Nanoparticle Contamination of Agricultural Crops

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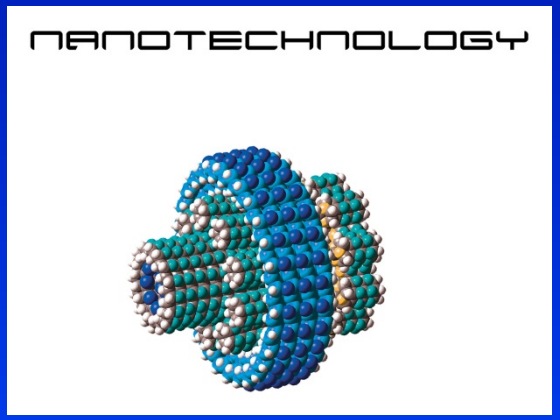
What is Nanotechnology?

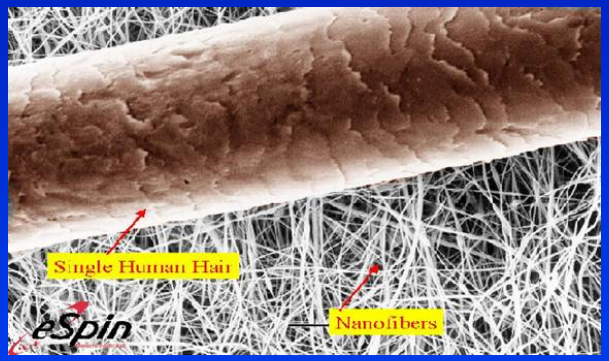
- Nanotechnology is a rapidly developing field of study involving the manipulation of materials on the atomic or molecular scale
- Typically nanotechnology deals with structures, devices, or systems in the size range between 1 and 100 nanometers (nm)

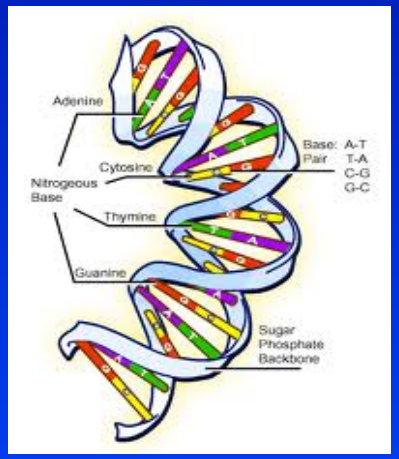


This nano-machine is 50 microns across and can only be viewed using a powerful Scanning Electron Microscope. For comparison, a human hair is about 100 microns across.

What is a Nanometer?

- A nanometer (nm) is a unit of length in the metric system, equal to one-billionth of a meter
- Nanometers are used to measure extremely small objects such as atoms or molecules
- A strand of DNA is about 2 nanometers wide
- A typical human hair is about 100,000 nanometers wide





What is a Nanoparticle?

- Nanoparticles can be defined as measuring less than 100 nanometers in at least two dimensions (length, width, height), nanomaterials have at least one dimension measuring less than 100 nanometers
- Nanoparticles can be produced by natural processes including volcanic activity, erosion, wave action and fire

What is a Nanoparticle?

- Nanoparticles can also be produced by human activities
- Anthropogenic sources of nanoparticles include the incineration of waste and coal combustion
- Also, the weathering of rubber car tires and paints

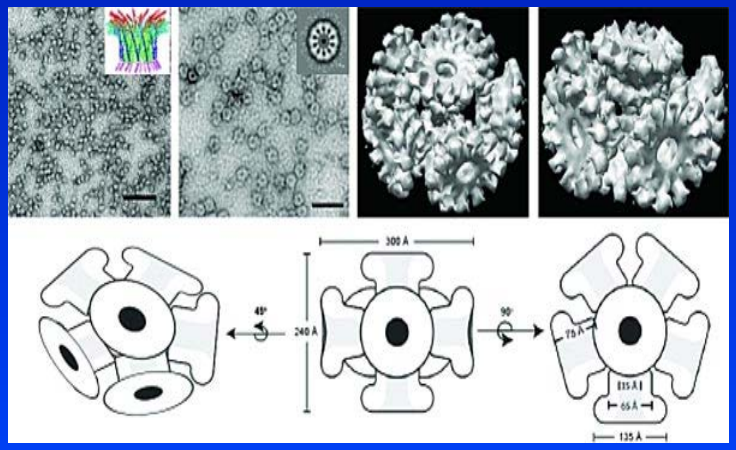
Unique Properties

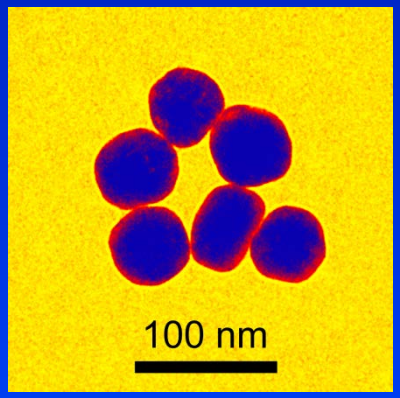
- The extremely small size of nanoparticles often results in unique chemical and physical properties that differ from the corresponding bulk or “non-nano” scale material
- Due to their greatly enhanced surface area, many nanoparticles are highly reactive
- This can lead to enhanced solubility or catalytic activities

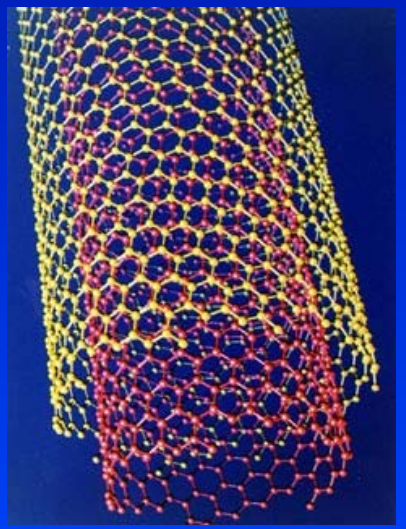
Material	Bulk Scale	Nano Scale
Copper	Malleable, ductile	Hard, stiff
Gold	Chemically inert	Chemically active
Silicon	Insulator	Conductor
Titanium Dioxide	White in color	Colorless

Engineered Nanoparticles

- As the field of nanotechnology has grown, our ability to design or engineer nanoparticles has also grown
- Engineered nanoparticles are often homogeneous with regular conformation, and designed with specific chemical and physical properties in mind
- The majority of nanotechnology products utilize engineered nanomaterials



