobile Research Institute, Tsukuba, Japan). The rarity of mortality, the fact that most sufferers manage with small loss of productivity, and the perceived difficulty of regulating the sources have kept the health burden of OC aeroallergens beyond the attention of most researchers. ---Few epidemiological studies have incorporated measures or estimates of exposures to carbonaceous species. BC is crudely indexed by the BS filter darkening method for estimating PM levels that were linked to health by several studies (Muir & Laxen, 1995, 1996; Horvath, 1996; Penttinen et al., 2000; Anderson et al., 2001; Lai et al., 2006). Although BC strongly influenced the measure of exposure, such studies only suggest that BC and/or its associated organic compounds actually contributed to the health outcomes. A hospital admissions study in London (Atkinson et al., 1999) **found that both PM10 and BS were significantly associated with admissions for cardiovascular effects, but BS had the stronger association, as shown by the greater persistence of its association when multiple pollutants were included in the model**. This result strongly suggested, but did not confirm, that the carbonaceous components of PM were important contributors to admissions. Mar et al. (2000) incorporated further carbon speciation in their study of associations between organic aerosol and total nonaccidental mortality and cardiovascular mortality in Phoenix, AZ, during 19951997. Data from 9276 deaths of residents in ZIP codes near a U.S. EPA monitoring site were compared to concentrations of PM10 , PM2.5 , and CP gases at various lags (days) between exposure and outcomes. The PM2.5 was analyzed for OC, EC, and elements. Results for the components of PM2.5 and gases were only reported for cardiovascular mortality. **Positive significant associations of similar magnitude were found between mortality and CO, nitrogen dioxide (NO2), sulfur dioxide (SO2), PM10 , PM2.5 , soil-derived potassium, OC, EC, and TC**. Such results suggest that OC and EC can have health importance equal to, or greater than, those of commonly measured pollutants. Ostro et al. (2006) examined associations between daily mortality in nine California counties and components of PM2.5 , including EC. A greater range of

PM components was evaluated than in the Mar et al. study, but gaseous pollutants were not included. Significant associations were found between cardiovascular mortality and PM2.5 , OC, and EC**. None of these three variables was significantly associated with respiratory mortality**. The results from the Mar and Ostro studies suggest that OC and EC contributed to the effects, although they did not indicate that carbonaceous components of PM2.5 contributed more strongly to effects than the other components. OC and EC were significant in the Mar study at both 1- and 3-day lags, and in the Ostro study at a 3-day lag. **The fact that different pollutants can have different lags between increases in exposure and effect, as could occur due to different biological mechanisms, complicates the resolution of cause - effect relationships from epidemiological data**. Among recent studies, the impact of organic aerosol on public health is most comprehensively evaluated in the **Aerosol Research and Inhalation Epidemiology, study (ARIES),** which has operated continuously in Atlanta, GA, and other locations in the southeastern United States since the late 1990s (Klemm et al., 2004; Hansen et al., 2006). The ARIES exposure estimates are --based on data from an urban-scale monitoring site at which numerous PM and gaseous pollutants are measured. Associations with health outcomes are evaluated for both individual PM components and for selected species serving as indicators for physical-chemical classes. Klemm et al. (2004) included PM2.5 and PM10-2.5 mass as well as OC, EC, SO2- , and NO- . Gaseous pollutants included 4 3 NMHC and OHC. Daily mortality in persons aged >65 yr was associated with multiple pollutants. PM2.5 mass was significant for all causes of mortality, as were the CP gases. The association of mortality with PM2.5 TC was nearly, but not quite significant. Associations for NMHC and OHC were also positive, but not quite significant. In a time-series analysis, 3-day moving averages of **toluene (indicative of evaporative mobile source emissions) were significantly related to both cardiovascular and respiratory visits, and 2-butanone (indexing SOA) was significantly related to visits for ischemic heart disease** (Tolbert et al., 2001; Klemm et al., 2004**). VOCs were significantly related to all cardiovascular visits, with strongest associations for ischemic heart disease, myocardial infarctions, and congestive heart failure**. Both NMHCs and OHCs contributed to the associations, but the strongest associations were found for aromatic compounds, toluene, and alkenes. There was no significant association between total VOCs and total respiratory emergency visits, but NMHCs were significantly associated with asthma in the summer, upper respiratory disease in the winter, and chronic obstructive pulmonary disease (COPD**). Significant associations were also found between cardiovascular visits and several thermally derived carbon fractions** (see Figure 4), but few associations were found for respiratory visits. Initial conclusions include: Among VOCs, NMHCs appear to be of greater concern than OHCs; among **PM carbon, the more volatile** OC and low temperature EC (Figure 4) **appear to be of greater concern** than less volatile fractions; and **there is little evidence that polarity per se is an important determinant of hazard**. Lipfert et al. (2006) tested associations between mortality in U.S. military veterans and county-level data for PM2.5 (including OC

and EC), traffic density, and CP gases. Overall, traffic density was most closely **related to mortality in both single- and multipollutant models.** Among the PM components, EC was most significantly related to mortality; OC had a smaller, non significant relationship. These results suggest the importance of exposure to traffic emissions, and especially to the EC component. The study did not provide information on the importance of VOCs or other non-PM organic emissions that would have accompanied PM. There is a wide range of epidemiological evidence for **adverse health effects from occupational exposures to organic compounds that are also found in ambient air at lower concentrations**. Several HAPs are listed on this basis. Effects of occupational exposures can be taken as direct evidence for a potential environmental hazard, but they do not confirm that environmental exposures to lower concentration affect public health. Data for human dose-response relationships at environmental concentrations exist for few of these compounds. The U.S. EPA (2006) estimated unit risks in the environmental concentration range assuming linear, no-threshold responses. **These compounds included respiratory irritants (e.g., several carbonyls and acids), carcinogens (e.g., benzene, PAHs), neurotoxins (e.g., toluene, perchloroethylene), and reproductive and developmental toxins (e.g., ethylene dibromide, hexachlorobutadiene). The U.S. EPA (2006),** the International Agency for Research on Cancer (IARC, www.iarc.fr), and Klassen (2001) provide more detailed listings and literature citations.--Indirect Evidence for Effects of Carbon-Containing Mixtures Many epidemiological studies have associated health outcomes with exposures to mixtures containing carbonaceous pollutants having known toxicity and present in sufficient quantity to have plausibly contributed to the effects. **Exposure to engine emissions is an example of a combustion-derived mixture containing organic gases and particles**. **There are significant associations between the proximity of homes and schools to heavily used roadways and various adverse health outcomes in adults and children**. Brunekreef et al. (1997) **found that reduced lung function of school children was related to proximity of homes and schools to heavy traffic, with stronger associations for truck than for automobile traffic, and significant associations with BS measured at the school. Hoek et al. (2002) found that living near a major roadway was associated with relative risks of 1.95 and 1.41 for cardiopulmonary and total mortality, respectively, among 55- to 69-year old adults during the period 1986-1994**. **Several conceptually similar studies in multiple countries have yielded similar result**s. In a recent study, Tonne et al. (2007) **found significant associations between the proximity of Massachusetts residences to traffic and acute myocardial infarctions**. The associations with traffic emissions, although convincing in aggregate, do not directly implicate the carbonaceous components. The significance of BS in Brunekreef et al. (1997) suggested the importance of EC, but EC could have been serving as a marker for unmeasured emissions. However, because fresh diesel, gasoline, and natural gas engine emissions as encountered near roadways contain substantial amounts of gaseous and PM organics, and many of them are known to be toxic at some concentration, the results strongly

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Comment [247]: Is there a threshold below which no effects on health of PM are expected to occur in all people?
Answer:
Epidemiological studies on large populations have been unable to identify a threshold concentration below which ambient PM has no effect on health. It is likely that within any large human population, there is such a wide range in susceptibility that some subjects are at risk even at the lowest end of the concentration range.

suggest that OC and EC emissions probably contributed to the effects. There has been considerable attention to the potential for lung cancer risk from environmental exposures to diesel exhaust (reviewed in California Environmental Protection Agency, 1998; Lloyd & Cackette, 2001; U.S. EPA, 2002; Mauderly & Garshick, 2007), but the actual magnitude of risk remains uncertain. The **plausibility of cancer risk derives largely from the organic components** in diesel PM (DPM**) that are mutagenic to bacteria and carcinogenic to mouse skin when extracted and administered at high doses**. Evidence for lung cancer from animals exposed by inhalation to diesel emissions has been negative at environmental concentrations, and all but the most extreme occupational concentrations. Epidemiological studies of railroad workers, truck drivers, and other occupations having higher than environmental exposures to diesel emissions have demonstrated relative risks averaging 1.4 for lung cancer and ranging upward to over 2.0. To date, however, no published study has included actual measures of exposure of the study cohorts, and estimates of exposure based on job title and the time course of introduction of diesel engines into the workplace are uncertain. The California EPA (1998) and the South Coast Air Quality Management District (SCAQMD, 2000) have estimated cancer risks in the range of 300 additional cancers per million persons per microgram per cubic meter of lifetime exposure to DPM. In view of uncertainties, the U.S. EPA has not adopted a risk value, but has noted that risks may be in the above range (U.S. EPA, 2002). Several agencies and organizations have classified diesel emissions or DPM as probable or known carcinogens. Both OC and EC emissions from new on- and off-road diesel engines are falling rapidly in response to regulations (Lloyd & Cackette, 2001; Chow, 2001), and there are as yet no health data on the most recent emissions**. Overall, it can be reasonably assured that carbonaceous emissions from older diesel engines have contributed, and continue to contribute, to the health impacts of organic aerosols.** Smoke from vegetative burning is another mixture containing carbon in the particulate and gaseous phases and contributing to the ambient organic aerosol (McDonald et al., 2006; Laeher et al., 2007). There is considerable evidence that smoke from residential wood burning, controlled burning of vegetation, and wildfires (Jacobs, Kreutzer, & Smith, 1997; Kunzli et al., 2006; Laeher et al., 2007) has impacted health**. Exposures to wood smoke have been associated with mortality, reduced respiratory function, exacerbation of asthma, and respiratory symptoms**. Considering the organic composition of wood smoke and its content of known irritants, mutagens, and carcinogens, it is very likely that the carbonaceous components of smoke contribute to its health effects. Indoor-generated carbonaceous pollutants can also contribute to the environmental organic aerosol. **Carbonaceous combustion products are released to the environment from heating and cooking processes**. **Carbonyls such as formaldehyde and acrolein in substantial concentrations have been measured in restaurant exhaust, and may add to environmental concentrations (Ho et al., 2006). Fumes from heated vegetable and animal oils are known to be genotoxic and to cause oxidative stress in mammalian cells** (Dung, Wu, & Yen, 2006). Perhaps the most prevalent, and paradoxically

