Nanotechnology Use in Agriculture: Benefits and Potential Risks

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What are Nanoparticles (NPs)?

• Nanoparticles are generated *naturally* by erosion, fires, volcanoes, and marine wave action

• **A key point** - People have been exposed to nanoparticles for as long as there have been people; in other words, “nano” isn’t inherently bad

• Nanoparticles are also produced by human activities such as coal combustion, vehicle exhaust, and weathering rubber tires
What are Engineered Nanomaterials?

• Our ability to construct and manipulate materials at the nano-scale has increased dramatically in the last decade

• Why does this matter? Materials at the nano-scale behave *differently* than the same material at the bulk or non-nano scale

• Have higher surface area to volume; can engineer for surface reactivity or other desired characteristics

• Frequently, this unique behavior can be both useful and profitable

• Nanotechnology was a $1 billion industry in 2005; will be a $1 trillion industry in 2015

<table>
<thead>
<tr>
<th>Changes in properties</th>
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<tbody>
<tr>
<td></td>
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<tr>
<td><strong>Bulk-scale</strong></td>
</tr>
<tr>
<td><strong>Nano-scale</strong></td>
</tr>
<tr>
<td>Si</td>
</tr>
<tr>
<td>Insulator</td>
</tr>
<tr>
<td>Conductive</td>
</tr>
<tr>
<td>Cu</td>
</tr>
<tr>
<td>Malleable and ductile</td>
</tr>
<tr>
<td>Stiff</td>
</tr>
<tr>
<td>TiO₂</td>
</tr>
<tr>
<td>White color</td>
</tr>
<tr>
<td>Colorless</td>
</tr>
<tr>
<td>Au</td>
</tr>
<tr>
<td>Chemically inert</td>
</tr>
<tr>
<td>Chemically active</td>
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</tbody>
</table>

Different size gold NPs reflect different wavelengths of light.
Nanotechnology-based Products- “The Good”

- As of March 2011, over 1300 commercially available products contain nanomaterials (The Project on Emerging Nanotechnologies)
- Used in medical devices, electronics, fuel cells, air filters, water treatment technologies, pharmaceuticals

[Graphs and images showing trends and comparisons of products and materials.]
There has been significant interest in using nanotechnology in agriculture.

The goals fall into several categories:
- Increase production rates and yield
- Increase efficiency of resource utilization
- Minimize waste production

Specific applications include:
- Nano-fertilizers, Nano-pesticides
- Nano-based treatment of agricultural waste
- Nanosensors
Nanomaterials and Agriculture

- **Nano-fertilizers** often contain nutrients/growth promoters encapsulated in nanoscale polymers, chelates, or emulsions.
- Slow, targeted, efficient release becomes possible.
- In some cases, the nanoparticle itself can stimulate growth.

- **Nanosensors** can be used to detect pathogens, as well as monitor local, micro, and nano-conditions in the field (temperature, water availability, humidity, nutrient status, pesticide levels...)

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Nano-pesticides often follow a similar model to nano-fertilizers; active pesticidal (insecticide, fungicide, …) ingredient associated with or within a nanoscale product or carrier.

- Increased stability/solubility, slow release, increased uptake/translocation, and in some cases, targeted delivery (analogous to nano-based delivery in human disease research).
- Can result in lower required amounts of active ingredients.

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**Nanomaterials and Agriculture**

**Inhibition Effects of Silver Nanoparticles against Powdery Mildews on Cucumber and Pumpkin**

Kabir Lamsal¹, Sang-Woo Kim¹, Jin Hee Jung¹, Yun Seok Kim¹, Kyoung Su Kim¹ and Youn Su Lee²*

¹Division of Bio-Resources Technology, Kangwon National University, Chuncheon 200-701, Korea
²Department of Agricultural Biotechnology, Center for Fungal Genetic Resources and Center for Fungal Pathogenesis, Seoul National University, Seoul 133-721, Korea