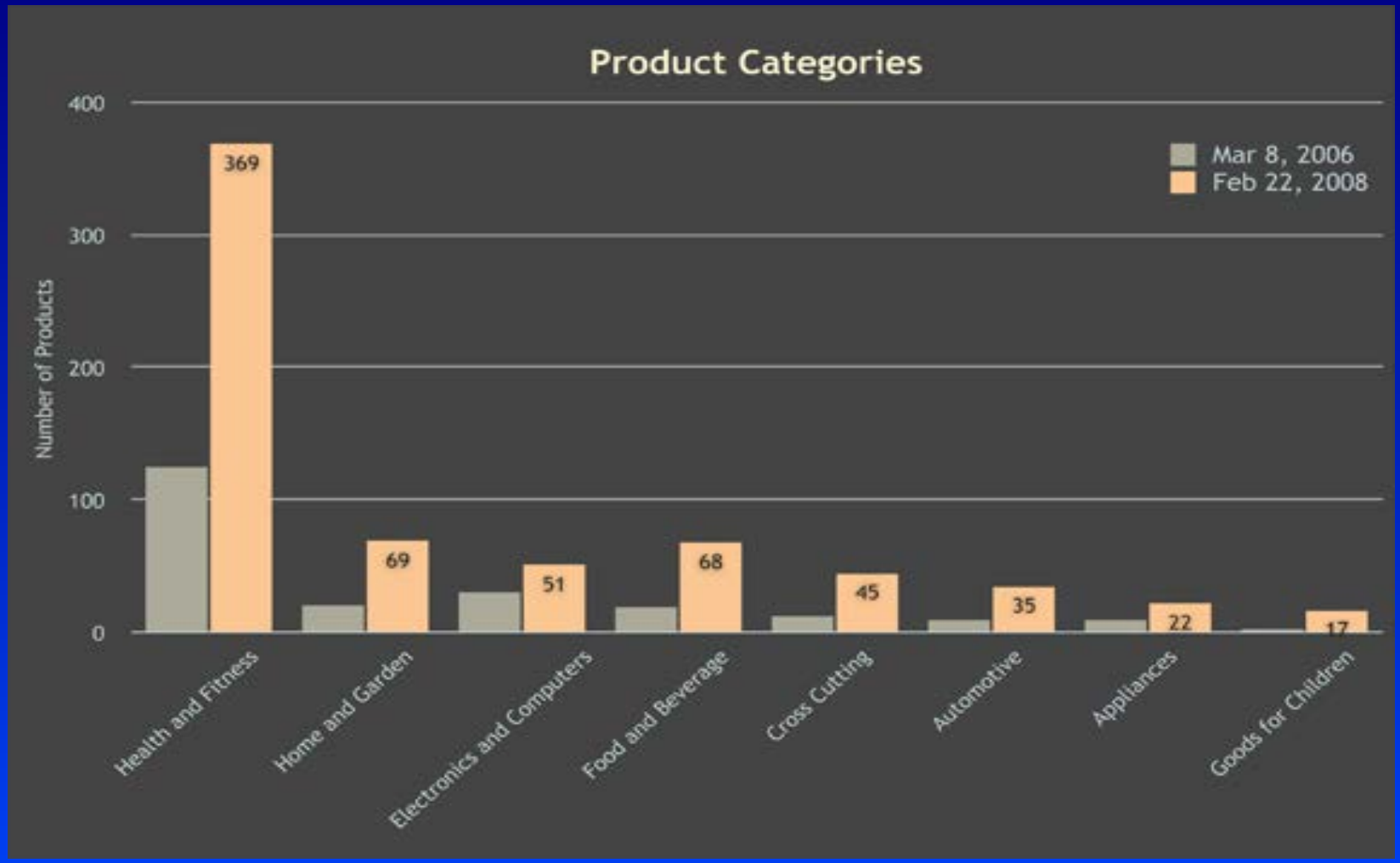




Nanotechnology Products

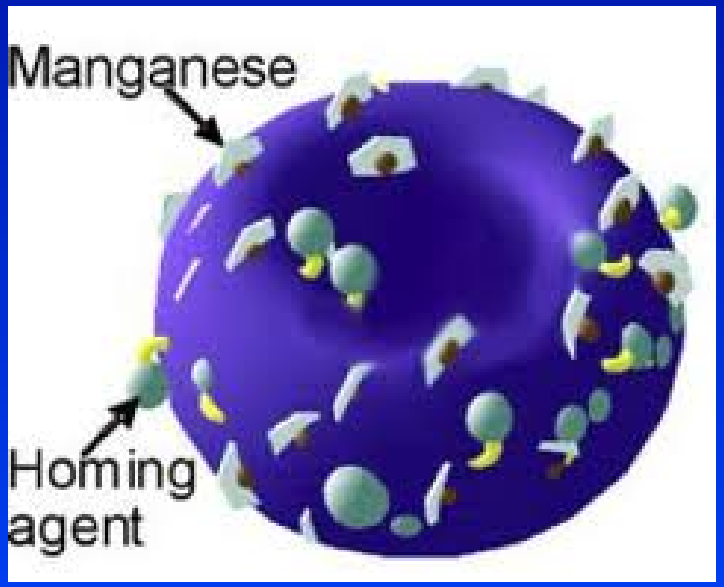
- In 2005 global investment in nanotechnology exceeded $4 billion; by 2015, the annual value of nanotechnology-related products is projected to be in excess of $1 trillion
- As of November 2010 over 1000 nanotechnology products were on the market in a variety of categories
- Nanotechnology based materials can be found in a number of consumer products including agricultural, automotive, cosmetics, electronics, fitness, food packaging, medical devices, pharmaceuticals, textiles, and water-treatment technologies