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Comment [248]: Fast foods that have there oils heated at 200+ degrees at the minimum and this would as well transfer over to the food

the most indirect, epidemiological evidence for the health importance of carbonaceous pollutants results from their constituting a portion of the ambient **PM mass. A large body of evidence associates PM mass and number concentrations with adverse health outcomes** (U.S. EPA, 2004b; Pope & Dockery, 2006). Figure 1 shows that all respirable PM size classes contain OC and EC. Because few epidemiological studies have included speciation of PM components, the default regulatory assumption remains that all components contributed to the effects in proportion to their relative mass. On that simple basis, 1050% of the total health burden from PM could be attributed to carbonaceous material because organic matter typically comprises 1040% of PM mass (U.S. EPA, 2004a). Current research programs, such as the U.S. EPA PM Research Centers (http://epa.gov/ncer) and the Health Effects Institute National Particle Components Toxicity (NPACT) program (www.healtheffects.org) are focusing on identifying causal components of PM, and may help to clarify the health impacts of OC and EC. Other Evidence Experimental exposures of humans, animals, and cells to components found in the environmental organic aerosol provide evidence for their health importance. These include realtime exposures to: (1) whole atmospheres (e.g., city air, on-road exposures); (2) atmospheric components (e.g., concentrated ambient particles [CAPs]); (3) simulated environmental mixtures (e.g., SOA created in the laboratory, combustion mixtures); and (4) specific organic compounds. **The results confirm the biological activity and potential health hazard of many carbonaceous species,** but only a few have confirmed that actual environmental exposures or doses cause harm. Real-Time Exposures to Environmental Aerosols or Components A few attempts have been made to expose animals directly to ambient air in locations having high pollution or representing different microenvironments. It has proven challenging to detect significant effects at ambient levels, and characterizations of exposures have seldom included OC or EC. Although the following studies exemplify those most likely to have been influenced by carbonaceous species, no study has provided explicit evidence that the carbonaceous components caused the observed effects. Elder et al. (2004a, 2007) exposed aged normal and spontaneously hypertensive rats, with and without prepriming with endotoxin or influenza virus, to on-road interstate highway atmospheres using a mobile laboratory. **Health endpoints included bronchoalveolar lavage (BAL) indicators of inflammation and inflammatory cell activation, plasma fibrinogen and endothelin, and electrocardiogram**. The PM number counts ranged from 0.95 to 3.13 × 105 /cm3 , PM mass was estimated (not measured) at 37 to 106 μg/m3 , and measured BC mass averaged 4.0 μg/m3 (Kittelson et al., 2004). OC was not measured. Concentrations of CO2 , CO and NO averaged 413, 8.6, and 0.19 ppmv, respectively. Since the UP in this study were composed primarily of volatile organic material (Kittelson et al., 2004), and gasoline and diesel emissions are known to contain VOCs and SVOCs in mass concentrations that can exceed PM (McDonald et al., 2004b, 2007), it may be assumed that OC comprised a substantial portion of the total exposure. Exposures of both normal and primed aged rats caused significant changes in indicators of inflammation and vascular endothelial activation. In a comparison using normal and endotoxin-primed rats,

Elder et al. (2007) found no difference between effects of exposure to whole and filtered atmospheres. Exposure to the whole atmosphere resulted in decreases in heart rate and heart rate variability (HRV; Elder et al., 2007). The focus was on UP; however, the comparison of filtered versus unfiltered atmospheres indicated that non-PM components caused the effect and both VOCs and SVOCs would have been present in substantial concentrations. Elder et al. (2004a) did not clarify the role of carbonaceous components, but it is reasonable to assume that they contributed to the effects. Although there have been many studies of animals exposed to CAPs, few have provided information on the chemical species most closely correlated to adverse effects. Real-time CAPs exposures also include exposure to non-PM pollutants, but they are not characterized in most studies. Saldiva et al. (2002) exposed normal rats and rats **with SO2 -induced bronchitis** to Boston CAPs collected near a busy street and measured lung inflammation by BAL and histopathology. The relationships between inflammation and OC and EC were significant, but not more significant than relationships with inorganic components **(silicon [Si], vanadium [V], bromine [Br], lead [Pb], SO2-)**. Using 4 the same system, Wellenius et al. (2003) examined the effects of exposure to Boston CAPs on the heart rate and electrocardiogram of dogs with experimental myocardial ischemia exposed to 345 μg/m3 PM for 6 h/day for 3 consecutive days. Significant associations were found for Si, which was assumed to derive from street dust. No significant association was found for either OC or EC, although together they comprised 26% of the mass. Non-PM OC was not measured in either study. Lippmann, Gordon, and Chen (2005) exposed atherosclerosis-prone ApoE-/- mice 6 h/day, 5 days/wk for 5 mo to an average of 113 μg/m3 CAPs at the rural New York University Sterling Forest laboratory. They examined relationships between heart rate and HRV and four source-oriented compositional categories based on factor analysis (Maciejczyk & Chen, 2005). The category containing OC and EC was termed "secondary sulfate," although the mass concentration of OC (15 μg/m3) was greater than that of sulfur (S; 12 μg/m3). The most significant associations were found for the category attributed to residual oil combustion on the basis of nickel (Ni) and V loadings. However, the category containing OC and EC was associated with reduced heart rate during the afternoon, and reduced HRV at night (outside of exposure hours). The study did not clarify the role of the carbonaceous PM components, but they could have contributed to the effects.

Exposures to Carbonaceous Materials Collected in the Environment Animals or cells have been exposed in the laboratory to carbonaceous materials collected in the environment. **The largest number of such studies has involved exposures of bacteria or mammalian cells to solvent extracts of the organic component of PM, most frequently using mutagenicity as an indicator of carcinogenic potential** (Lewtas, 1988, 2005). Most studies were aimed at one of three goals: **(1) estimating carcinogenic potential; (2) determining the causal chemical species; or (3) evaluating the impacts of different combustion conditions**. Relative mutagenicity has also been used to develop potency rankings for known human carcinogens, in order to estimate hazards or risks from emissions or materials for which

epidemiological data were insufficient. A classic example was the estimation of carcinogenic risks from diesel exhaust based on the comparative mutagenicity of diesel, coke oven, and roofing tar emissions, and tobacco smoke extracts (Albert et al., 1983). An approach termed "**biodirected fractionation**" has long been used to identify the most mutagenic chemical classes and compounds in extracts of combustion PM. Extracts are divided iteratively into subclasses and individual compounds by chemical fractionation, **and the fractions are tested for specific mutagenic activity per unit of mass** (Schuetzle & Lewtas, 1986). A recent example was the study by DeMarini et al. (2004), who showed that the chemical classes **driving the bacterial mutagenicity of DPM extracts** differed among samples from different sources. Tests have also been done to determine the influences of engine type, fuel, and operating condition on the mutagenicity of DPM extracts (Clark et al., 1984; Bechtold et al., 1984). Although the biological activity of extracts provides evidence for the importance of PM-borne OC, there are caveats. **The doses applied to the biological system are typically high compared to ambient levels**. The extent to which material extracted using solvents, ultrasound, heat, etc. mimics material that would be released from PM in vivo (i.e., "bioavailability") is uncertain. Extrapolation from single-cell systems to human hazards and risks is uncertain. **Moreover, the extracts can also contain inorganic elements and compounds** (Molinelli et al., 2006). Many investigators have conducted laboratory studies of PM collected in the environment, but few studies were designed to determine the influence of the carbonaceous components. The largest effort to date was the Respiratory Allergy and Inflammation Due to Ambient Particles program that evaluated numerous PM samples collected in multiple European cities during different seasons (Steerenberg et al., 2006). PM was extracted in methanol from collection media, analyzed chemically, and tested using several in vivo and in vitro assays of inflammatory, cytotoxic, and adjuvant potentials that yielded 13 response variables. The fractional contents of OC and EC were not reported, but 28 extracted OC species were measured. Correlations between each composition and response variable were reported, and composition-based cluster analysis was used to infer major PM sources. Correlations varied among both composition and response variables in a complex manner that defies simple summary; however, correlations with the OC components were found. Li et al. (2003) exemplifies a different approach that implicated OC. UP (<0.15 μm), PM2.5 , and PM10-2.5 CAPs were collected from two urban sites in the Los Angeles area. The UP fraction averaged 70% OC and 12% EC; the PM2.5 and PM10-2.5 fractions contained progressively lower carbon fractions. The content of PAH and the potential for forming reactive oxygen species (determined using the dithiothreitol assay) were greatest for the UP fraction. The UP fraction also caused the greatest oxidative stress in a mouse macrophage cultured cell line. Li et al. (2003) speculated that PAHs were largely responsible for the oxidative injury. Both UP and PM2.5 were internalized by cultured bronchial epithelial cells. However, PM2.5 was found only in the cytoplasm while UP penetrated into the mitochondria where it caused damage. Although the most toxic chemical species were not resolved, the study presents evidence for the toxicity of ambient OC. Laboratory studies have also included