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**Parameters Affecting the Efficient Delivery of Mesoporous Silica Nanoparticle Materials and Gold Nanorods into Plant Tissues by the Biolistic Method**

Susana Martin-Ortígosa, Justin S. Valenstein, Wei Sun, Lorena Moeller, Ning Fang, Brian G. Trewyn, Victor S.-Y. Lin, and Kan Wang*  
In memory of Professor Victor S.-Y. Lin, deceased May 4, 2010

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**Role of nanotechnology in agriculture with special reference to management of insect pests**

Mahendra Rai - Ashok Ingle

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**Antifungal activity of zinc oxide nanoparticles against Botrytis cinerea and Penicillium expansum**

Lili He¹, Yang Liu¹, Azlin Mustapha, Mengshi Lin*
Nanomaterials and Agriculture

- Finding out the status of some of this research is difficult.
- The existing regulatory framework does not require particle-size specific data; EPA exception for NP silver in pesticides (2011)
- At SETAC Europe in May 2013, there were over 150 abstracts on nanotoxicology; only 3 were on plants (2 were mine)
- A lecture entitled “State of knowledge on nano-pesticides and implications for environmental exposure assessment in the EU”
- Over 3000 “nano-pesticide” patents have been filed globally
- More than 100 peer-reviewed papers (most in the last 3 years)

EPA Proposes Policy on Nanoscale Materials in Pesticide Products

Release date: 09/09/2011
Contact Information: Dale Keneavy (News Media Only) keneavy.dale@epa.gov 202-564-7839 202-564-4355

WASHINGTON — The U.S. Environmental Protection Agency announced today it plans to obtain information on nanoscale materials in pesticide products. Under the requirements of the law, EPA will gather information on what nanoscale materials are present in pesticide products to determine whether the registration of a pesticide may cause unreasonable adverse affects on the environment and human health. The proposed policy will be open for public comment.

“We want to obtain timely and accurate information on what nanoscale materials may be in pesticide products,” said Steve Owens assistant administrator for EPA’s Office of Chemical Safety and Pollution Prevention. “This information is needed for EPA to meet its requirement under the law to protect public health and the environment.”

A number of organizations, as well as government, academic and private sector scientists, have considered whether the small size of nanoscale materials or the unique or enhanced properties of nanoscale materials may, under specific conditions, pose new or increased hazards to humans and the environment.

EPA also recognizes that nanoscale materials have a range of potentially beneficial public and commercial applications, including pest control products. The agency will continue to encourage responsible and innovative development of products containing nanoscale materials to realize those benefits while also addressing health or environmental concerns.

The new proposed policy options will be published in the Federal Register shortly. The notice will also propose a new approach for how EPA will determine whether a nanoscale ingredient is a “new” active or inert ingredient for purposes of scientific evaluation under the pesticide laws, when an identical, non-nanoscale form of the nanoscale ingredient is already registered under FIFRA. This approach will help ensure that EPA is informed about the presence of nanoscale ingredients in pesticide products and allows a more thorough review of the potential risks.

Comments on the Federal Register notice will be accepted until 30 days after publication. The notice will be available at www.regulations.gov in docket number EPA– HQ–OPP–2010–0197.

More information or to read the proposed notice:
http://www.epa.gov/pesticides/regulating/nanotechnology.html
Nanotechnology Products— “The Questionable?”

- NMs are also used in pesticides, fertilizers, food packaging, cosmetics, and toys.
- Are the risks of nanotechnology fully appreciated?
- Current regulatory guidelines assume a nanoparticle is toxicologically equivalent to the corresponding bulk material.
- A valid assumption? If a substance at the nano-scale behaves chemically and physically different, what about biologically/toxicologically?
- Concerns have been raised from the beginning that the same attributes of NPs that make them useful, may lead to novel risks to human health and the environment. Those concerns are now becoming more mainstream.
Nanotoxicology and Agriculture

- Data on NM toxicity to plants is not abundant. Most early studies (2007-2010) looked only at NPs with no bulk material/ion comparison.

