FAST BREAKING PAPERS - 2009

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Anne Kahru talks with *ScienceWatch.com* and answers a few questions about this month's Fast Breaking Paper in the field of Environment/Ecology.

Article Title: Toxicity of nanosized and bulk ZnO, CuO and TiO_2 to bacteria Vibrio fischeri and crustaceans Daphnia magna and Thamnocephalus platyurus

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SW: Why do you think your paper is highly cited?

This research paper is novel and very timely considering that there are widespread urgent concerns regarding the environmental implications of nanotechnologies/nanoparticles (NP). First, we provided valuable and reliable quantitative information on the ecotoxicity of three metal oxide nanoparticles (TiO_2, ZnO and CuO) that could be used for environmental risk assessment purposes. Secondly, this paper presents the first evaluation of the toxicity of TiO_2, ZnO, and CuO to bacteria *Vibrio fischeri* and crustaceans *Thamnocephalus platyurus*. Also, this was the first study of nano ZnO and nano CuO toxicity for crustaceans *Daphnia magna*—one of the "gold standard" organisms in aquatic toxicology.

Does it describe a new discovery, methodology, or synthesis of knowledge?

It is also one of the first papers that showed that the solubilisation of metal-containing nanoparticles is the key factor in their (aquatic) toxicity. Metal oxide particles do not necessarily have to enter the cells to induce the toxic effects but the solubilised ions will "do the job." That, of course, does not necessarily mean that the particles themselves are harmless—the toxic effects can be combined.

To demonstrate that, we used a novel approach based on a combination of traditional ecotoxicological methods (comparing the ecotoxic effects of nanosize metal oxides with their bulk analogues and soluble zinc and copper salts) and metal-specific recombinant biosensors. This combined approach allowed us to differentiate the toxic effects of metal oxide NPs *per se* and solubilised metal ions.

Would you summarize the significance of your paper in layman's terms?

Synthetic NPs are defined as particles with one dimension less than 100 nm and they can be organic (C60 fullerenes, carbon nanotubes, dendrimers) or inorganic (metal oxides, nano-silver, quantum dots). Metal oxide NPs, especially TiO_2 and ZnO, are increasingly used in various consumer products such as cosmetics and sunscreens, dental fillings, self-cleaning coatings, and textiles. CuO NPs have potential in gas sensors, catalysis, and as antimicrobials.

As the use of NPs is constantly increasing in broad applications, sooner or later the NPs will end up in the environment, either due to accidental spills or *via* various waste streams analogously to other industrial chemicals. Therefore, information on the ecotoxicological effects of NPs is urgently needed. Usually the first level evaluation of hazardous environmental effects of chemical compounds is performed on simplified food-chain model organisms (crustaceans, algae, fish).

In our paper, we showed that differently from TiO_2 NPs that were not toxic, ZnO and CuO NPs were remarkably toxic to bacteria and crustaceans, showing acute toxic effects to *Daphnia magna* at level of ~3 mg per liter. For another crustacean, *Thamnocephalus platyurus*, the toxicity of ZnO was even about 19 times higher (LC50=0.18 mg/l). Thus, the environmental impact of manufactured NPs should be carefully assessed in order to ensure the sustainable development of nanotechnology.

"Being the Chair-person of Estonian Society of Toxicology, I have learned that companies or industries are interested in the expertise of the specialists in toxicological issues when there is a law to apply and thus assistance needed."

How did you become involved in this research, and were there any problems along the way?

My first experience with NPs was when I was participating with my group in the evaluation of the potential toxic effects of polyamidoamine (PAMAM) dendrimers and polyethylenimine dendrimers (PEI)—organic NPs studied mainly for the gene delivery applications—on various non-vertebrate ecotoxicological test organisms concurrently with mammalian cell cultures *in vitro* and for mice *in vivo*.