multiple components of ambient organic aerosols. Seagrave et al. (2001) compared the lung inflammatory potential of SVOC, PM, and the combined masses collected from a traffic tunnel. SVOC was collected in a resin trap (PUF/XAD) and PM was collected by filter. Rats were instilled intratracheally with the combined SVOC-PM mass in their original collection ratio or with either material separately, **and lung inflammation was measured by BAL at 24 h**. **Both fractions were inflammogenic, and their combined effect was approximately additive; however, the potency of the SVOC per unit of mass was fourfold that of PM, attesting that SVOC in the vicinity of fresh engine emissions has inflammatory potential that may equal or exceed that of PM**. Of course, the caveats concerning dose, bioavailability, and extrapolation to human hazard pertain. A less direct approach (Seagrave et al., 2006) employed multivariate statistical analysis to impute biological effects to carbonaceous components of ambient PM rather than testing individual fractions. PM2.5 from urban and rural areas of the southeastern United States in winter and summer was extracted from filters by sonication using both organic solvent and water. The recombined material from each sample was analyzed chemically and tested for lung inflammatory potential by instillation into rat lungs followed by BAL (as in Seagrave et al., 2001). Principal component analysis linked to partial least squares regression was used to determine the chemical components that co-varied most closely with toxicity. A chemical mass balance method was used to assign source contributions to each sample, so relationships between source and toxicity could also be explored. The results indicated that OC and EC, and combustion sources (primarily vehicle exhaust) **were most strongly associated with lung inflammatory potential.** Although this approach does not confirm the roles of the carbonaceous components or sources and is attended by the usual extrapolation caveats, the results strongly implicate OC and EC, and their predominant sources. Exposures to Laboratory-Generated Organic Aerosols Studies of **ambient PM have provided little distinction between the effects of primary OC and EC emissions and effects of SOA formed by condensation of SVOCs or chemical reactions involving anthropogenic or biogenic VOCs**. Studies of laboratory-generated aerosols, however, have provided evidence that **SOA can exert health effects**, although only a small range of chemical species has been studied to date. Studies of mice **exposed to aerosols formed by reacting terpenes (-pinene, d-limonene, and isoprene) with O3 demonstrated that the reaction products caused airway irritation and airflow limitation that were not caused by the precursor gases alone** (Clausen et al., 2001; Rohr et al., 2002; Rohr, Shore, & Spengler, 2003; Wilkins et al., 2003). **Eye-only exposures of humans to similar precursors and reacted mixtures also demonstrated the formation of irritant aerosols, using blink frequency as an indicator** (Klena & Wolkoff, 2004). The reacted mixtures contained **volatile aldehydes, ketones, and carboxylic acids as well as secondary PM.** However, the correspondence of the magnitude of the irritant effects with the extent of formation of UP among the mixtures suggests that PM OC dominated the effects**. The studies just mentioned addressed common indoor VOCs**, but similar reactions between oxidant gases and both

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Comment [249]:
➢Particulate matter (PM)—
➢Airborne particulate matter represents a comple x mixture of organic and inorganic substances. ➢ Mass and composition in urban environments tend to be divided into two principal groups: coarse particles and fine particles. ➢The barrier between these two fractions of particles usually lies between 1 µm and 2.5 µm-- ➢Particulate air pollution is a mixture of solid, liquid or solid and liquid particles suspended in the air. These suspended particles vary in size, composition and origin
➢The size of suspended particles in the atmosphere varies over four orders of magnitude, from a few nanometres to tens of micrometres

anthropogenic and biogenic VOCs occur outdoors. Oxidation by O3 may also enhance the toxicity of PM-borne OC, as indicated by the finding that treatment of DPM with O3 enhances its biological activity in animals (Madden et al., 2000) and cultured cells (Kafoury & Kelley, 2005). The largest body of information on the health effects of laboratory-generated organic aerosols derives from studies of animals and humans exposed to engine emissions and wood smoke. Although these combustion mixtures contain substantial portions of OC in the PM, SVOC, and VOC phases (McDonald et al., 2004b, 2006, 2007), none of these studies has isolated effects of the OC components. A broad range of adverse health outcomes has resulted from exposures to whole emissions from diesel engines of many types burning diverse fuels and operated either at steady state or on variable duty cycles. **Short, single exposures of humans have caused transient airway and lung inflammation and increased airway response to broncho constricting drugs, with little difference in response between normal and asthmatic subjects** (Stenfors et al., 2004). Similar exposures have caused changes in peripheral arterial flow (Mills et al., 2005). Lung inflammatory responses also occur in rodents, which can develop chronic, progressive noncancer disease at extreme exposure concentrations. **Near lifetime exposures to extreme concentrations have caused increased lung tumors in common laboratory strains of rats, but not in mice or Syrian hamster**s (Mauderly & Garshick, 2007). This difference led to the understanding that the carcinogenicity in rats was a **species-specific response to heavy, progressive loading of the lung with poorly soluble particles, could be duplicated with EC or noncarbonaceous PM alone, and had little or nothing to do with PM-borne OC** (Mauderly & McCunney, 1996). Repeated exposures of rodents have also **amplified allergic responses to antigens, reduced resistance to lung infections, altered heart rate and rhythm, and induced reproductive and developmental effects**. These findings support the plausibility that environmental exposures to diesel emissions contribute to these health hazards; however, few of these effects have been demonstrated at even an order of magnitude higher than environmental exposure levels. There have been fewer studies of laboratory-generated whole gasoline emissions (McDonald et al., 2007) than of diesel emissions, and the studies have been limited to animals. Extreme exposures to gasoline exhaust emissions have induced a broad range of respiratory, cardiovascular, and extrapulmonary effects. Naeher et al. (2007) reviewed studies of animals and humans exposed experimentally to wood smoke. As for the other combustion emissions, **exposures to high concentrations have been shown to induce irritant and inflammatory effects and to amplify allergic responses**. Few studies have attempted to determine the physical chemical species causing the effects of combustion emissions, and none (except the association of rat lung tumors with diesel EC) has isolated effects to the carbonaceous components. **The plethora of irritant, O2 radical-generating, hormone- and antigen-mimetic, mutagenic, and carcinogenic species among the PM, SVOC, and VOC phases of fresh combustion emissions makes it plausible that all of them could contribute to adverse effects**. Although the overwhelming focus of pollutant research during the past decade

has been on PM, substantial and **growing evidence confirms that non-PM components of combustion emissions are important to many health effects**. The irritant and inflammatory effects of single exposures of humans to diesel emissions were not altered by insertion of a ceramic PM trap (Rudell et al., 1999). Removal of PM by filtration did not significantly reduce the effects of repeated exposure of rodents to diesel emissions on: (1) amplification of allergic responses (Maejima et al., 2001; Watanabe & Ohsawa, 2002); (2) reduction of sperm and Sertoli cell counts (Watanabe, 2005); (3) retardation of testis, ovary, and thymus development (Watanabe & Kurita, 2001); or (4) changes in heart rhythm (Campen et al., 2005). The effects of on-road exposures on lung inflammatory responses of mice were not significantly affected by filtration (Elder et al., 2004b). Lund et al. (2007) found that the effects of repeated exposure to gasoline emissions on oxidative stress and pro-atherosclerotic tissue changes in aortas of mice were not reduced by removal of PM using high-efficiency particulate air (HEPA) filtration. Although these results do not confirm involvement of SVOCs or VOCs, they indicate that research on organic aerosols should not be restricted to PM-borne OC. Exposure to Components of Laboratory-Generated Organic Aerosols Components of organic aerosols generated in the laboratory and studied as single fractions or simple combinations can exert a wide range of biological effects. Although these studies do not evaluate environmental aerosols directly and typically employ unrealistically high doses, they provide evidence for the plausibility that the same components in the environment contribute to the effects of air pollution. An example of such studies was a series that used multivariate analysis to explore the components of engine emissions driving lung inflammation and cytotoxicity. Zielinska et al. (2004) collected PM (filter) and SVOC (PUF/XAD trap) from the exhaust of normal and high-emitting gasoline and diesel vehicles recruited from public use and tested on a chassis dynamometer in the laboratory. The two fractions were extracted, chemically analyzed, recombined in their original mass ratios, and tested by instillation into rat lungs followed by BAL (Seagrave et al., 2002). **The seven resulting samples differed in both chemical composition and toxicity, providing a database suitable for multivariate analysis**. Principal component analysis linked to partial least squares regression yielded chemical-based models that closely predicted differences in toxicity, and showed hopanes and steranes to covary most closely with toxicity (McDonald et al., 2004b). The predominance of those oil-derived OC species in the explanatory models was consistent with the finding that emissions from high-emitting vehicles, largely oil burners, were more toxic per unit of mass than emissions from properly functioning vehicles. A subsequent study using emissions from new, mid-life, and retired compressed natural gas city buses also pointed toward hopanes and steranes (Seagrave et al., 2005). Although the statistical relationships in this and the earlier mentioned studies did not confirm that hopanes and steranes actually caused the toxicity, they pointed to the importance of PM-borne OC in general, and crankcase oil emissions specifically, in the lung toxicity of engine emissions. Campen et al. (2005) obtained results more directly confirming the importance of the vapor-phase SVOC and VOC components of engine emissions. They bubbled fresh diesel