- This is a key point. It is somewhat irrelevant whether a NP/NM is toxic. The key questions are is that NM/NP more toxic than the bulk/ion and if so, is it by a different mechanism?

- Are nanomaterials an emerging class of contaminants?

- There have been a number of recent studies assessing the effects of specific NPs on germination, root elongation, and other physiological parameters.

- These studies have tended to focus on acute toxicity; relatively short exposure to high concentrations. This is where we start in toxicology but as is frequently the case, chronic low dose exposure may be more important.

- Larger issue may be food chain contamination and an uncharacterized pathway of human exposure.
The entire program is based on a simple question- From a regulatory standpoint, bulk/ion and NMs are considered equal. Is that true? Or are there important instances where they “behave” differently?

USDA NIFA Grant 1- 3/15/11 “Addressing Critical and Emerging Food Safety Issues.” A 5-year $1.5 million grant entitled “Nanomaterial contamination of agricultural crops”

Obj. 1: Determine the uptake, translocation, and toxicity of NM to crops.
Obj. 2: Determine the impact of environmental conditions on NM uptake, translocation, and toxicity to crops.
Obj. 3: Determine the potential trophic transfer of NMs.
Obj. 4: Quantify the facilitated uptake of pesticides through NM-chemical interactions
Objective 1 - Determine the uptake, translocation, and toxicity of NM to crops

- 12 plant species: corn, soybean, wheat, alfalfa, rye, rice, pea, bean, zucchini, spinach, lettuce, tomato
- 12 particles: S/MW CNTs, fullerenes, Ag, CuO, Si, ZnO, Au, TiO₂, CeO₂, SiO₂, Al₂O₃
- Batch hydroponic screen with 10 day exposure to 0, 50, or 500 mg/L bulk, ion, and NP/NM. Measure biomass, transpiration, particle content. Select assays on others.
- 12 plants have been exposed to Ag; 11 to CeO₂; 11 to CuO; 6 to TiO₂; 4 to ZnO; 4 to MWCNT or C₆₀ fullerenes; 3 to Al₂O₃; 3 to SiO₂
- Toxicity and accumulation potential are species-, particle-type-, and concentration-specific. **Most importantly, lots of particle size-specific toxicity/accumulation.**
- Not the most exciting of experiments to run but critical to isolating sensitive plant-nanoparticle combinations for more detailed study. Thorough evaluation of the screen will only be possible when full data set is available.
Effect of activated carbon, MWCNTs (top) or Fullerenes (bottom) on zucchini biomass under hydroponic conditions. All present at 1000 mg/L.

Root epidermal cell walls entrapped CuO NPs (A, B) and translocation of CuO NPs across epidermal cell walls (H).

Magnified view (B) of the square in (A). CuO NPs near the interface between the plant cell wall and the plasma membrane (H).

Endocytosis-like structure in the cells (C,D). CuO NPs in cell and intracellular space of cortical cells (E-G). Magnified views (F,G) of the circled region and squared region in (E).

Energy-dispersive spectroscopy (EDS) spectra of dark regions confirm Cu.

**Effects of 0-100 mg L\(^{-1}\) CuO NPs, 0.15 mg L\(^{-1}\) Cu\(^{2+}\) ions and 100 mg L\(^{-1}\) CuO BPs on root morphology after 15 days exposure.**

