NANOTOX 2006

KAROLINSKA INSTITUTET
NOBEL FORUM

MINI-SYMPOSIUM ON NANOTOXICOLOGY: POTENTIAL RISKS AND SAFETY ASSESSMENT

NOVEMBER 27, 2006

Karolinska Institutet

Swedish Society of Toxicology

Welcome to the Nobel Forum and the Mini-Symposium on Nanotoxicology: Potential Risks and Safety Assessment. This 1-day symposium will cover a wide spectrum of topics of relevance to our understanding of the potentially hazardous effects of nano-sized particles (i.e. particles in the range of 1-100 nm). Leading scientists from Europe and the United States will present their work on toxicity of nanoparticles, including so-called single-walled carbon nanotubes, which are of emerging importance in technology and medicine. The speakers will also highlight the topics of risk assessment and regulatory issues related to nano-sized particles.

The current symposium on Nanotoxicology is hosted by the Institute of Environmental Medicine at Karolinska Institutet, and is sponsored, in part, by the Swedish Society of Toxicology. We are very grateful for this support. We also wish to thank all the invited speakers for agreeing to share their insights into the nanoworld, and we look forward to an exciting and interactive day of lectures at the Nobel Forum.

Bengt Fadeel, M.D., Ph.D.

Program

09.30	**Registration and coffee**
10.00	Opening of the meeting (*Göran Pershagen*, Institute of Environmental Medicine, Karolinska Institutet, Stockholm)
10.15	Nanopromises and nanothreats: an introduction (*Bengt Fadeel*, Institute of Environmental Medicine, Karolinska Institutet, Stockholm)
	Chairperson: Valerian Kagan
10.30	Nanomaterials: definitions and standardization of methods for the evaluation of toxicity (*Harald Krug*, Institute of Toxicology and Genetics, Karlsruhe)
11.15	Human studies: inhalation of carbon particles; implications for nanotoxicology (*Magnus Svartengren*, Department of Public Health Sciences, Karolinska Institutet, Stockholm)
12.00	**Lunch**
	Chairperson: Harald Krug
13.15	Animal studies: pulmonary toxicity of nanomaterials/carbon nanotubes (*Anna Shvedova*, National Institute of Occupational Safety and Health, Morgantown)

14.00	Cellular studies: carbon nanotubes; recognition by macrophages, inflammatory responses, oxidative stress (*Valerian Kagan*, Department of Environmental and Occupational Health, University of Pittsburgh, Pittsburgh)
14.45	**Coffee**
	Chairperson: Anna Shvedova
15.15	Risk assessment of nanoparticles; methods and models (*Lang Tran*, Institute of Occupational Medicine, Edinburgh)
16.00	Nanotoxicology: regulatory aspects; looking to the future (*Eva Hellsten,* Environment Directorate-General, European Commission, Brussels)
16.45	Closing remarks (*Lars Wiklund*, Swedish Society of Toxicology, Stockholm)

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Opening remarks

Göran Pershagen

Chairman

Institute of Environmental Medicine, Karolinska Institutet, Stockholm, SWEDEN

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Nanopromises and Nanothreats: an introduction

Bengt Fadeel

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Nanosciences are currently receiving a great deal of attention. The prefix "nano" is derived from the Greek word *nanos*, meaning dwarf, and refers to the nanometer (nm), i.e. one billionth of a metre. Physicist and Nobel laureate Richard Feynman was the first to predict the opportunities presented by the manipulation of matter at the level of individual atoms and molecules (1). The term "nanotechnology" was coined in 1974 by Japanese engineer Norio Taniguchi, and the development of the scanning tunneling microscope 25 years ago, followed by the arrival of the atomic force microscope 5 years later, were crucial in propelling the field of nanoscience (2). Today, nanotechnologies have the potential to bring about major changes in human health (3). However, the very properties that make nano-sized materials so promising from a technological perspective, such as their high degree of reactivity and their ability to cross barriers, could also make them hazardous to humans and to the environment. Studies of these toxicological effects of nanoparticles are of considerable importance. Some examples of the future promises and potential threats of nanoparticles will be discussed in this brief presentation.