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Comment [250]: Organic Carbon-OC Volatile organic compounds (VOCs) semi- volatile organic compounds (SVOCs)

emissions through saline solution that was then filtered and shown to contain numerous volatile carbonyls and alkanes, but only traces of semivolatile PAHs. Fresh preparations of mouse coronary arteries were perfused with control or diesel-treated saline, and vascular responses to vasoconstricting or vasodilating drugs were measured. Exposure to the diesel-treated saline did not alter baseline vascular tone, but caused increases and decreases in responses to vasoconstrictors and dilators, respectively. Campen et al. (2005) hypothesized that VOC components caused the effects. A broad range of biological effects of laboratory-generated DPM collected from filters has been studied in the absence of other emission components. DPM has been experimentally administered to humans, animals, or cells, often with little characterization of the material and only a vague description of its source. Some caution is warranted in interpreting these results because of artifacts that can occur during filter collections, including absorption or desorption of VOCs and SVOCs, and oxidative transformation or nitrosylation of PM-borne OC species (Arey et al., 1988; Khalek, 2004). Few of these studies provide insight into the components causing the effects. Results of studies using a DPM sample collected at the Japanese Institute for Environmental Studies suggest the likely importance of the OC component for noncancer health outcomes, and also illustrate the diversity of composition and toxicity that can occur among different DPM samples (Arey, 2004). This sample was collected under relatively cool conditions, consisted of 50% OC and 9% EC (Singh et al., 2004), and was found to enhance allergic responses of humans (e.g., Diaz-Sanchez et al., 1997) and rodents (e.g., Muranaka et al., 1986) to various antigens (i.e., an "adjuvant" effect; reviewed in Mauderly & Garshick, 2007). The finding by a UCLA research group that this DPM, solvent extracts, and pure phenanthrene caused similar stimulation of lymphocytes in vitro (Takanaka et al., 1995; Tsien et al., 1997) was taken to infer that the OC component (inaccurately termed "PAHs" in the papers) drove the effects. Singh et al. (2004) found the lung toxicity of this sample to differ from that of a National Institute of Standards and Technology (NIST) diesel forklift-derived reference sample (SRM 2975) comprised of only 5% OC and 60% EC. When administered to mice by aspiration, the Japanese sample resulted in a primarily macrophage cellular response, while the NIST sample showed a primarily neutrophil response. The Japanese sample caused greater increases in proinflammatory cytokines. Solvent extracts of neither sample produced significant effects in mouse lungs. DeMarini et al. (2004) also found that although extracts from both samples **were mutagenic to bacteria**, the greatest activity was in the hexane-extracted fraction (low polarity) of the Japanese sample and the methanol fraction (high polarity) of the NIST sample. In aggregate, these results suggest the potential importance of PM-borne OC in noncancer effects and show the variability in composition and biological activity that can occur within a single source category.

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Comment [251]: polycyclic aromatic hydrocarbons (PAHs)

Studies of Single Organic Classes and Compounds

There is a large literature demonstrating that many individual organic classes and compounds found in the environment are toxic in some biological system at some dose. The ranges of compounds studied and biological responses demonstrated are too great to review here, but numerous examples are

summarized in toxicology texts (e.g., Klassen, 2001). It is sufficient to note that confirmation of the toxicity of single compounds may establish that a potential health hazard exists, but that information alone does not lead to the conclusion that meaningful health risks result from environmental exposures. Biological activity must be linked **to environmental exposure conditions (i.e., concentration × time) in order to place environmental risks in context**. As noted in preceding sections, the majority of the 188 HAPs are organic compounds or classes, and each is listed because its toxicity has been demonstrated in some manner. Although some also have natural sources, most HAPs have been studied primarily because of their anthropogenic, and thus potentially manageable, sources. **These include: (1) common species such as formaldehyde, naphthalene, and toluene; (2) widespread but more localized species such as methylene chloride or perchlorethylene; and (3) less widespread and more localized species such as hydrazine or the herbicides, insecticides, and rodenticides**. The toxicity of most species is an unintended consequence, but some are used explicitly because of their toxic properties. The environmental organic aerosol also contains many individual biogenic components, such as the terpenes and other species that participate in forming secondary PM, pollens, fungal spores, bacteria, and viruses. With the exception of pollens, the effects of environmental exposures to noninfectious biogenic OC have received little attention. The hazards of most infectious agents are well established. However, although outdoor exposures to infectious agents undoubtedly occur, indoor exposures are thought to be a much more important mode of transmission.

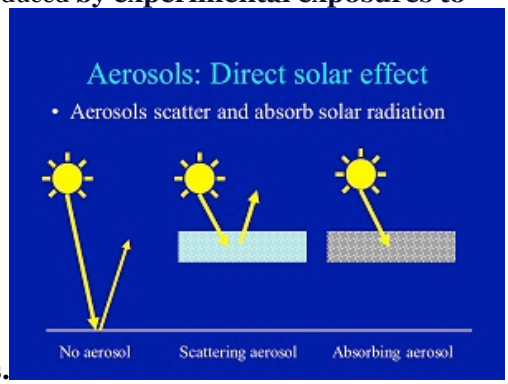
KEY KNOWLEDGE GAPS

This section summarizes gaps in knowledge constituting key limitations to understanding the nature and importance of environmental organic aerosols, and **the most appropriate approaches to minimizing their effects**.

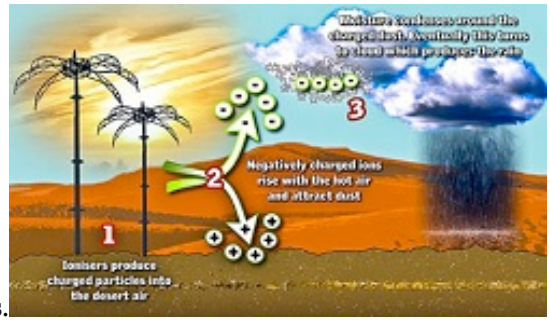
Characterization and Exposure Spatial and Temporal Scales Most networks are compliance oriented and do not represent impacts from nearby sources. Besides the urban CSN and rural IMPROVE networks, the U.S. EPA compliance networks only measure mass, not organic aerosol. Long-term speciation networks lack source-oriented sites that would help to estimate exposures. Monitoring sites to represent human exposure should be selected to represent different scales (U.S. EPA, 1997; Chow et al., 2002), including: (1) indoor- or collocated-scale or ducted emissions (110 m, distance indicates the diameter of a circle with the monitor at its center); (2) microscale (10100 m); (3) neighborhood scale (500 m4 km); (4) urban scale (4100 km); (5) regional-scale (1001000 km); (6) continental-scale background (100010,000 km); and (7) global-scale background (>10,000 km). Collocated scales are used to define precision of the monitoring method. Concentrated emissions for effluent pipes and smoke stacks are used to establish emission profiles and emission inventories. Micro- and middle-scale monitors are often used for short-term human exposure studies to evaluate contributions from nearby sources such as busy roadways and to evaluate the zones of representations for compliance sites. These scales can also be used to estimate emission rates and compositions from nonducted sources such as road dust (e.g., Gillies et al., 1998). Vertical resolution of pollutant concentrations at

micro- and middle-scales provide information on particle formation, nighttime chemistry, carryover, and transport that cannot be achieved by surface measurements (Watson & Chow, 2002a). The neighborhood scale represents the size of emissions and modeling grids used for air quality modeling in urban areas. Neighborhood-scale sources consist of vehicle exhaust, residential wood combustion or vegetative burning, and geological material (e.g., paved and unpaved roads, construction dust). Most ambient monitoring networks are represented by urbanscale monitors such as those in compliance networks and CSN. Regional- or continental-scale background monitors are represented in the IMPROVE network (Watson, 2002), which is located in nonurban or rural areas away from local sources, often at high elevations. These sites are used as regional transport sites to obtain upwind pollution concentration levels. Global-scale background monitors are used to address pollution transport between continents. For human exposure assessment, different monitoring scales need to be considered. **While secondary ammonium sulfate [(NH4)2 SO4] and ammonium nitrate (NH4 NO3) are regional in nature with less spatial variability, large spatial inhomogeneities are found for OC and EC due to localized vehicle exhaust and vegetative burning.** Both zones of representation for the receptors and zones of influence for the sources need to be addressed. Resolving the spatial inhomogeneity of organic pollutant concentration and composition will require denser deployment of simpler, less-expensive samplers that can distinguish exposure in different microenvironments. Assessment of human exposure also needs to address different temporal scales (from minutes to years). Highly sensitive fast-response monitors provide particle characterization by fractions of a minute, but is costly in data validation and analysis (Chow et al., 2008). In situ, high time resolution (preferably 1 h) of PM mass and specific components is desired. **Information is needed on both "typical" pollution and special events such as forest fires or other instances of abrupt changes in the nature and level of organic pollutants.** Available resources need to be considered in designing an effective network for epidemiological studies. Trade-offs among measurement variables, number of sampling sites (i.e., spatial scales), sampling frequency, duration, and periods need to be considered. Limitations on every third- to sixth-day 24-h measurement for the compliance network, CSN, and IMPROVE network need to be addressed. Chemical Speciation Hundreds of PM organic compounds have been identified, but in a relatively small number of samples owing to the high cost of sampling and analysis. On the other hand, over 100,000 OC and EC measurements are made each year. A middle ground is needed to distinguish among fresh versus aged aerosol, primary versus secondary organic aerosol, organic classes, oxidized compounds (e.g., WSOC), and biomarkers. Standardization of a subset of organic markers is desirable for risk assessment and source apportionment. Organic speciation should move away from labor-intensive solvent extraction to thermal evolution with more FIG. 6. The m/z 57 ion thermal desorption gas chromatograms show n-alkane distributions for residential wood burning, cooking, gasoline, and diesel profiles based on information in Chow et al. (2004b). specific detectors (Chow et al., 2007b). Retrospective analyses using thermal desorption gas chromatography/mass