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<table>
<thead>
<tr>
<th></th>
<th>control</th>
<th>Cu(^{2+}) ions</th>
<th>10 NPs</th>
<th>100 NPs</th>
<th>100 BPs</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Length/cm</strong></td>
<td>818 ± 158 b</td>
<td>806 ± 133 b</td>
<td>291 ± 24.2 a</td>
<td>121 ± 8.2 a</td>
<td>970 ± 45.0 b</td>
</tr>
<tr>
<td><strong>SurfArea/cm(^2)</strong></td>
<td>103 ± 16.9 b</td>
<td>91.5 ± 18.6 b</td>
<td>39.6 ± 6.4 a</td>
<td>23.5 ± 4.7 a</td>
<td>99.6 ± 10.9 b</td>
</tr>
<tr>
<td><strong>AvgDiam/mm</strong></td>
<td>0.36 ± 0.04 ab</td>
<td>0.37 ± 0.01 ab</td>
<td>0.45 ± 0.04 b</td>
<td>0.62 ± 0.10 c</td>
<td>0.34 ± 0.02 a</td>
</tr>
<tr>
<td><strong>Tips/No</strong></td>
<td>2271±50e</td>
<td>1314±246c</td>
<td>618±11b</td>
<td>211±7a</td>
<td>1817±178d</td>
</tr>
</tbody>
</table>

Split-root experiments with maize seedlings exposed to NP CuO or ions

- CuO NPs present in shoots
- Presence of CuO NPs in non-exposed roots suggests phloem transport from shoot to root
- During phloem transport to roots, CuO reduction to Cu$_2$O and Cu$_2$S is evident by interplanar crystal spacing as calculated by fast Fourier transformation (FFT)

Collaborative experiments with the Institute of Experimental Botany, Czech Republic

- Focus is on changes in *Arabidopsis thaliana* gene expression after exposure to metal oxide NPs and fullerene soot.
- Specifically, microarrays were used to study the effect of 7-day exposure to 100 mg/L ZnO, TiO$_2$, or Fullerenes (FS) NPs on gene expression in *A. thaliana* roots.
- Subsequent up/down regulated gene expression monitored; functionality mapped.

Landa et al., 2012 *J. Hazard. Mat.* 241/242:55-62
Total numbers of up/down regulated genes, the fold change in expression, and functionality

- ZnO NPs induced most change in gene expression
- Changes in gene expression upon TiO2 exposure were mild
- Some overlap but clear particle-specific changes in gene expression is evident
- Relatively more stress responsive genes induced for ZnO NPs and fullerene soot
- Relevance of these findings to agricultural crops is unknown


[Graphs and diagrams showing gene expression changes]
Obj. 4: Nanomaterial interactions with co-existing organic chemicals

- Nanomaterials may represent a novel class of contaminants entering agricultural systems directly (pesticide/fertilizers) or indirectly (biosolids)
- Agricultural systems contain a number of other organic chemicals
- Interactions between nanomaterials and these co-existing contaminants/chemicals are unknown
  - Could bioavailability of legacy pesticides be affected? A food safety issue?
  - Could efficacy of intentional pesticides be affected? An economic issue?
- Several sets of experiments to date
  - Impact of C$_{60}$ fullerenes and Ag on DDE accumulation by crops in a model system (vermiculite)
  - Impact of C$_{60}$ fullerenes on weathered DDE/chlordane accumulation from soil by crop and worm species
Obj. 4: Quantify the facilitated uptake of pesticides through NM-chemical interactions

- Initial experiment using zucchini, tomato, and soybean grown in C₆₀-amended vermiculite
- Watered with DDE-containing solution (100 ng/mL)
- Measuring DDE root and shoot (GC-ECD or GC-MS) content upon co-exposure with C₆₀ fullerenes
- LC-UV and LC-MS/MS method for fullerene detection in plants
Quantify the facilitated uptake of pesticides through NM-chemical interactions

- Zucchini shoot, root, and total plant content of DDE
- Fullerenes enhance DDE accumulation in both roots and shoots.
- Suggests interaction between DDE-fullerenes
- Fullerenes clearly present in and on roots; shoots?

De La Torre Roche et al. 2012. Environ. Sci. Technol. 46, 9315–9323
Membrane damage and fullerene uptake

- Soybean and tomato had significantly greater MDA formation (lipid peroxidation) upon DDE and/or C$_{60}$ exposure.
- Zucchini had 60-4400 ppb C$_{60}$ in over half the stem samples.

