SIZE MATTERS. COMPARING THE TOXICITY OF MICRO- AND NANO PARTICLES

There is a slowly growing body of work that investigates the toxicity of synthesized nanoparticles to plants, aquatic invertebrates, algae, bacteria and different cell lines. Although the potential negative effects of nanoparticles on organisms and the environment have raised concerns, limited studies so far have examined the difference between nanoparticles and bulk particles with the same chemical composition and mineral phase, or addressed the toxicity of dissolved metal ions from the nanoparticles.

In new work by scientists at the University of Massachusetts, the toxicity of bulk oxide particles and the released ions were assessed along with four oxide nanoparticles, which clearly showed that size matters.

Baoshan Xing, a professor in the Department of Plant, Soil & Insect Sciences at the University of Massachusetts, and his group examined nanoparticulate aluminum oxide (60 nm), silicon dioxide (20 nm), titanium dioxide (50 nm), and zinc oxide (20 nm) – nanoparticles that are common industrial additives and have various applications ranging from cosmetics to electronics.

"We performed our experiments to better understand whether the bacterial toxicity of nanoparticles was size- or composition-related," Xing tells us. "We did this by comparing the nanoparticles to their bulk counterparts, and by evaluating the contribution of dissolved metal ions to the overall toxicity. In addition, we examined the interaction between nanoparticles and bacteria and their surface properties."

Xing’s team conducted their toxicity assessments using three model bacteria species: gram-positive Bacillus subtilis; gram-negative Escherichia coli and Pseudomonas fluorescens.

Since bacteria perform many critical roles in ecosystem function and productivity, the potential impact on them by nanoparticles released into the environment deserves particular attention.

"Interactions between bacteria and nanoparticles may provide us with more information about the impact of nanoparticles on the ecosystem once released," says Xing. "At the same time, bacteria as single cell organisms are good test models to study the toxicity of nanoparticles and to explore how nanoparticles affect the cell/organism function."

In this work, which has been published online in Environmental Pollution ("Bacterial toxicity comparison between nano- and micro-scaled oxide particles"), the team (working with Xing were Wei Jiang as first author and Hamid Mashayekhi) reports three major findings:

1) Aluminum oxide, silicon dioxide, and zinc oxide nanoparticles were toxic to the three bacteria species tested, while titanium dioxide nanoparticles did not show any observable toxicity. The zinc oxide nanoparticles showed the highest toxicity.

2) Aluminum oxide, silicon dioxide, and zinc oxide nanoparticles showed higher toxicity compared to their bulk particle counterparts.

3) Toxicity of nanoparticles was not only from the dissolved metal ions, but also from their greater tendency to attach to the bacterial cell walls.

"What we found shows that nanoparticles that were toxic to bacteria behave differently in bacterial suspension than the non-toxic ones" explains Xing. "The toxic nanoparticles could cover the whole bacteria surface, while the non-toxic ones under our experimental condition could not cover the bacteria cells but

Continued on page 4
The OECD has developed a global resource which collects research projects that address environmental, human health and safety issues of manufactured nanomaterials.

**OECD LAUNCHES DATABASE ON RESEARCH INTO THE SAFETY OF MANUFACTURED NANOMATERIALS**

The OECD Database on Research into Safety of Manufactured Nanomaterials is a global resource which collects research projects that address environmental, human health and safety issues of manufactured nanomaterials. This database helps identify research gaps and assists researchers in future collaborative efforts. The database also assists the projects of the OECD’s Working Party on Manufactured Nanomaterials (WPMN) as a resource of research information.

**Database on Research into Safety of Manufactured Nanomaterials: General Information**

As part of the OECD activities to promote international cooperation in addressing human health and environmental safety aspects of manufactured nanomaterials, the OECD has developed a global resource which collects research projects that address environmental, human health and safety issues of manufactured nanomaterials. This database holds details of completed, current and planned research projects on safety, which are to be updated (electronically) by delegations. This database is also intended to be an inventory of information on research programmes to help the other projects of the WPMN by identifying relevant research projects or storing information derived from the projects of the WPMN, including the sponsorship programme on the testing of manufactured nanomaterials.