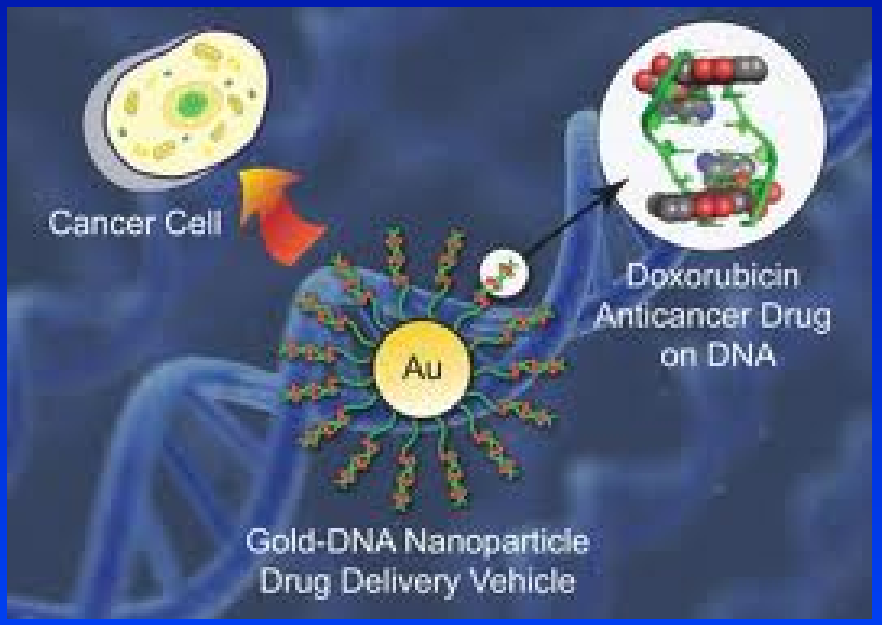
Nanotechnology Products (http://www.nanomedicinecenter.com)



Nanotechnology Products- "The Good"

- Nanoparticle based products are being used for efficient drug delivery and to fight cancer
- Clinical trials are underway using nanoparticles that recognize and attach to cancerous cells for targeted cell destruction while leaving healthy cells unharmed





Nanotechnology Products- "The Questionable"

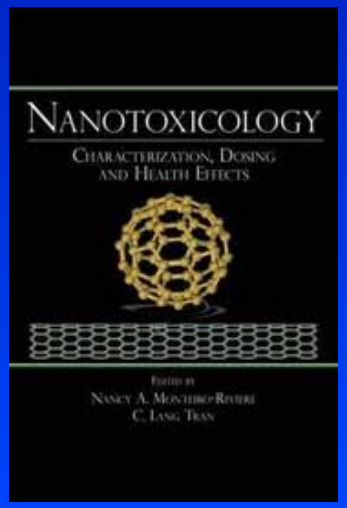
- Nanoparticles are being used in cosmetics, food packaging, textiles and even children's toys
- We and others feel that the risks associated with these types of uses are poorly understood

Nanotechnology Products- "The Questionable"

- Currently regulatory guidelines assume that nanoparticle toxicity is equivalent to that of the corresponding bulk material
- This may not be a valid assumption; if a nanoparticle behaves differently chemically and physically from the "non-nano" material, it may behave differently biologically (toxicity)

Nanoparticle Toxicology

- Some of the unique properties that make nanoparticles attractive may lead to new and unforeseen risks to humans and the environment
- There is limited knowledge of the biological and environmental fate of materials containing nanoparticles
- Nanotoxicology is an emerging field of science studying the potential for nanoparticle-based materials to cause adverse effects



Nanoparticle Toxicology

- The effects and toxicity of nanomaterials on living organisms has only recently been explored
- Preliminary toxicological studies have focused on a number of species including bacteria and algae, invertebrates such as nematodes and crustaceans, and vertebrates such fish and rats

Nanoparticles and Agriculture

- Nanoparticles are being used in pesticides and fertilizers
- Little is known about the effects, toxicity, or fate of nanoparticles on agricultural crops and the associated environment
- Also, nanoparticle contamination of agricultural crops and the environment may be an uncharacterized pathway of human exposure

Nanoparticle Research at CAES

- As we have already learned, many engineered nanoparticles behave differently from their corresponding non-nano scale or bulk form
- We have begun to focus on the impacts of nanoparticles on agricultural crops
- Most studies investigating the impact of specific nanoparticles on plants have failed to compare the effects of exposure to nanoparticles alongside exposure to the same material in bulk form
- CAES was one of the first to directly compare the effects of exposure to both nanoparticles and the corresponding bulk materials

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Assay-Dependent Phytotoxicity of Nanoparticles to Plants

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The effects of five nanomaterials (multiwalled carbon nanotubes [MWCNTs], Ag, Cu, ZnO, Si) and their corresponding bulk counterparts on seed germination, root elongation, and biomass of *Cucurbita pepo* (zucchini) were investigated. The plants were grown in hydroponic solutions amended with nanoparticles or bulk material suspensions at 1000 mg/L. Seed germination was unaffected by any of the treatments, but Cu nanoparticles reduced emerging root length by 77% and 64% relative to unamended controls and seeds exposed to bulk Cu powder, respectively. During a 15-day hydroponic trial, the biomass of plants exposed to MWCNTs and Ag nanoparticles was reduced by 80% and 75%, respectively, as compared to control plants and corresponding bulk carbon and Ag powder solutions. Although bulk Cu powder reduced biomass by 69%, Cu nanoparticle exposure resulted in 90% reduction relative to control plants. Both Ag and Cu ion controls (1−1000 mg/L) and supernatant from centrifuged nanoparticle solutions (1000 mg/L) indicate that half the observed phytotoxicity is from the elemental nanoparticles themselves. The biomass and transpiration volume of zucchini exposed to Ag nanoparticles or bulk powder at 0−1000 mg/mL for 17 days was measured. Exposure to Ag nanoparticles at 500 and 100 mg/L resulted in 57% and 41% decreases in plant biomass and transpiration, respectively, as compared to controls or to plants exposed to bulk Ag. On average, zucchini shoots exposed to Ag nanoparticles contained 4.7 greater Ag concentration than did the plants from the corresponding bulk solutions. These findings demonstrate that standard phytotoxicity tests such as germination and root elongation may not be sensitive enough or appropriate when evaluating nanoparticle toxicity to terrestrial plant species.

Introduction

In 2005, total global investment in nanotechnologies exceeded $4 billion, and the estimated annual value for nanotechnology-related products is expected to reach $1 trillion by 2015 (*1*). The manufacture and use of particulate material in the size range of a few nanometers (nm) is the driving force behind this growth (*2*). The extremely small size, structure, and surface characteristics of nanoparticles result in unique physicochemical properties not observed with larger or bulk particles of the same material. For example, materials with dimensions less than 5 nm exhibit unique electronic states, magnetic/optical properties, and catalytic reactivities that differ from corresponding atomic and bulk scale counterparts (*3*). In addition, insoluble substances can exhibit drastically enhanced solubility when the particle size is less than 100 nm. Nanoparticles also have a greater surface area than larger particles with equivalent mass, yielding a greater proportion of atoms on the surface relative to the interior of the structure and resulting in higher surface reactivity (*4*). Surfactants and other additives can modify the surface active properties of nanomaterials and may prevent particle aggregation (*5*). Such effects can be particularly important for hydrophobic particles such as carbon nanotubes and fullerenes.