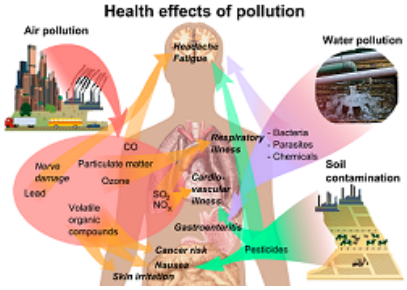
spectrometry (TD-GC/MS) can be performed on archived quartz-fiber ambient and source samples, which have the potential to identify and quantify source contribution. Examples of TD-GC/MS thermograms are shown in Figure 6. More than 130 nonpolar organic compounds (e.g., alkanes, alkenes, PAHs, hopanes, steranes) can be quantified with this method. Figure 6 shows alkane patterns for residential wood burning, cooking, gasoline, and diesel profiles. Residential wood burning has the highest Cmax at C29 with significant odd number alkanes dominant. Both cooking and gasoline have a Cmax of C25 . The lowest Cmax (C21) is found for the diesel profile. The position and pattern of the hump containing unresolved complex materials (UCM) can also be used to distinguish different sources. Large humps are found for vehicle emission sources; gasoline exhaust showed a much broader hump than diesel exhaust, but the humps for residential wood burning and cooking samples are not as pronounced. Health Impacts Health Outcomes Predominated by Carbonaceous Pollutants Ultimately, it is desirable to know which of the many adverse health outcomes associated with air pollution are driven either completely or in large part by carbonaceous air contaminants. This is true for both the statistical associations established by epidemiology and the effects produced **by experimental exposures to carbon-containing mixtures.**



The effects of exposure to many individual carbonaceous species are well known from toxicological and occupational studies. This allows the assumption, for example, that OC is an important contributor to cancer risks associated with air pollution. The evidence summarized above indicates that OC can have many other effects. Owing to the infrequency of organic speciation measurements, however, we have modest knowledge of the range of health outcomes that might be driven by carbonaceous pollutants. It is possible that few, if any, adverse health outcomes are attributable solely to carbonaceous pollutants. The few exclusive effects might **include protein-specific allergies, binding to specific cellular receptors, or compound-specific covalent binding with biomolecules such as the formation of DNA**



adducts. Carbonaceous components of environmental aerosols most likely contribute in some way to many outcomes associated with air pollution. Most health responses tend to be integrative; i.e., many combinations of causal agents (air pollution and other), exposure patterns, biological mechanisms, **and susceptibilities can result in lung inflammation, reduced lung function, allergic responses, asthma exacerbations, heart arrhythmias, or myocardial infarctions**.



There are few outcomes to which OC, metals, acids, or CP gases cannot be reasonably hypothesized to contribute in some way. The goal is not necessarily to identify OC- or EC-specific health effects, although such effects may be found. **The goal is to confirm which effects are attributable in part to OC and EC and to determine which, if any, effects they predominate**. This knowledge would help place the roles of carbonaceous and other pollutants, and their sources, in their proper context, as a foundation for more accurately assessing and managing sources of risk. Causal PhysicalChemical Classes, Species, and Combinations Closely related to, but different from, the preceding need is the **necessity to better understand the physical chemical classes and individual species of carbonaceous pollutants that are the most important**

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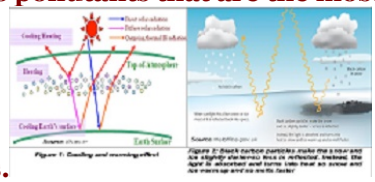
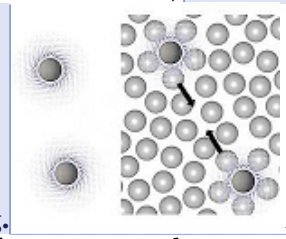
Comment [252]: elemental carbon (EC, also called black carbon [BC] or soot)



drivers of health effects. **The current state of science is rudimentary in identifying causal components of complex mixtures, and environmental exposures are always complex. Increasing the number, location, and frequency of measurements of**

pollutant species will be required for epidemiology to do a better job of parsing causal pollutants. For practical reasons, detailed dissection of causal species will inevitably remain the province of laboratory studies. It remains unfortunately novel at present to even evaluate the relative contributions of PM and non-PM components to the effects of mixtures, much less the classes and species within those components. The level of detail with which we need to understand the causal components is not clear, although it is clear that our present understanding is not adequate. Identification of causal pollutants by class is a useful first step and may suffice in some cases, **but will often not be sufficient for decision making.**  An illustrative example is the frequent assertion that PAHs as a class are responsible for carcinogenicity, promotion of allergic response, oxidative injury, etc. While it is true that certain PAH species can have those effects, it is often unrecognized that PAH is a very diverse structural class occurring in the VOC, SVOC, and PM phases. For example, PM-borne mutagenic PAHs in diesel emissions have received much attention, but their emitted mass is miniscule compared to that of the less-studied naphthalenes (McDonald et al., 2004a), which are present mostly as VOCs and are also a known carcinogenic hazard (Lin et al., 2006)). Among the pyrene subclass, BaP is a strong carcinogen, but pyrene is not carcinogenic. Phenanthroquinone is a strong oxidant (Kumagai et al., 2002), but many other PAHs have little oxidative capacity. Assignment of hazard by general chemical class may be sufficient for classes that are largely consistent in source, physical phase, and biological effects across individual species, but it may not be sufficient for more diverse classes. A noteworthy research need is the health importance of UCM in the chromatogram, representing the portion of OC mass which is not readily speciated or attributable to common sources by conventional GC/MS methods. Not only do the hundreds of organic compounds measured by an array of the analytical techniques specified in Table 2 **pertain to a minority (often 20%)** of the total OC mass, but there is also basis for believing that UCM is biologically active. The analytically unwieldy material is comprised of large OC polymers, including HULIS. Although little studied by the air pollution health research community, they are known to have biological reactivity in water-dwelling organisms (e.g., Meems, Steinberg, & Wiegand, 2004; Timofeyev et al., 2007). HULIS' ability to complex **iron has been hypothesized to play a role in coal worker's pneumoconiosis (Ghio & Quigley, 1994) and lung injury of tobacco smokers** (Ghio, Stonehuerner, & Quigley, 1994). Although HULIS are currently difficult to isolate for study, advances in separation and analytical methods (e.g., Gora & Hutta, 2005; Limbeck et al., 2005) create increasing potential for evaluating their contribution to the biological effects of PM-borne OC. An important subset of this knowledge gap is the causality of

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Comment [253]: because of the lack of information no one can say that the chemtrailing in the air –since these are also particle pollutants exiting the plain and the streams that come out ergo a chemtrail-for those who are chemtrail dysfunctional and with this type of clarity you now have a fuller understanding that anything that is being "exhausted out of a airplane" is a chemical is safe and with all the other pollutants that have been released in the atmosphere since post 40's=from nuclear explosions to industrial dumping to the natural releases from the earth and space pollutants with this mix and with this objective perspective all the chemtrails are doing is adding to the burden of the planets ecosystem

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Comment [254]: Organic Carbon—so what about the other 80% and you have to realize certain chemicals by themselves maybe be inert normally and even in light amounts if harmful do neglible if any damage but when combined with other chemicals can then become more reactive more volatile or create a different chemical reaction that can excessively damaging or create compounds not listed in the periodic table 

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Comment [255]: Interesting implication that it is a iron not the tobacco that maybe the cause of respiratory issues with smokers

combinations of OC and EC, different OC species, and mixtures of carbonaceous species with other pollutants. **This is part of our general ignorance of the importance of combinations of pollutants with or without other factors** (Mauderly, 2006). **In part, this gap results from the predominance of single-pollutant research strategies in the past**. It is also because the current state of the art of research strategies and tools has not yet evolved to the sophistication of evaluating combinations. **The premise underlying this research need is that there are combinations of pollutants that, together, produce health effects that are not linear, independent sums of the individual effects.** There is enough information to know that this is plausible and sometimes true, but we don't know how often it might be true**. The range of plausible nonadditive interactions spans synergism, antagonism, potentiation, inhibition, and masking** (U.S. EPA, 2000). It is not feasible to examine the effects of the myriad possible combinations in a systematic, factorial manner. However, it should always be considered, and occasionally tested, that combinations of physical chemical species may be causing effects we ascribe to individual species, or effects that we do not expect or cannot explain from our understanding of single species effects. **Other than testing simple combinations, the most practical approach is to examine multipollutant effects by multivariate analysis of data sets containing multiple variations of exposure composition and response**. Exposure-Dose-Response and Thresholds To assess risks from carbonaceous pollutants accurately, we need to know the relationship between internalized dose and health responses, including whether actual or practical thresholds exist. Our generally **poor understanding** of **exposure-dose response relationships holds for carbonaceous, as well as other pollutants**. The default assumption for risk assessment is that the relationship is linear, yet it is biologically unlikely that most dose-response curves are actually linear. For most pollutants, we do not have the information necessary to depart from the default assumption. Epidemiology has low power to do this because personal exposures are seldom known with accuracy and outcomes are predominantly measured on a population, rather than an individual, basis. **Variations in susceptibility among the population mitigate against the identification of thresholds**. Laboratory studies have greater ability to examine dose-response functions, but there have been few attempts to do so by extending the dose range downward to the point of no observable effects. Studies using unrealistically high doses are commonly accepted as confirming hazard and supporting plausibility for effects of environmental exposures. A corollary issue is the lack of a systematic, widely accepted framework for judging when biological responses should be considered "physiological" (i.e., "normal," "compensatory," "normative," or "homeostatic") and thus not worth management attention, and when they should be considered "adverse" and thus worthy of the expenditures necessary to reduce or avoid them (Samet, 2000). **Mammalian biology is dynamic; we are in a continual state of adjusting physiologically to our environment (including what we breathe) to maintain normal (homeostatic) function**. We now have the ability to monitor this "biological traffic" in detail. The goal is not to stop this