**What sort of information is included in the database?**

The OECD Database on Research into the Safety of Manufactured Nanomaterials (the database) is an inventory of safety research information on manufactured (engineered) nanomaterials.

Manufactured nanomaterials are those intentionally produced to have specific properties or specific composition, a size range typically between 1 nm and 100 nm and material which is confined in one, two, or three dimensions at the nanoscale (nano-object) or having an internal or surface structure at the nanoscale (nanostructured).

The database contains information relevant to research on the environmental, health, and safety of nanomaterials for projects that are planned, underway or completed.

The following information is stored in distinct fields:

- Project Title; Start date; End date;
- Project Status (Current; planned; or completed);
- Country or organisation;
- Funding information (where available, on approximate total funding; approximate annual funding; and funding source);
- Project Summary; Project URL; Related web links;
- Investigator information: name, research affiliation, contact details;
- Categorisation by material name, relevance to the safety, research themes, test methods;
- Overall outcomes and outputs.

Guidance for using this database is available as “pop-up” information or as guidance manual accessible at [to be provided].

**How can I access and search the database?**

Users can access the database through an internet interface at: [http://webnet.oecd.org/NanoMaterials](http://webnet.oecd.org/NanoMaterials). The website is compatible with MS Internet Explorer (6 and 7) and Mozilla Firefox (1.5 and 2.0).

The database is searchable either through:

- Simple search that enables interrogation of the database using keywords and Boolean operators: AND (space, &), OR (|) , NOT(-), Wild cards(*);
- Advanced search that allows users to search by different criteria such as keyword text, project information, country and category.

The simple search also enables users to access other linked databases: ‘Environmental Health and Safety Database’ by International Council on Nanotechnology (ICON) and ‘Nanoparticle Information Library’ by US National Institute for Occupational Safety and Health (NIOSH).

Users of the database are bound by the terms and conditions for OECD Website Usage and Privacy Policy.

**How has the database been established?**

This database builds on the database of the Woodrow Wilson International Center for Scholars: Nanotechnology Health and Environmental Implications: An Inventory of Current Research. The database was pre-populated with research projects transferred from the inventory. OECD Delegations have been updating or adding new information.

**How can I contribute to the database?**

If you wish to contribute to the database, please contact the OECD Secretariat (nanosafety@oecd.org). The Secretariat will contact respective representatives to the OECD to register you as a Data Provider for your country or organisation.

Data Providers enter and revise information through a password protected online web portal. The respective representatives to the OECD and the OECD Secretariat will facilitate individual access to the database.

**How will the integrity of the database be maintained?**

Ensuring data quality is critical to the integrity of the database. The process for data entry and checking is completely within the control of individual delegations.

- Data Providers are responsible for ensuring the quality of the data entered.
- Designated Contact Points are responsible for checking the accuracy and internal consistency of data reporting.

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New work demonstrates that Raman spectroscopy can be used to detect and monitor circulating carbon nanotubes in vivo and in real time

NOVEL TECHNIQUE FOR REAL-TIME DETECTION OF CARBON NANOTUBES IN THE BODY

New work at the University of Arkansas at Little Rock has, for the very first time, demonstrated that Raman spectroscopy can be used to detect and monitor circulating carbon nanotubes in vivo and in real time. These findings could have a significant impact on the knowledge of how nanomaterials interact with living biological systems.

Carbon nanotubes (CNTs) can be used for various advanced bio-medical applications. Before any clinical application of nanoparticles, it is imperative to determine critical in vivo parameters, namely pharmacological profiles including nanoparticle clearance rate from the circulation and their biodistribution in various tissue and organs. Until now, their distribution was only monitored by collecting samples after various time intervals, but this new research shows the ability of monitoring their concentration in vivo and in real time, while the animal is alive. Moreover, this work can be extended to the detection of circulating cancer cells that have been tagged by carbon nanotubes.