The U.S. EPA organizes engineered nanomaterials into four categories, including (1) carbon-based materials in tubes, spheres, or ellipsoids; (2) metal-based materials such as Au, Ag, but also including metal oxides and quantum dots; (3) dendrimers or nanosized polymers; and (4) composites integrating nanoparticles with other bulk scale materials. Hundreds of nanotechnology-based products are currently in the marketplace, with common applications being found in electronics, optics, food packaging, textiles, medical devices, cosmetics, water treatment technology, fuel cells, catalysts, biosensors, and components for environmental remediation (*6*−*8*). For example, nanoscale zerovalent iron can be used to detoxify halogenated molecules such as polychlorinated biphenyls or to reduce nitrates in groundwater (*9*). The antimicrobial properties of Ag nanoparticles have found use in a range of products, including textiles, bandages, air filters, and vacuum cleaners. Carbon-based nanomaterials are integrated into plastics, catalysts, battery/fuel cell electrodes, water purification systems, orthopedic implants, adhesives/composites, and electronics (*10*).

Nanoparticulate matter can be produced by naturally occurring processes such as volcanic activity, fire, and erosion; as such, organisms have long been exposed to and have evolved with these materials. However, the current magnitude of exposure and the unique nature of engineered particulates warrant caution. Manufactured nanoparticles can enter the environment unintentionally through atmospheric emissions, domestic wastewater, agriculture, and accidental release during manufacture/transport; or through intentional releases such as during remediation efforts (*11*). The interaction and impact of nanomaterials, with their unique physical and chemical properties, on living systems has only recently been explored (*12*). Obviously, some direct data on human response to nanoparticle exposure has been acquired (*13*). A range of species have been investigated in nanotoxicology studies, including bacteria (*14*, *15*), algae (*16*), invertebrates such as nematodes and crustaceans (*17*, *18*), and vertebrates such as fish and rats (*19*, *20*). However, this literature is far from complete and is plagued by shortcomings, with many studies failing to directly compare bulk and nanoparticle toxicity for a given material. In terms of ecotoxicity, there has been significantly greater focus on aquatic rather than terrestrial species, and very little work has focused on terrestrial plants. Some studies have reported the toxic effects of nanoparticles on the germination and/or root growth of some plant species (*21*, *22*). A recent study

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Nanoparticle Research at CAES

- Five engineered nanoparticles were chosen for study:
  - Silver
  - Gold
  - Silicon
  - Copper
  - Carbon

Nanoparticle Research at CAES

- Many studies investigating the impact nanoparticles on plants have used traditional EPA phytotoxicity assays; seed germination and seedling root elongation
- Using these traditional assays we have seen negligible differences between exposure to nanoparticles and the corresponding bulk material
- However, we have shown significant reductions in growth, as measured by biomass and transpiration, of zucchini plants grown hydroponically and exposed to nanoparticle carbon, silver, and copper when compared to corresponding bulk materials

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Toxicity of Silver and Copper to *Cucurbita pepo*: Differential Effects of Nano and Bulk-Size Particles

AQ2 **Craig Musante, Jason C. White**

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ABSTRACT: The phytotoxicity of bulk and nanoparticle Cu and Ag was directly compared. NP Ag reduced biomass and transpiration by 66–84% when compared with bulk Ag. The Ag ion concentration was 4.4–10-times greater in NP than bulk particle solutions. The Cu ion concentration was 1.4–4.4-times greater in bulk than NP amended solutions. Humic acid (50 mg/L) decreased the ion content of bulk Cu solution by 38–42% but increased ion Cu content of NP solutions by 1.4–2.9 times. Bulk and NP Cu were highly phytotoxic; growth and transpiration were reduced by 60–70% relative to untreated controls. NP Cu phytotoxicity was unaffected by solution type, but humic acid (50 mg/L) completely alleviated phytotoxicity caused by bulk Cu. The data demonstrate differential toxicity of Ag NP relative to bulk Ag. The finding that humic acid and solution chemistry differentially impact bulk and NP behavior highlights the importance of evaluating nanoparticles under environmentally relevant conditions. © 2010 Wiley Periodicals, Inc. Environ Toxicol 00: 000–000, 2010.

Keywords: nanoparticles; phytotoxicity; Ag; Cu; engineered nanomaterials; nanotoxicology

INTRODUCTION

The term nanotechnology describes a rapidly developing discipline involving the characterization and use of structures, devices, and systems with size ranging between 1 and 100 nm. As of 2005, the total global investment in nanotechnologies exceeded $4 billion; by 2015, the annual value for all nanotechnology-related products will be in excess of $1 trillion (Roco, 2005; Aitken et al., 2006). Although nanoparticulate matter can be produced by naturally occurring processes such as volcanic activity, fire, and erosion, engineered nanomaterials (NM) differ in a number of key respects from those produced unintentionally. Engineered particles are monodispersed/homogeneous, often with regular conformation, and designed to have specific surface characteristics that may vary greatly from those of the corresponding bulk material.

For example, nanoparticles have higher reactivity due to a greater proportion of atoms on the surface relative to the interior of the structure (Handy et al., 2008a,b). As such, insoluble substances can exhibit drastically enhanced solubility when the particle size is less than 100 nm. In addition, materials with dimensions less than 5 nm exhibit unique magnetic/optical properties, electronic states, and catalytic reactivities that differ from corresponding bulk materials (Auffan et al., 2009).

As of November 2009, in excess of 1000 nanotechnology products were on the market. Common applications include electronics, optics, textiles, medical devices, cosmetics, food packaging, water treatment technologies, fuel cells, catalysts, biosensors, and components of environmental remediation (Roco, 2003; Zhang and Elliot, 2006; Klaine et al., 2008). For example, nanoparticulate Ag has well-known antimicrobial properties that have directly led to incorporation into products ranging from bandages to socks to vacuum cleaners (Geranio et al., 2009). Carbon-based NM such as single/multiwalled nanotubes are

Additional Supporting Information may be found in the online version of this article.