continuous flow of responses, which would cause death, but to determine which and at what level responses should be avoided. As for other pollutants, it is important to understand fundamental biological responses to carbonaceous species not in isolation, but in the context of their contribution to clinical outcomes. Developing a more rigorous framework for evaluating the regulatory implications of biological responses is a research and risk assessment need. Susceptibility Factors There is a wide range of susceptibility to the outcomes statistically associated with air pollution. Although this is another subset of the dose-response issue, it is worthy of special note, and particularly in light of the difficult-to-define Clean Air Act mandate to protect the most sensitive populations. **Some differences are obvious, such as the greater impact on mortality among the elderly than young adults, and the greater impact on lung function among asthmatics than normals**. The growing field of **environmental genetics is progressively revealing less obvious genetic factors, such as the greater susceptibility of individuals without the glutathione S-transferase allele** (Schwartz et al., 2005) or without polymorphisms in the hemachromatosis gene (Park et al., 2006) to pollution-related reductions of HRV. **Animals of different strains have different susceptibilities to pollutants, and animals are often genetically engineered to enhance differences in susceptibility. There is substantial variation in response even among animals of the same strain, gender, and age**. Ironically, researchers tend to diminish the importance of that variability by averaging rather than exploring causes of the extremes. **The issue of susceptibility is not fundamentally different for carbonaceous pollutants than for other pollutants, but susceptibility factors differ for different chemical species or source mixtures**. We are dealing with a diverse range of compounds that will act via a daunting spectrum of mechanisms. **Some of these mechanisms will be common to other pollutants, but some may be dominated by the organic aerosol. For example, some organic species such as BaP have little toxicity as inhaled, but form reactive, toxic metabolites after deposition**. **Some nonbiogenic organic species will likely be found to cause biological responses because they mimic biogenic allergens. Other organic species are known to mimic hormones or bind competitively to hormone receptors** (Capen, 2001), **and more will likely be found. Airborne OC will not escape the burgeoning and controversial attention to "endocrine disruptors**" (Yang, Park, & Lee, 2006; Martin et al., 2007). As we parse the effects of pollutants between carbonaceous and other species, we will also be in the business of parsing susceptibilities is not enough. It is becoming prevalent to relate health effects to **PM components aggregated into OC, EC, metals, and other elements**, but that will not suffice for OC. Much of the foregoing information deals with disaggregating OC and EC into smaller physical and chemical subclasses assuming that subclasses have different health effects and sources, and thus different management priorities and options. There is a great practical need to understand hazards and risks by class, but broad chemical classes may not bear sufficient information to differentiate health outcomes. The issue is whether or not other approaches to aggregation might be

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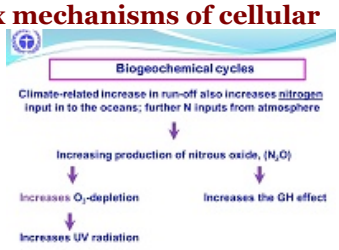
Comment [256]: Interesting take on the genetics of the animals in the wild they would hunt or forage and as a result would have adapted to there environment as a result of either the animals hunted or plants that would have been consumed to have developed a immunobody to save them or allow them to thrive in the polluted areas—domestication of these animals may have diminished this ability

useful. It should be considered whether carbonaceous (and other) pollutants might be aggregated by the nature of their initial, or critical, biological interactions regardless of their source or structure. **There are many possible pathways (mechanisms) by which initial contact of pollutants with the body can be linked to observable health outcomes,** and understanding them all may never be within our grasp. However, there is likely a much smaller number of fundamental ways that foreign molecules can interact with biomolecules, and summarizing them categorically may be possible. For example, Gregus and Klaassen (2001) group initial reactions into covalent binding, noncovalent binding, hydrogen abstraction, electron transfer, enzymatic reactions, etc. **They group effects on target molecules into dysfunction, destruction, and neo antigen formation, and then go on to group more complex mechanisms of cellular**



dysregulation. From similar perspective but using different language, air pollution researchers now speculate that oxidative injury may be a unifying hypothesis in the form of an initiating mechanism for many of the PM effects (U.S. EPA, 2004a). **There may be utility in grouping carbonaceous and other pollutants by classes of initiating reactions**, and perhaps developing instruments that could index the potency of airborne materials for engaging in those different types of reactions. Indeed, some groups are exploring detectors of gross oxidant potential (Chung et al., 2006; Venkatachari et al., 2007). **The utility of grouping pollutants by common pathogenetic pathways or common health outcomes should also be explored more actively, and other aggregation structures will undoubtedly be identified**. We are faced with understanding the health implications of an overwhelmingly complex and variable mixture of air contaminants and contributing factors. It is almost certain that advancing our understanding of the relationships among sources, pollutants, and effects will depend in part on furthering our ability to systematically group pollutants in different ways. RESEARCH TOOL NEEDS Cross-Disciplinary Glossaries No single research discipline can achieve the gains in knowledge of organic aerosols that are desired. This is true in most research arenas, but the complexity of organic aerosols and human exposures confers special value on collaboration. **There is a need to assemble glossaries of medical, physiological, biological, Fundamental Mechanisms Initiating Biological Responses** Although this information gap overlaps the preceding, it is highlighted for a different reason. We will likely never have a full understanding of the health impacts of individual carbonaceous air pollutant species, and could never manage them individually even if we did. We are inevitably forced to lump, or aggregate,

pollutants into groups. At a very crude level, for example, **we have been aggregating all nongaseous, non vapor pollutants into a physical class termed PM**. That has proven informative, but toxicological, and atmospheric technical terminology to facilitate multidisciplinary research. Several carbon workshops (e.g., http://www.wrapair.org/APACE) serve as a good starting point for assembling such a glossary, but this type of effort needs to be materialized by the U.S. EPA or some other agency to ensure the continuance and to provide periodical updates. Standardization of Compounds, Terminology, and Naming Conventions There are thousands of organic compounds present in the atmosphere. For each functional group, Table 2 gives examples of individual compounds, their International Union of Pure and Applied Chemistry (IUPAC) nomenclature, common names, and associated Chemical Abstracts Services (CAS) number. Chemists prefer to use IUPAC nomenclature, which names chemical compounds and describes the science of chemistry in general. Atmospheric scientists prefer a shorter common name. However, the use of common names is inconsistent. As shown in Table 2, **there are seven common names associated with BaP and six common names associated with 2,6,10,14tetramethylpentaecane, which further complicates the issue**. A standardization of marker or indicator compounds, terminology, and naming convention is needed to avoid confusion. Common name with CAS number is a helpful method of identification. Standardization of Sampling and Analysis Methods Most organic compounds attain a gas/particle equilibrium with their environment. Changes in temperature, relative humidity, or VOC vapor pressure during sampling cause SVOCs to evaporate, resulting in negative sampling artifacts (Eatough et al., 1989, 1990; McDow & Huntzicker, 1990), whereas VOC and condensable SVOC may adsorb onto filter media and deposited particles, resulting in positive sampling artifacts (Turpin, Huntzicker, & Hering, 1994; Turpin, Saxena, & Andrews, 2000; Kirchstetter, Corrigan, & Novakov, 2001; Watson & Chow, 2002b). Differences in sampler flow rates or face velocity cause different pressure drops across the filter that may also affect the vapor pressure over volatile particles and affect OC collection (McDow & Huntzicker, 1990). For PM OC and EC, different sampling configurations have been used with or without preceding organic denuders (e.g., XAD resin or parallel plate with charcoal-impregnated glassfiber filters [CIGF]), followed by tandem filter packs (e.g., quartz behind quartz, quartz behind Teflon, or CIGF behind quartz) to evaluate organic artifacts. There is no consensus regarding the extent of organic sampling artifacts, and they certainly vary by location, time of day, and time of year. Huebert and Charlson (2000) estimate 3050% and 50% for positive and negative artifact, respectively. The two major networks in the United States (CSN and IMPROVE) will begin to use the same type of sampler (URG 3000N carbon sampler; URG Corporation, Chapel Hill, NC) and report eight carbon fractions (OC1 to 4, EC1 to 3, and pyrolytic OC [OP]) following the IMPROVE A TOR temperature protocol (Chow et al., 2005, 2007a) starting May 2, 2007. Differences in carbon fractions between the two networks will be minimized in the future. **These carbon fractions are highly correlated with PAH** and are useful in source apportionment and health studies. Most of the nonoxygenated VOCs can be collected in canisters and measured using GC/MS.

OHCs can be collected on sorbent tubes (e.g., charcoal, Tenax, silica gel), thermally extracted, and followed by GC/MS analysis (U.S. EPA, 1999c) or by sorbent derivitization coupled with liquid elution high performance liquid chromatography (HPLC) or TD-GC/MS analyses (U.S. EPA, 1999a; Ho & Yu, 2002, 2004a). Commonly applied analytical methods for organic compounds by functional groups are specified in Table 2. A standardized set of procedures and calibration standards are needed for comparability. Standard Reference Materials (SRMs) A common set of calibration standards is needed for thermal/optical carbon analysis. Sucrose and potassium hydrogen phthalate (KHP) are used for OC, but there are no common EC standards. Acetylene flame and **graphitic arc soot are commonly used in animal studies and should be used as part of carbon calibration standards**. To reduce uncertainty in variations of thermocouple temperature (i.e., temperature sensor in carbon analyzers), temperature calibration and tests of trace O2 in the combustion atmosphere are also essential to ensure adequate carbon fractions (Chow et al., 2005). There are 200-300 organic standards, most of which (e.g., PAHs, n-alkanes, organic acids) are commercially available. However, many of the standards are costly (e.g., thousands of U.S. dollars for 10 mg of some PAHs) and have short shelf-lives (i.e., influenced by moisture, O2 , ultraviolet light) due to complicated synthesis processes and their instability**. Many organic chemists prefer to synthesize their own organic standards. Accurate weighing and preparation of calibration standard solutions are necessary to determine organic concentrations**. **Their accuracy should be examined with certified reference materials, such as SRMs available from the NIST**. A few organic compounds (e.g., anteiso-/iso-alkanes, some hopanes and steranes) are not commercially available because no proper separation method from raw materials or no precise synthesis schemes in the generation of pure compounds exist. **Therefore, their response factors are commonly assumed to be the same as the respective compounds that have authentic standards (e.g., compounds in the same organic family, optical isomers, or compounds with the same carbon number**). This increases uncertainties in the determination of real concentrations in the samples. In addition to improved and expanded calibration standards, there is a need to broaden the range of standardized materials for biological studies. This need is amplified for OC because of the large spectrum of compounds and mixtures that need to be studied, the broad range of health outcomes to be examined, and the analytical complexity involved. The establishment and maintenance of a selection of widely available, well-characterized study Downloaded By materials is a wise investment that will pay off well beyond the cost. SRMs facilitate systematic research by providing consistent benchmarks for comparisons of biological reactivity within and between studies, and among laboratories. The availability of SRMs in sufficient quantity and at nominal cost expands the investigative range by supplying scientists having little ability or insufficient funding to generate them on their own. Providing a full physical chemical characterization saves the cost of investigators conducting their own analyses. Centralized maintenance ensures that material is stored under optimal conditions and can be analyzed periodically to monitor for storage-related shifts in composition. SRMs enhance