"We were motivated by the desire of being able to visualize individual carbon nanotubes moving in lymph, blood and tissues," Alexandru S. Biris tells us. "Prior to our work it was not possible to visualize carbon nanotubes in vivo in real time or to observe how cancer cells migrate through circulation."

Biris, Chief Scientist and Assistant Professor at the University of Arkansas Nanotechnology Center, and his collaborator Vladimir Zharov, Professor and Director of the Philips Classic Lasers and Nanomedicine Laboratory at the University of Arkansas for Medical Sciences believe that in vivo flow cytometry using Raman detection technique is very promising for counting and identification of individual circulating nanoobjects with strong Raman scattering properties, and this technique can be supplementary or alternative to existing fluorescent and photoacoustic methods.

The researchers have reported their findings in the April 28, 2009 online edition of Journal of Biomedical Optics ("In vivo Raman flow cytometry for real-time detection of carbon nanotube kinetics in lymph, blood, and tissues").

"Previously, Raman spectroscopy has been applied to monitor CNTs in vivo in static condition, demonstrating large signal-to-noise ratios and molecular specificity due to the strong Raman scattering signals from CNTs and their specific vibrational spectra fingerprints, respectively," Biris explains. "Raman spectroscopy was successfully used also in vitro flow cytometry using surface-enhanced Raman scattering (SERS) nanoparticles. However, the application of Raman flow cytometry (RFC) for in vivo studies as we...

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instead lumped together. The bacteria could attract the positively charged individual particles or small aggregates from the solution. The dispersion may increase both the mobility and toxicity of nanoparticles. Therefore the nanoparticles staying in aggregates were not toxic, while the dispersed nanoparticles could be attracted by bacteria and subsequently showed toxicity."

The reason why only titanium dioxide didn't show toxicity among the four tested nanoparticle species is that it was the only highly negatively charged nanoparticle – the negatively charged bacterial cells repel the titanium dioxide nanoparticles.

Xing notes that bacteria will attract positively charged nanoparticles from aquatic systems, increasing the chance of such nanoparticles entering the aquatic food chain.

Since the the three bacteria species used in the experiments are widely present in soil and aquatic systems, they may be developed as indicators for nanoparticle toxicity.

"The relationship between nanoparticle toxicity and attachment to bacteria found in our research may inspire the manufacturers of nanoparticles to develop environment-friendly nanoparticles by adjusting their surface properties," says Xing. "On the other hand, the nanoparticles' antimicrobial activity may be applied in developing self-sterilizing materials."

So far the least understood aspects of nanoparticle toxicity with bacteria have to do with the mechanism of this toxicity. Most researchers believe that the toxicity to bacteria is caused by damage to the cell wall.

The main challenge to mechanistic studies like the one conducted by the University of Massachusetts scientists is the lack of effective and sensitive enough methods to probe the changes to the bacterial cell wall (which is a very complicated and integrated structure).

"It is very difficult to determine in which cell component the changes occur when we study the nanoparticles' effects using living cells," says Xing. "For most techniques, it is difficult to differentiate if the instrument signals are from the cell wall or the cell interior. And if instead we work on components extracted from bacterial cells, the observed effect may not represent what is occurring in integrated living cells."

**REAL-TIME DETECTION OF CARBON NANOTUBES IN THE BODY…**

have demonstrated, especially for single cell detection, is a novel approach."

Using single-walled CNTs, the research team demonstrates the nanotubes' ability to serve as excellent in vivo Raman contrast agents with unique Raman scattering spectrum. Raman spectra of CNTs contain characteristic bands in two spectral regions. The researchers found that the G band has the highest intensity (referred to as Raman signals) and therefore they used it to dynamically evaluate the presence of CNTs in the biological environments in vivo.

Zharov says that this technique in time-resolved mode may provide high sensitive detection of a limited number of nanoparticles in the small irradiated volume of blood and lymphatics on animal models, as well as selective detection of single cancer cells targeted by these nanoparticles.

"We believe that with new technique it would be possible to integrate detection of circulating tumor cells with almost simultaneous their laser killing using the same nanoparticles as photothermal agents," says Zharov. "Basically, it should be possible also to real-time monitoring the circulation of various nanomaterials and their accumulation into normal tissue and potentially into the tumor using Raman spectroscopy in dynamic and conventional 'static' modes, respectively."