We showed that a battery of simple tests can be used, not only to assess ecotoxicological properties, but also for high-throughput screening for biological effects NPs. My interest in the environmental hazard issues of NPs was confirmed by a reading in the *New Scientist* (2004) the discovery of Dr. Eva Oberdörster concerning the toxicity of C60 fullerenes for fish and daphnids already at sub milligram per liter level.

The observation of a relatively high toxicity of something that is just a different form of carbon was scientifically really exciting. Thus, the same year I wrote an application titled "Biological effects of modified fullerenes and nano-size metals" to the Howard Hughes foundation call for "Grants for Biomedical Research Baltics, Central and Eastern Europe, Russia, and Ukraine." Unfortunately this application was not granted, probably due to the fact that this Foundation is oriented on the funding of fundamental biomedical research.

However, in this project application I had summarized a lot of very interesting ideas and hypothesis and wrote a sound research plan. Thus, I managed to convince the Head of the Scientific Council of my Institute (Prof. Mart Saarma, University of Helsinki) and the Directors (Dr. Ago Samoson in 2005 and Dr. Raivo Stern since 2006) to support this innovative research from the general budget of our Institute.

In addition to the ideas and seeding money, I had a group of young and enthusiastic scientists trained in ecotoxicology, environmental chemistry, microbial physiology, and molecular microbiology. Currently, the nanoecotoxicological studies of my group are also supported by other funding sources of the Estonian Government.

Hereby I would like to recognize the work of my group—Prof. Henri-Charles Dubourguier, Dr. Angela Ivask, Dr. Irina Blinova, and Dr. Kaja Kasemets. Currently, my research team also involves six Ph.D. students who are interested in this novel and highly competitive area of research—Margit Heinlaan, Villem Aruoja, Monika Mortimer, Imbi Kurvet, Mariliis Sihtmäe and Olesja Bondarenko—in addition to several students at M.Sc. and B.Sc. level.

Where do you see your research leading in the future?

With my group of researchers and Ph.D. students, we have already published five papers which are listed in *Web of Science*® journals and four more papers which are currently in press or submitted. One interesting current topic is the different mechanisms involved in the toxicity of different types of NPs modulated by the physiology of the target organisms and environmental parameters.

"...one of the first papers that showed that the solubilisation of metal-containing nanoparticles is the key factor in their (aquatic) toxicity."

Research on the hazard of NPs has to integrate or at least to take into account both human and environmental impacts. I am currently involved as a managing Guest Editor in the preparation of a Special Issue of *Toxicology* entitled "Potential hazard of nanoparticles: from properties to biological & environmental effects." The interest of Elsevier to publish this Special Issue shows the need for integrated approaches in the hazard evaluation of synthetic nanoparticles.

Do you foresee any social or political implications for your research?

Absolutely—synthetic NPs have been already included in various consumer products (cosmetics, sunscreens, socks, and even underwear). However, relevant toxicological and ecotoxicological information is often misleading. Thus, human exposure to NPs is a real concern.

Naturally, as is the case of all industrial chemicals, workplace exposures at manufacturing sites of nanoparticles/materials may pose the greatest risk to humans, but also the potential effects to ecosystems and environment have to be studied. For example, NPs included in sunscreens applied by humans get washed into bodies of water and thus, aquatic organisms will be also exposed to these NPs.

Thus, till we do have enough—how much is enough?—information on the potential adverse effects of NPs (on all their life cycle levels), precautionary principles should be applied. Fortunately, we have lessons to learn from the past (PCBs, asbestos, GMOs), to avoid other potentially costly mistakes.

Being the Chairperson of the Estonian Society of Toxicology, I have learned that companies or industries are interested in the expertise of the specialists in toxicological issues when there is a law to apply and thus assistance is needed. For example, the implementation of the REACH directive, a new European Community Regulation on chemicals and their safe use, has remarkably increased the interest of the industry and legislative bodies on toxicological research. Currently, assessing the safety of synthetic NPs has become a worldwide issue and I am confident that soon the synthetic NPs will be regulated analogously to bulk chemicals.

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