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Comment [257]: More Hidden or misdirect since graphite is a form of carbon and is 2-3 times harder and denser then diamond and is a carbon –not be listed as such would cause a lot of misdirect and misinfo ont the actual impact it would have on even vegetation

overall scientific productivity because results can more readily compared across studies and over time. Moreover, the selection of the available SRMs can have a substantial influence on the nature and scope of research that follows. This review does not attempt to list desirable SRMs, **but they should include both single compounds and complex, sourcebased materials**. The argument for the utility of source-based SRMs is made by noting the widespread use of NIST standard diesel soot samples in biological research. The chief difficulty in establishing source-based SRMs, of course, is the considerable variation in composition that occurs within each source type. Still, representative source-based SRMs are very useful, as long as the nature of the source is well-documented. A prioritized list (e.g., HULIS) could be developed by a small group of informed atmospheric, analytical, and biological scientists. There has been **almost no study of the biological importance of HULIS**, in large part because biologists have not had material to study. Collecting (or creating) a large stock of representative material and analyzing its composition is a challenge that has not been undertaken. Standardization of Health Response Assays The diversity of interests, hypotheses, research tools, and funding incentives among health researchers is a strength in the exploration of fundamental biological phenomena, but often mitigates against the systematic development of an integrated understanding of the relative impacts and importance of different air contaminants (NRC, 2004; Mauderly, 2004). **The variety of animal and cellular models and response measures is a subset of this issue**. The huge range of classes, compounds, and combinations of carbonaceous pollutants **accentuates the problem**, because it ensures the need to compare and combine results from many studies conducted in multiple laboratories. We do not recommend repression of the exploration of new models and methods, but it would be of benefit to standardize and crosscalibrate approaches among studies and laboratories to a greater extent than at present. This is most practical within programs in which multiple laboratories are funded simultaneously to address complementary research questions (e.g., the U.S. EPA PM Research Centers or NPACT). A corollary issue is the need for more systematic comparisons of responses to a given exposure material among different health outcome models. This is distinct from the preceding need because it is a matter of standardizing the types of health models and assays applied to components of the organic aerosol, rather than standardizing the nature of the models. Finding that an aerosol component has a certain effect (e.g., lung inflammation) is very useful, but it is **even more useful to know whether that component also causes other effects (e.g., cardiac arrhythmia, amplification of allergic responses, DNA adduction, etc.)**. It is also useful to confirm biological activity in more than one type of assay before drawing conclusions. Sometimes information from multiple assays yields concurrence, such as the similar inflammatory potential of ambient PM samples from the Utah Valley in humans, animals, and cells (Ghio, 2004). However, sometimes different assays yield conflicting results, such as the nearly opposite rankings of the toxicity of the same engine emission samples in animals (Seagrave et al., 2002) and cells (Seagrave, Mauderly, & Seilkop, 2003). Steerenberg et al. (2006) compared the inflammatory, cytotoxic, and adjuvant potentials of numerous ambient PM

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Comment [258]: humic-like substances (HULIS; a group of macromolecular-size polycarboxylic acids- HULIS are ubiquitious in soil and ambient PM, and can be formed as atmospheric reaction products (Surratt et al., 2007). **HULIS can alter the hygroscopicity of PM**

samples from multiple European cities, and found good concurrence between in vivo and in vitro assays of inflammation and cytotoxicity, but a different ranking for adjuvant activity. Moreover, they did not find good concurrence between two different models of adjuvant activity. These examples illustrate that without attention to the uniformity with which different effects are assessed, research on the biological effects of the dauntingly complex organic aerosol could become a "random walk" that produces a kaleidoscopic assortment of findings that are difficult to forge into a basis for decision making. CONCEPTUAL FRAMEWORK The complexity of evaluating the health importance of environmental organic aerosols is not only daunting, but it also forces consideration of systematic frameworks by which it might plausibly be pursued. **The chemical species are diverse, not all yet identified, span multiple overlapping physical phases (gases, liquids, and solids) and sources (virtually all of them),** and are addressed by multiple existing regulatory structures (e.g., NAAQS, HAPs) and historic research programs. It is fair to begin by considering whether **"organic aerosols" can be, or should even be, addressed as an aggregate category**. Many of the practically measurable components are being addressed by different programs, although with highly variable degrees of emphasis and funding. Current attention is not necessarily in proportion to the importance of the different components and sources, overlooking some classes and sources altogether, and providing scant basis for integrating the pieces of the tapestry of knowledge into a meaningful whole. These caveats present a good case for considering organic (carbonaceous) air contaminants as a group. Another argument for focusing some portion of the air pollution monitoring and research effort on carbonaceous air contaminants as a group derives from the recent attention to shifting air quality management toward multi-pollutant strategies. In accordance with recommendations in NRC (2004), the U.S. EPA is exploring the potential for moving progressively away from single-pollutant risk assessment and regulation toward managing many pollutants and sources to obtain the greatest aggregate human and ecological health benefit. Such a movement will not only engender, but will also necessitate, a more balanced assessment of the impacts of all air contaminants. **Many of the historically under evaluated and poorly understood air contaminants are components of the organic aerosol**. If we are to pursue a course of inquiry different from the status quo, **a key goal will be to avoid conducting predominantly single pollutant or single-source research**. **Another will be to identify matrices of pollutants, potential health outcomes, and research approaches that can be pursued systematically in a manner that facilitates intercomparisons supporting integrative hazard and risk assessments**. What naturally follows is greater emphasis on standardization of terminology, reference materials, health indicators, and other research tools as described earlier. It also follows that there would be more thought given to the most appropriate use of different experimental strategies and study designs (Mauderly, 2004). Few scientists and research tools will be excluded. There will be appropriate roles for studies of single compounds, classes, and combinations. **There will be appropriate roles for factorial comparisons of simple combinations and multivariate analysis of complex-composition,**

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Comment [259]: in today's time that would have to be due to the overwhelming load the atmosphere has been exposed to ~ the time these particles have remained in the atmosphere and the integration or accumulation this chemistry has made ~ and with the mixture of the ultrafine or NANOPARTICLES which not only inegrates but is either assimilated or assimilates (agglomerate or aggregate) there is no longer any separate entity of chemistry or process

concentration response databases. There will be appropriate roles for epidemiology, experimental human and animal exposures, and studies of cells and nonbiological systems. Ideally, the difference will be that the complexity of the challenge will force a greater degree of thoughtful strategic planning and oversight to ensure that funds are directed, and research resources are deployed, in a manner that most efficiently produces useful information. **More than any other pollutant class, studying environmental organic aerosols places a premium on true interdisciplinary research strategies.** Nearly all sizable research programs today are multidisciplinary, in that they involve researchers from different disciplines working on a common issue. Atmospheric chemists collect and analyze, exposure scientists measure and model, epidemiologists collect and analyze, laboratory scientists expose and measure, but often these are parallel, largely independent efforts. Although more challenging, it might often be more productive to forge multidisciplinary research teams that actually conduct interdisciplinary research by combining their talents in the same studies. Not only does this enhance the scope of capabilities that can be brought to bear in a study, but it necessitates a greater level of cross-disciplinary education that will ultimately result in improved and more innovative study designs. Many air pollution biologists today, for example, have modest understanding of the composition of combustion emissions (e.g., whole diesel exhaust is still sometimes equated solely to the PM phase), or the existence, nature, and potential importance of SVOCs or HULIS. Many atmospheric chemists, for example, have modest understanding of the strengths and weaknesses of biological models or understand the importance of chemical **characteristics of pollutants to the likelihood and nature of biological reactivity or pathogenetic pathways**. Collaborating directly within studies causes communication and joint thinking well beyond that engendered by joint participation in research programs or scientific meetings. To the extent that the preceding is true, the responsibility for success will fall heavily on the research managers to ensure that the research community is provided with incentives that match the information needs and appropriate research strategies. The funding of research on a pollutant-by-pollutant basis, for example, would ensure that little attention would be given to other species or classes, that competition for funding would bias researchers toward the importance of the funded species or class, and that substantial gaps would remain in the larger matrix of information needs. Researchers can advise and respond to solicitations, but it is the sponsors who ultimately determine the direction and nature of research. REFERENCES

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DNA-NANO- cages 'can survive inside living cells'

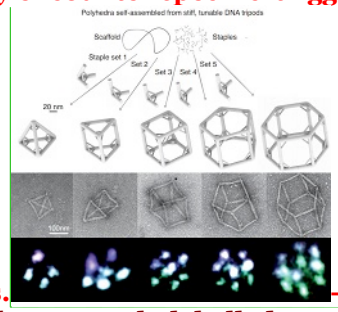
Date:
August 25, 2011
Source:
University of Oxford

Human embryonic kidney cells were used to test the DNA cages.