A major challenge for nanomedicine researchers is to use nanomaterials bio-conjugated with antibodies, proteins and other ligands to target selectively both single circulating tumor cells and tumors with many cancer cells. The interdisciplinary team of physicists, biomedical engineers, chemists, biologist, and physicians from the University of Arkansas at Little Rock and the University of Arkansas for Medical Sciences team are working on further improvement of this technique by increasing its sensitivity, time-response and specificity as well as by combination with photoacoustic flow cytometry (see NIH webpage: [Listening for One Cell in a Billion](https://www.nih.gov/)) and thermal ablation of individual cancer in real biological background.
OECD DATABASE…

Continued from page 2

Heads of OECD Delegations will identify Data Providers and Designated Contact Points in their respective countries. Delegations can nominate multiple Data Providers to facilitate data submission, but the number of Designated Contact Points in one country will be limited to ensure the integrity of data entry.

Following are some examples of processes built into data entry and checking that will assist in maintaining the integrity of the database:

• To minimise duplicate entries, the designated project coordinator will act as Data Provider for research projects that involve multiple researchers, e.g. transnational projects;
• To ensure accuracy projects entered into the database as planned or underway must be updated regularly and on completion;
• Detailed guidance is provided to users through the Guidance Manual and information contained within the interface;
• Data entry occurs in a secure environment and the Data Provider has control over when the entry is made public (i.e. published). Entries can be “saved as draft” or “saved and published”; and
• “Checking” rights are only awarded to Designated Contact Points. Designated Contact Points can only edit/check entries from their own country.

How will countries coordinate activities?

Individual delegations to the OECD are responsible for coordinating the work of their Data Providers and Designated Contact Points. Individual delegations may also use the “comments” field in the database for communication between Data Providers and Designated Contact Points.

The Guidance Manual includes an annex that describes modus operandi adopted by certain OECD delegations. While the modus operandi will vary between delegations, these are provided as examples that may be adopted or varied.

What features assist with data entry?

Several mechanisms have been built into the database to facilitate data entry and checking. These include:

• Multiple mechanisms for reporting research results:
  A free text box, URL; Upload files – word format and PDF
• A secure “comments” field that can be used as a communication tool between Designated Contact Points, Data Providers and OECD secretariat.

Criteria have been developed to preserve the confidentiality of commercially sensitive information and the privacy of individuals.

A Guidance Manual is available to provide a step-by-step guide for end users, Data Providers and Designated Contact Points. The OECD secretariat can be contacted if further clarification is required.

Can I provide feedback on its operation and content?

Users are encouraged to provide their feedback to the OECD Secretariat to ensure that the database continues to be a user friendly tool in the overall research effort. Avenues available for feedback include:

• e-mailing nanosafety@oecd.org; or
• using the “contact” function in the database

Communication and feedback between Data Providers and Designated Contact Points can occur via the secure comments field in the database.

Additional Information on the Database

Additional informational material has been developed which can be freely used for communication purposes.

• Guidance manual for using the database [to be provided]
• Overview slides on the database [to be provided]
• Brochure [to be provided]

Other OECD Activities on the Safety of Manufactured Nanomaterials

The outcomes from past OECD activities are also available at: Publications in the Series on the Safety of Manufactured Nanomaterials.

FUNDING OPPORTUNITY: ENVIRONMENTAL BEHAVIOR, BIOAVAILABILITY AND EFFECTS OF MANUFACTURED NANOMATERIALS

The U.S. Environmental Protection Agency (US EPA), as part of its Science to Achieve Results (STAR) program, in conjunction with the UK Environmental Nanoscience Initiative (UKENI), is seeking joint applications from US and UK partners that:

1. propose integrated model(s) of fate, behavior, bioavailability and effects for several important and representative nanomaterial classes over key environmental pathways using intrinsic material properties and life cycle analysis as a starting point for model development;
2. validate and refine these model(s) through interdisciplinary research, addressing key assumptions and areas of uncertainty; and
3. develop effective methods and tools to detect, assess, and monitor the presence of nanomaterials in biological and environmental samples.