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CAES Hydroponic Assay-Methods

- Zucchini (*Cucurbita pepo* ssp *pepo*) and Squash (*Cucurbita pepo* ssp *ovifera*) were germinated from seeds
- Seedlings were grown hydroponically in Hoagland's Modified Basal Salt Solution for 1-2 weeks prior to exposure to nanoparticle or bulk material (Hoagland's is a widely used plant nutrient solution necessary for hydroponic growing)

Methods-Exposure

- Hydroponic assays consisted of exposure to solutions amended with nanoparticle or bulk material of only one element at varied concentrations for 14-16 days. Incubation was under controlled conditions of light and temperature
- Biomass and transpiration were monitored

Methods-Exposure

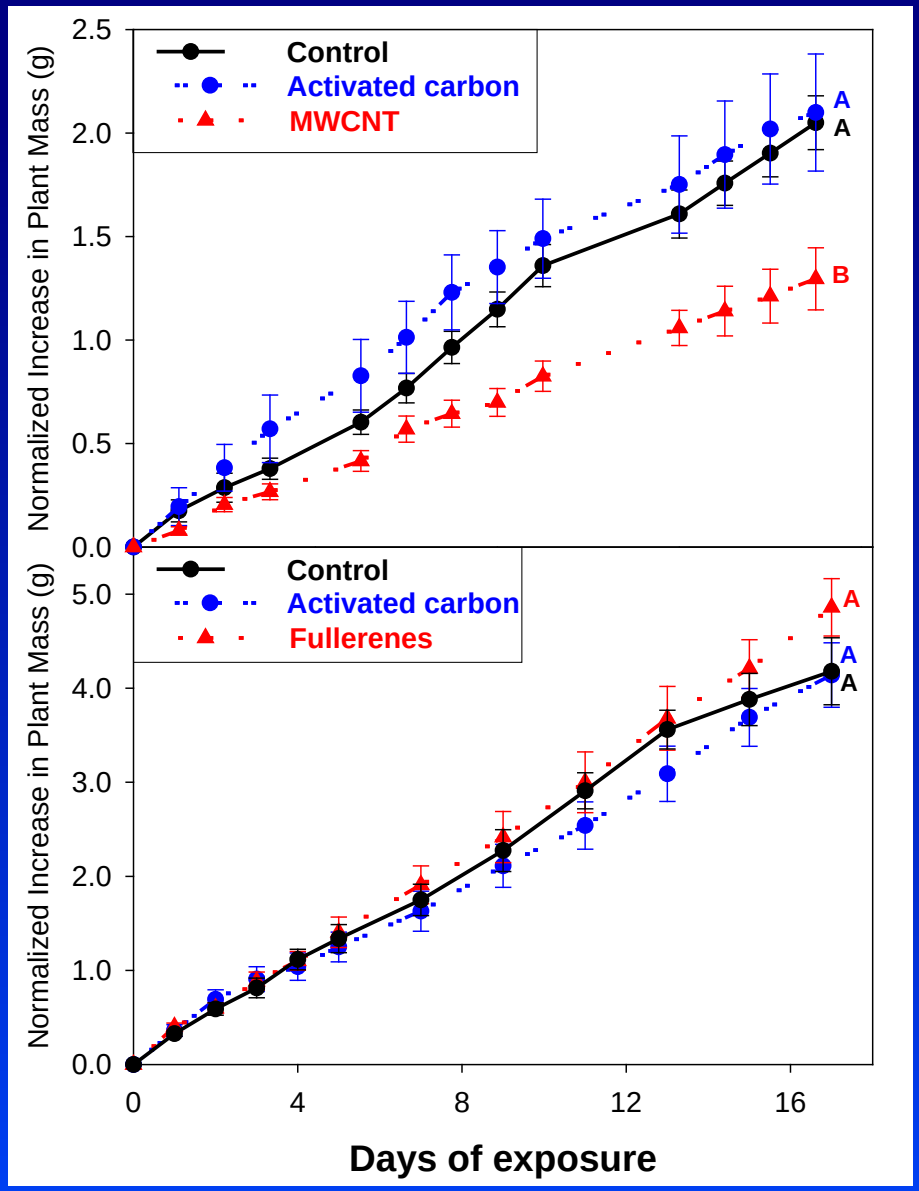
- Also, parallel hydroponic assays (specific material and concentration) were prepared and amended with humic acid
- Humic acid is a natural substance found in soils and has been shown to reduce nanoparticle aggregation (increase exposure)?
- Treatment with humic acid makes these hydroponic assays more relevant to real world environmental applications of nanoparticle materials

Methods-Exposure

- After hydroponic exposure for 14-16 days, aerial plant tissues not in direct contact with contaminated solution were harvested for examination of nanoparticle or bulk material uptake into plants

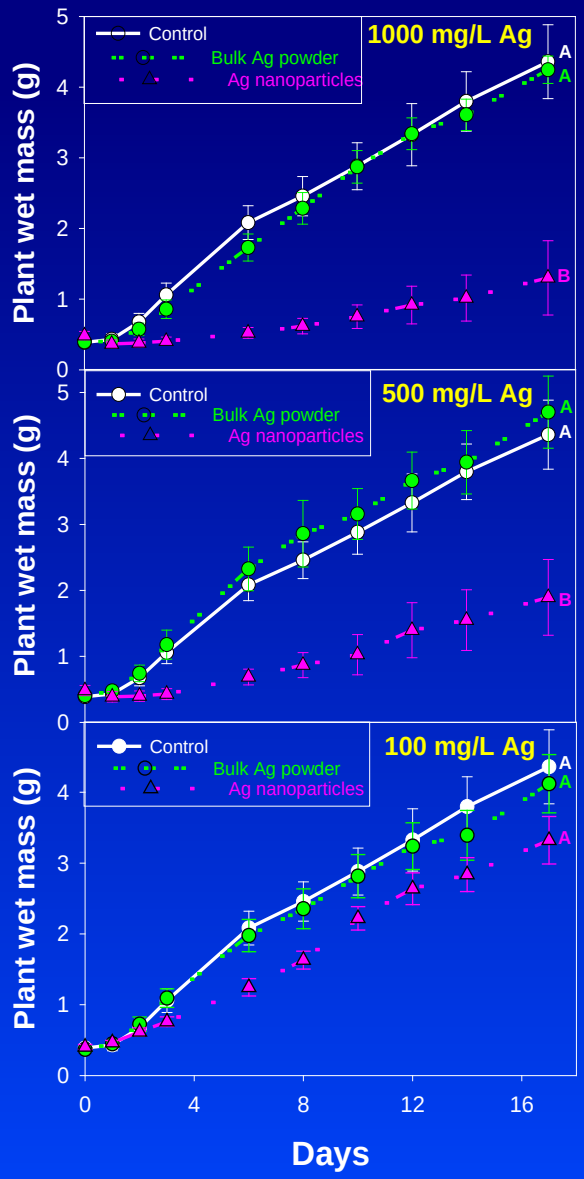
Results

- Effect of activated carbon, MWCNTs or Fullerenes on zucchini biomass under hydroponic conditions; all present at 1000 mg/L