Scientists at Oxford University have shown for the first time that molecular cages made from DNA can enter and survive inside living cells.

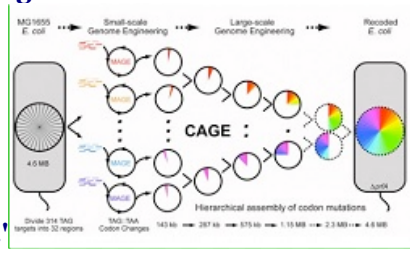
The work, a collaboration between physicists and molecular neuroscientists at Oxford, shows that artificial DNA cages that could be used to carry cargoes of drugs can enter living cells, potentially leading to new methods of drug delivery.--A report of the research is published online in the journal *ACS Nano*.--The cages developed by the researchers are made **from four short strands of synthetic DNA. These strands are designed so that they naturally assemble themselves into a tetrahedron** (a pyramid with four triangular faces) around

7 nanometres tall.--The Oxford researchers have previously shown that **it is possible to assemble these cages around protein molecules, so that the protein is trapped inside, and that DNA cages can be programmed to open when they encounter specific 'trigger' molecules that are found inside cells.**



--In the new experiment they introduced fluorescently-labelled DNA tetrahedrons into human kidney cells grown in the laboratory. **They then examined the cells under the microscope and found that the cages remained substantially intact, surviving attack by cellular enzymes,** for at least 48 hours. This is a crucial advance: to be useful as a drug delivery vehicle, a **DNA cage must enter cells efficiently and survive until it can release its cargo where and when it is needed**.

'At the moment we are only testing our ability to create and control cages made of DNA,' said Professor Andrew Turberfield of Oxford University's Department of Physics, who led the work. 'However, these results are an important first step towards proving that **DNA cages could be used to deliver cargoes, such as drugs, inside living cells.'**



Professor Turberfield said: 'Previous studies have shown that the size of particles is an important factor in whether or not they can easily enter cells, with particles with a radius less than 50 nanometres proving much more successful at gaining entry than larger particles. At 7 nanometres across our DNA tetrahedrons are compact enough to easily enter cells but still large enough to carry a useful cargo. More work is now needed to understand exactly how these DNA cages manage to find their way inside living cells.'--**Story Source--**The above story is based on materials provided by **University of Oxford**. *Note: Materials may be edited for content and length*.**Journal Reference**--Anthony S. Walsh, HaiFang Yin, Christoph M. Erben, Matthew J. A. Wood, Andrew J. Turberfield. **DNA Cage Delivery to**

FREEDOM 4/17/2017 10:45 AM

Comment [260]: There are three main classes of biopolymers, classified according to the monomeric units used and the structure of the biopolymer formed: polynucleotides (RNA and DNA), which are long polymers composed of 13 or more nucleotide monomers; polypeptides, which are short polymers of amino acids; and polysaccharides, which are often linear bonded polymeric carbohydrate structures. [

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Comment [261]: DNA nanotechnology is the design and manufacture of artificial nucleic acid structures for technological uses. In this field, nucleic acids are used as non-biological engineering materials for nanotechnology

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DNA nanotechnology is sometimes divided into two overlapping subfields: structural DNA nanotechnology and dynamic DNA nanotechnology. Structural DNA nanotechnology, sometimes abbreviated as SDN, focuses on synthesizing and characterizing nucleic acid complexes and materials that assemble into a static, equilibrium end state. On the other hand, dynamic DNA nanotechnology focuses on complexes with useful non-equilibrium behavior such as the ability to reconfigure based on a chemical or physical stimulus. Some complexes, such as nucleic acid nanomechanical devices, combine features of both the structural and dynamic subfields

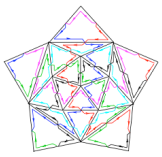
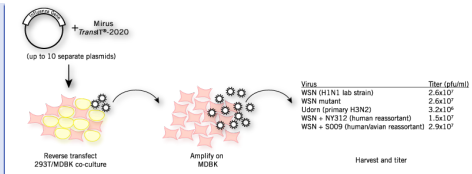
FREEDOM 4/17/2017 10:45 AM

Comment [262]: Does anyone else smell –vaccine orrrrrr a outbreak in a bio fashion or even possibly inert materials?

Mammalian Cells. *ACS Nano*, 2011; 110628154938010 DOI: 10.1021/nn2005574

DNA cage delivery to mammalian cells.

Walsh AS., Yin H., Erben CM., Wood MJ., Turberfield AJ.

DNA cages are nanometer-scale polyhedral structures formed by self-assembly from synthetic DNA oligonucleotides.  Potential applications **include in vivo imaging and the targeted delivery of macromolecules into living cells**. We report an investigation of the ability of a model cage, a **DNA tetrahedron, to enter live cultured mammalian cells**. Cultured human embryonic kidney cells were treated with a range of fluorescently labeled DNA tetrahedra and subsequently examined using confocal microscopy and flow cytometry**. Substantial uptake of tetrahedra into cells was observed both when the cells were treated with tetrahedra alone and when the cells were treated with a mixture of tetrahedra and a transfection reagent**. 

Analysis of the subcellular localization of transfected tetrahedra using confocal microscopy and organelle staining indicates that the cages are located in the cytoplasm. **FRET experiments indicate that the DNA cages remain substantially intact within the cells for at least 48 h after transfection**. **This is a first step toward the use of engineered DNA nanostructures to deliver and control the activity of cargoes within cells**.

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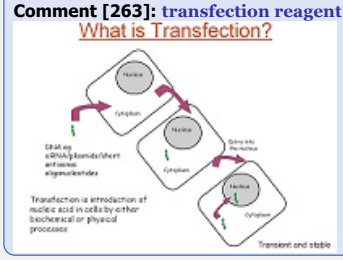
Comment [263]: transfection reagent


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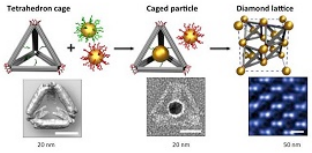
DNA cages 'can survive inside living cells

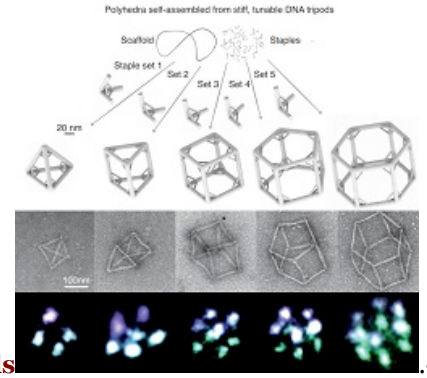
Scientists at Oxford University have shown for the first time **that molecular cages made from DNA can enter and survive inside living cells.--** The work, a collaboration between physicists and molecular neuroscientists at Oxford, shows that artificial **DNA cages that could be used to carry cargoes of drugs can enter living cells,** potentially leading to new methods of drug

delivery. A report of the research is published online in the journal *ACS Nano*.--The cages developed by the researchers are made from four short strands of synthetic DNA. These strands are designed so that they naturally assemble themselves into a **tetrahedron (a pyramid with four triangular faces)**

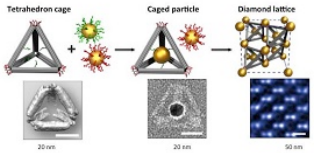


around 7 nanometres tall.

The Oxford researchers have previously shown that it is possible to assemble **these cages around protein molecules, so that the protein is trapped inside, and that DNA cages can be programmed to open when they encounter specific 'trigger' molecules that are found inside**

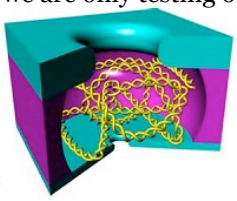


cells.--In the new experiment they introduced **fluorescently-labelled DNA**



tetrahedrons into human kidney cells grown in the laboratory. They then examined the cells under the microscope and found **that the cages remained substantially intact, surviving attack by cellular enzymes, for at least 48 hours**. This is a crucial advance: to be useful as a drug delivery vehicle, a **DNA cage must enter cells efficiently**

and survive until it can release its cargo where and when it is needed.--'At the moment we are only testing our **ability** **to create and control cages**



made of DNA,' said Professor Andrew Turberfield of Oxford University's Department of Physics, who led the work. 'However, these results are an important first step towards **proving that DNA cages could be used to deliver cargoes, such as drugs, inside living cells.'**

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Comment [264]: **Cargoes Such as drugs orrrrr other materials that could be genetic in nature ~ this basically being a weaponized tech which can carry "cargo " of drugs or bio agents**

Professor Turberfield said: 'Previous studies have shown that the size of particles is an important factor in whether or not they can easily enter cells, with particles with a radius less than 50 nanometres proving much more successful at gaining entry than larger particles. **At 7 nanometres across our DNA tetrahedrons are compact enough to easily enter cells but still large enough to carry a useful cargo**. More work is now needed to understand exactly how these DNA cages manage to find their way inside living cells.'--**Story Source-**The above post is reprinted from materials provided by **University of Oxford**. --**Journal Reference**-Anthony S. Walsh, HaiFang Yin, Christoph M. Erben, Matthew J. A. Wood, Andrew J. Turberfield. **DNA Cage Delivery to Mammalian Cells**. *ACS Nano*, 2011; 110628154938010 DOI: 10.1021/nn2005574 -University of Oxford. "DNA cages 'can survive inside living cells'." ScienceDaily. ScienceDaily, 25 August 2011. <www.sciencedaily.com/releases/2011/07/110714100319.htm>.