The outputs of this program will be used to further scientific understanding of the fate, behavior, bioavailability and effects of nanomaterials and risk management policy development.

EUROPE LAUNCHES NOVEL INTEGRATED APPROACH TO NANO-PARTICLE RISK ASSESSMENT

The Institute of Occupational Medicine (IOM) has announced the launch of ENPRA - a major new European Framework 7 project to develop and implement a novel integrated approach for engineered nanoparticle (ENP) risk assessment.

With an estimated economic impact of €292 billion by 2010 across industrial, consumer and medical products, nanotechnology is already one of the key industries within Europe and worldwide. Key to its long term growth and sustainability is establishing end-user confidence that the technologies developed are safe. ENPRA (Engineered NanoParticle Risk Assessment) aims to support long term growth and sustainability of nanotechnologies by expanding the classic exposure-dose-response paradigm of risk assessment, to develop an effective approach for the assessment and management of potential health risks from exposure to engineered nanoparticles.

The 3½ year IOM-led project, worth €3.7 million, harnesses the knowledge and capabilities of 15 European and 6 U.S. partners including three U.S. Federal Agencies: EPA, NIOSH and NIH-NIEHS. Under the coordination of Dr Lang Tran, IOM’s Director of Computational Toxicology, ENPRA will utilise the latest advances within in vitro, in vivo and in silico approaches to nanotechnology environment, health & safety (EHS) research to realise its aims.

“ENPRA also has a strong British element, with the participation of three UK research organisations, the IOM, Edinburgh Napier and Edinburgh Universities,” said Dr Tran. “In addition, the in vitro and in silico approaches to be developed within ENPRA will also help to reduce the need for animal experimentation in nanotoxicology” he added.

Harnessing the latest advances in toxicology to nanotechnology EH&S issues, the fundamentally novel rationale of ENPRA goes beyond traditional toxicity assessment of ENP and seeks to:

- identify the critical ENP physico-chemical characteristics responsible for the observed toxicity;
- investigate the cellular and molecular mechanisms underlying the observed association;
- develop systems, verifiable with in vivo experiments, which could be used as potential high throughput alternative toxicity tests;
- use a Structure-Activity method to facilitate such identification and use this to predict the hazard of new materials;
- extrapolate the results from in vitro to in vivo and to other relevant occupational or consumer situations;
- incorporate all possible data as weight-of-evidence for a risk assessment of ENP

“Our ability to disseminate the results of ENPRA is strengthened considerably by our pre-existing management of SAFENANO, one of the world’s key information portals on nanotechnology health and safety issues” Dr Tran commented.
IN SHORT – PAPERS, INITIATIVES & UPDATES

**PAPER:** Comparative Photoactivity and Antibacterial Properties of C60 Fullerenes and Titanium Dioxide Nanoparticles

Bacterial (Escherichia coli) toxicity tests suggest that, unlike nano-TiO2 which was exclusively phototoxic, the antibacterial activity of fullerene suspensions was linked to ROS production. Nano-TiO2 may be more efficient for water treatment involving UV or solar energy, to enhance contaminant oxidation and perhaps for disinfection. However, fullerol and PVP/C60 may be useful as water treatment agents targeting specific pollutants or microorganisms that are more sensitive to either superoxide or singlet oxygen. doi: 10.1021/es803093t

**PAPER:** Uptake, Translocation, and Transmission of Carbon Nanomaterials in Rice Plants

Scientists at Clemson University characterized the dynamic uptake, compartment distribution, and transformation of fullerene C70 in rice plants and have detected the transmission of C70 to the progeny through seeds. The integration of nanoparticles by plant species may result from the nanoparticles’ small dimension and self-assembly and from the nanoparticle interactions with plant organelles and natural organic matter. The potential impacts of these processes on both food safety and the environment are important subjects to understand. doi: 10.1002/smll.200801556