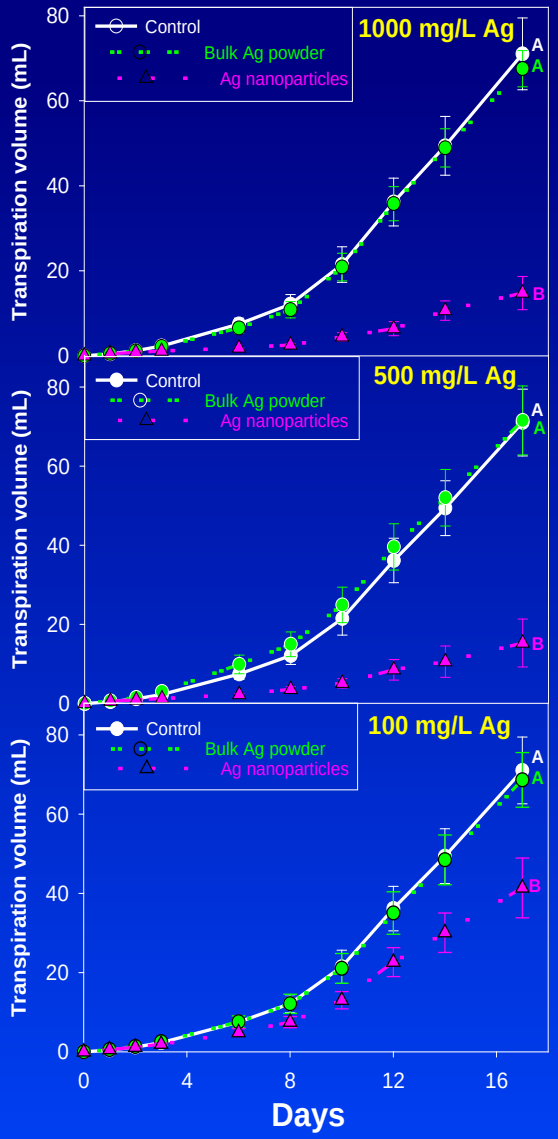
Results

Biomass



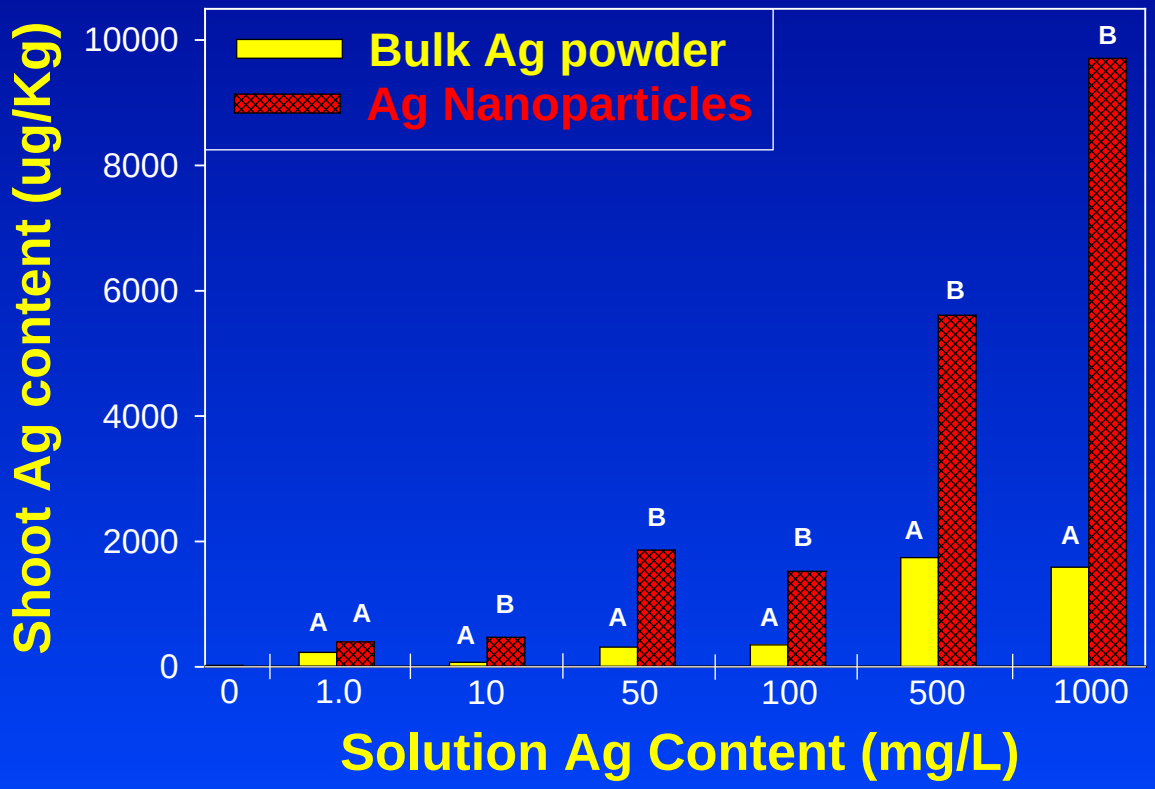
Zucchini dose-uptake study (0-1000 mg/L) assessing effect of nanoparticle or bulk Silver (Ag) on biomass and transpiration