**Concentration of malondialdehyde (µM MDA) produced by plant root and shoots upon exposure to DDE and C$_{60}$ by the TBARS method. MDA is produced during the degradation of fatty acids.**

<table>
<thead>
<tr>
<th>Plant</th>
<th>Control</th>
<th>DDE</th>
<th>C60</th>
<th>DDE + C60</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Tomato</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Shoot</td>
<td>0.123 A</td>
<td>0.175 B</td>
<td>0.134 A</td>
<td>0.182 B</td>
</tr>
<tr>
<td>Root</td>
<td>0.132 A</td>
<td>0.139 A</td>
<td>0.170 B</td>
<td>0.168 B</td>
</tr>
<tr>
<td><strong>Soybean</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Shoot</td>
<td>0.451 A</td>
<td>0.590 B</td>
<td>0.684 B</td>
<td>0.462 A</td>
</tr>
<tr>
<td>Root</td>
<td>0.489 A</td>
<td>0.755 B</td>
<td>0.924 B</td>
<td>0.674 AB</td>
</tr>
<tr>
<td><strong>Zucchini</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Shoot</td>
<td>0.190 A</td>
<td>0.183 A</td>
<td>0.166 A</td>
<td>0.188 A</td>
</tr>
<tr>
<td>Root</td>
<td>1.03 A</td>
<td>0.909 A</td>
<td>0.956 A</td>
<td>1.19 A</td>
</tr>
</tbody>
</table>

Soybean DDE Content in the presence of 500-2000 mg/L bulk or nanoparticle Ag. Ionic Ag was present at 5 and 20 mg/L.

Soybean Ag Content in the presence of 100 ng/mL DDE

De La Torre Roche et al. 2013. Environ. Sci. Technol. 718–725
CAES Nanotoxicology Program

- USDA NIFA Grant 2- 3/1/12 “Nanotechnology for Agricultural and Food Systems.” A 3-year $473,000 grant “Nanoscale Interactions between Engineered Nanomaterials and Black Carbon (Biochar) in Soil
  - **Obj. 1:** To quantify and mechanistically model the binding of NMs to biochar
  - **Obj. 2:** To determine the impact of biochar nanostructure and weathering on the effects of engineered nanomaterials on crop and earthworm species.

- Formal/informal collaborations with the National Institute of Standards and Technology (NIST), University of Texas El-Paso (UTEP), Institute of Experimental Botany (Czech Republic), University of Parma (Italy), Hasselt University (Belgium), and the Ocean University of China

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Conclusions

- Nanotechnology clearly has the potential to dramatically impact and improve agriculture
- However, the current degree of understanding of nanomaterial fate and effects in agricultural systems is poor
- It is possible that engineered nanomaterials may represent an emerging class of contaminants
- Exposure on agricultural crops may occur directly through NM-containing pesticide/fertilizer formulations, as well as spills, or indirectly through the application of NM-containing biosolids
- Lots of particle size-specific toxicity; not really supposed to happen
- Very little known in the area of co-contaminant interactions but it appears that some nanoparticles may significantly alter co-contaminant fate.
- Soil may minimize many of these co-contaminant interactions; more work currently being done here.
Acknowledgements

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- Wang et al - Ocean University of China
- J. W. Kelsey - Muhlenberg College
- T. Vanek - Czech Republic
- At CAES - Dr. Roberto de La Torre-Roche, Dr. Helmi Hamdi, Joe Hawthorne, Craig Musante

Funding -

- 2011-2016: USDA AFRI -Addressing Critical and Emerging Food Safety Issues- “Nanomaterial contamination agricultural crops”
- 2012-2015: USDA AFRI –Nanotechnology for Agricultural and Food Systems- “Nanoscale interactions between engineered nanomaterials and black carbon (biochar) in soil”