**GOVERNMENT:** UK House of Lord Hears Evidence on Food Nanotechnology

In a hearing held yesterday in the U.K. House of Lords, Professor Ken Donaldson from the University of Edinburgh and Dr Qasim Chaudhry from the Food and Environment Research Agency presented evidence to the Select Committee on Nanotechnologies in Food. The hearing focussed specifically on nanoparticles in food. As part of the evidence given, several key points were raised including: 1) the ability of nanoparticles to cross the gut wall and other cellular barriers; 2) the potential novel toxic effects of such nanoparticles in the human body; 3) the possibility that other materials in the gut (e.g. bacteria or other contaminants) may attach to the nanoparticles and be transported across the gut wall (the Trojan Horse Effect). Other important concerns included the ability of nanoparticles to interfere with cellular processes in the body e.g. oxygen metabolism, and the antimicrobial properties of some nanoparticles which may result in further harmful effects. Click here to watch a webcast of the full proceedings.

**REPORT:** OECD Publishes New Report in its Series on the Safety of Manufactured Nanomaterials

"Preliminary Analysis of Exposure Measurement and Exposure Mitigation in Occupational Settings: Manufactured Nanomaterials" is the eighth publication in the Series on the Safety of Manufactured Nanomaterials. This report provides preliminary analyses and recommendations as well as brief summaries of background documents listed in the operational plan relevant to phase 1 of the project on exposure measurement and exposure mitigation. This document is published on the responsibility of the Joint Meeting of the Chemicals Committee and the Working Party on Chemicals, Pesticides and Biotechnology of the OECD.

**INITIATIVE:** SAFENANO Team Completes Global Review of Nanomaterial EHS Research

EMERGNANO – the first global review of active research into the environment, health and safety risks of nanotechnology has been published today by Defra, the UK Government Department for Food & Rural Affairs. The review, led by the SAFENANO initiative at the Institute of Occupational Medicine in Edinburgh, UK, provides a unique identification and analysis of research carried out worldwide on nanotechnology safety, including that relating to hazard, exposure, risk assessment & regulation. EMERGNANO identified more than 670 projects from around the world, and after careful selection assessed more than 260 unique, relevant projects completed, close to completion or in progress since 2004. The final report provides a comprehensive listing of projects, alongside detailed evaluation of their outputs. It also provides an assessment of the extent to which these projects contribute towards meeting the 18 Research Objectives for nanotechnology risks laid out by the UK Nanotechnology Research Coordination Group, and highlights the gaps still remaining.

**INITIATIVE:** New Website nanoSMILE Offers Information About Nanotechnology Risk Issues

NanosMILE has been developed under the FP6 European program Nanosafe2. The website offers training activities and communicates information about the potential risks of nanomaterials.

**INITIATIVE:** NIOSH Updates its Guidance 'Approaches to Safe Nanotechnology'

The National Institute for Occupational Safety and Health (NIOSH) recently issued an updated and expanded edition of its document, "Approaches to Safe Nanotechnology". The updated document reiterates NIOSH’s standing interim recommendation that employers take prudent measures to control occupational exposures in the manufacture and industrial use of engineered nanomaterials, as research advances for determining if such materials pose work-related health and safety risks.

**PAPER:** Characterizing Manufactured Nanoparticles in the Environment: Multimethod Determination of Particle Sizes

Sizes of stabilized nanoparticle suspensions were determined using several state-of-the-art analytical techniques (transmission electron microscopy; atomic force microscopy; dynamic light scattering; fluorescence correlation spectroscopy; nanoparticle tracking analysis; flow field flow fractionation). Theoretical and analytical considerations were evaluated, results were compared, and the advantages and limitations of the techniques were discussed. No “ideal” technique was found for characterizing manufactured nanoparticles in an environmental context as each technique had its own advantages and limitations. doi: 10.1021/es900249m
The nanoRISK newsletter is dedicated to providing objective and accurate information about critical issues and developments related to the risks arising from engineered nanomaterials. nanoRISK appears bi-monthly (ISSN 1931-6941). For a complete list of all published nanoRISK newsletters please go to www.nanorisk.org.

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