Transpiration



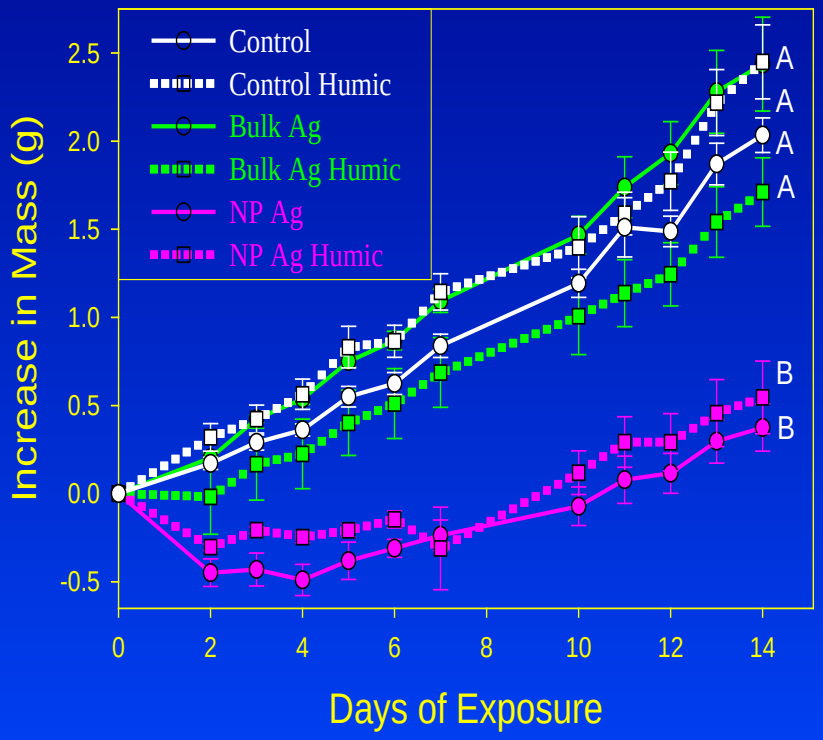
Results

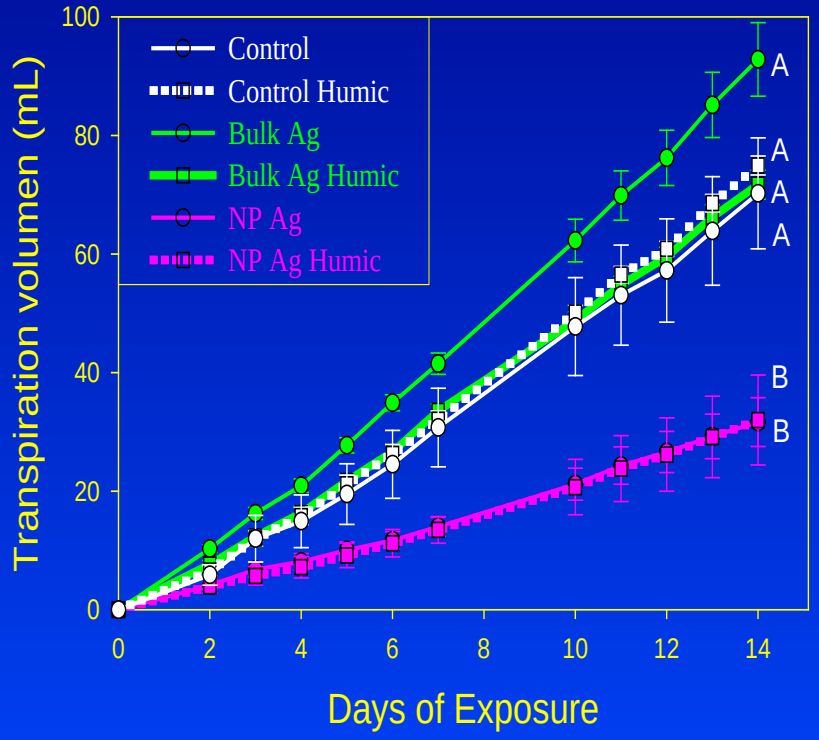
- Silver (Ag) content of zucchini shoots grown in silver nanoparticle or bulk solutions (1-1000mg/L)
- Elemental content of plant tissue was determined using Inductively Coupled Plasma Mass Spectroscopy (ICP-MS)



Results

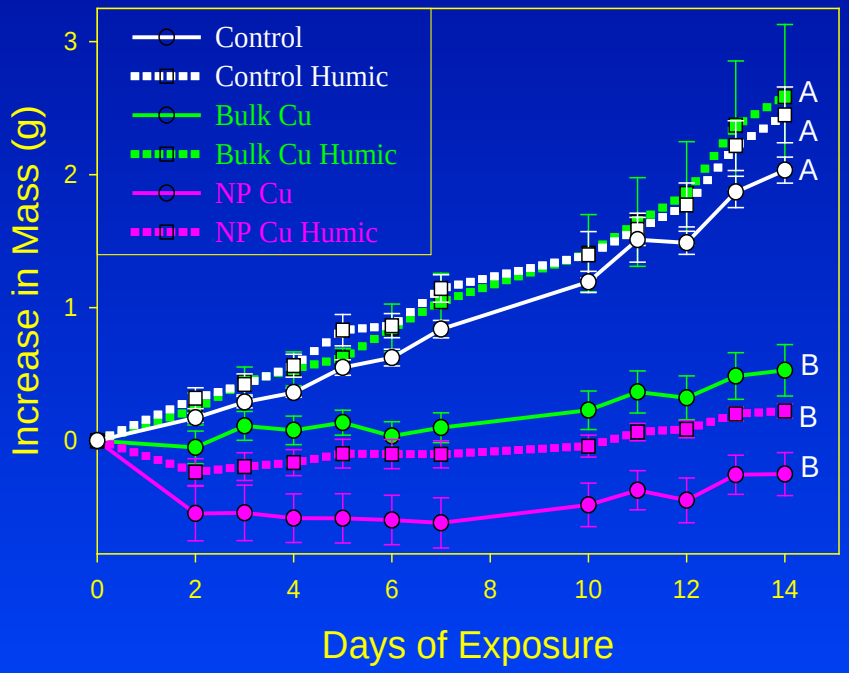
- Squash biomass and transpiration upon exposure to 500 mg/L bulk or nanoparticle silver (Ag) in the presence or absence of 50 mg/L humic acid

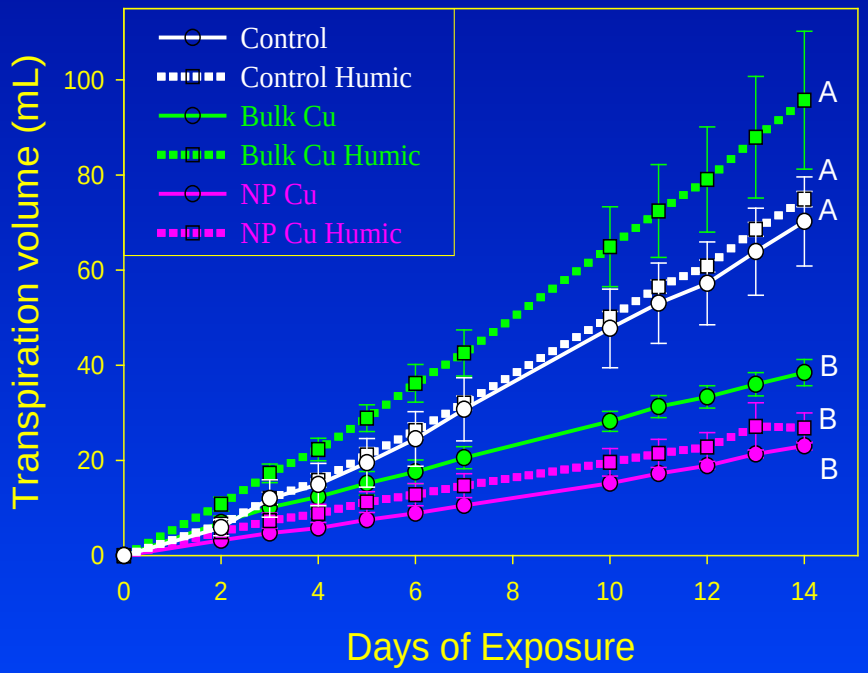




Results

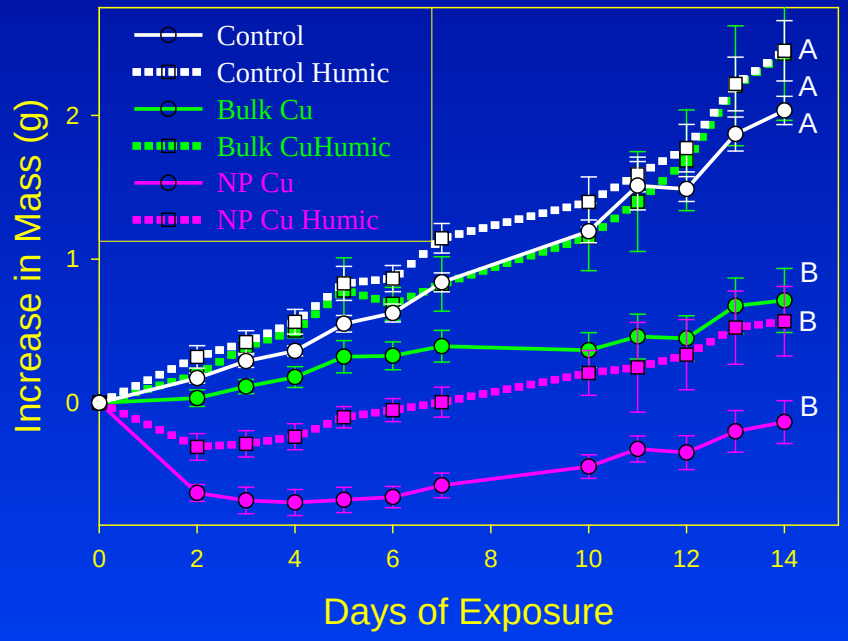
- Squash biomass and transpiration upon exposure to 500 mg/L bulk or nanoparticle copper (Cu) in the presence or absence of 50 mg/L humic acid

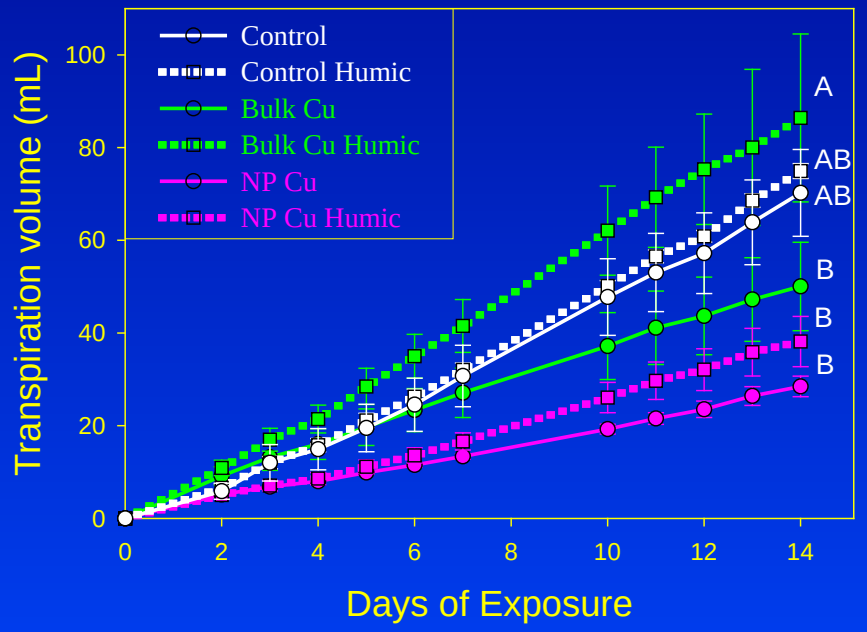




Results

- Squash biomass and transpiration upon exposure to 100 mg/L bulk or nanoparticle copper (Cu) in the presence or absence of 50 mg/L humic acid





Conclusions

- The use of nanomaterials in consumer products is increasing at a very rapid pace
- Some of the unique properties that make nanoparticles attractive may lead to new and unforeseen risks to humans and the environment
- There is limited knowledge of the biological and environmental fate of materials containing nanoparticles
- Exposure of plants to certain nanoparticle based materials can be more toxic when compared to corresponding bulk materials
- It is not currently known whether nanomaterials in pesticides and fertilizers pose a risk to humans
- More research on the interaction of nanoparticle material with important food crops and their fate in the environment is needed

USDA NIFA Grant

- In February of 2011, Dr. Jason C. White was awarded a 5-year $1.5 million federal grant from USDA
- The project is entitled “Nanoparticle contamination of Agricultural Crops” and was funded through the “Addressing Critical and Emerging Food Safety Issues” program
- CAES is the primary awardee; co-investigators include Professors at the University of Massachusetts, State University of New York College of Environmental Science & Forestry, and Southern Illinois University-Carbondale

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- Professor Lee A. Newman, Department of Environmental and Forest Biology, SUNY College of Environmental Science and Forestry

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