Literature

(1) Feynman RP. There's plenty of room at the bottom; an invitation to enter a new field of physics. *Engineering & Science* 1960;23 (22 Feb).

(2) Gerber C, Lang HP. How the doors to the nanoworld were opened. *Nat Nanotech* 2006;1:3-5.

(3) Moghimi SM, Hunter AC, Murray JC. Nanomedicine: current status and future prospects. *FASEB J* 2005;19:311-330.

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Nanomaterials: Definitions and Standardization of Methods for the Evaluation of Toxicity

Harald F. Krug

Forschungszentrum Karlsruhe, GERMANY

Most elements of living cells, including proteins, nucleic acids, lipids and even cellular organelles are on the same scale as synthetic nanomaterials. Therefore it is a logical consequence that these small particles can interact with biological systems leading possibly to a variety of alterations on the molecular level. Moreover, nanosystems are specifically engineered to interact with biological systems for particular medical or biological applications. Thus, there is no doubt that nanomaterials, especially nanoparticles, can interact or interfere with the experimental set-up producing false-positive or false-negative results. This is especially of great relevance when assessing the biological effects of nanoparticles with relation to occupational exposure or human health in general. Recently, it has been published by several authors, that specific toxicity test systems have been falsified by the investigated nanoparticles leading to false-positive outcome (1;2). These findings strongly suggest verifying cytotoxicity data with at least two or more independent test systems for each new class of materials or newly synthesized nanoparticles. Moreover, several preparations of specific nanomaterials contain not only the desired particles but often contaminants can also be found within such materials. Especially, carbon nanotubes are frequently contaminated with metals, amorphous carbon and other materials (3). Most toxicity assays therefore tested for the toxic effects of the contaminants and not for the original materials that should be tested as has been published recently (2;4). With regard to these remarks it should be recommended to standardize not only the methods but also the materials that were tested. In any case, a complete analytic of the nanoparticles should be included to all toxicity assays.

Literature

(1) Monteiro-Riviere NA, Inman AO. Challenges for assessing carbon nanomaterial toxicity to the skin. *Carbon* 2006; 44:1070-1078.

(2) Wörle-Knirsch JM, Pulskamp K, Krug HF. Oops they did it again! Carbon nanotubes hoax scientists in viability assays. *Nano Lett* 2006; 6:1261-1268.

(3) Donaldson K, Aitken R, Tran L, Stone V, Duffin R, Forrest G, et al. Carbon Nanotubes: a review of their properties in relation to pulmonary toxicology and workplace safety. *Toxicol Sci* 2006; 92:5-22.

(4) Kagan VE, Tyurina YY, Tyurin VA, Konduru NV, Potapovich AI, Osipov AN, et al. Direct and indirect effects of single walled carbon nanotubes on RAW 264.7 macrophages: role of iron. *Toxicol Lett* 2006; 165:88-100.

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Studies of Translocation of Inhaled 100 and 35 nm Carbon Particles to the Circulation in Humans

Magnus Svartengren

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There are data in the literature indicating fast and substantial systemic uptake of ultrafine particles from the lung. We have developed an improved method to produce ultrafine particles with more stable radiolabelling and defined particle size range. Two experiments using 35 and 100 nm particles were conducted. Both experiments used a gamma camera imaging technique. A) Fifteen subjects inhaled technetium 99m (99mTc)-labelled carbonaceous particles of 100 nm in size. Radioactivity over the lung was followed for 70 h. Lung retention at 46 h was mean ± SD 99 ± 4.6%. The 24-h activity leaching in urine was 1.0 ± 0.55%. B) Nine healthy subjects and four asthmatics inhaled the test particles. Retention of 35 nm 99*m*Tc-labeled carbonaceous particles, was followed for 24 h. One additional subject was exposed to particles with unstable labeling. There were no significant differences between healthy and asthmatic subjects in any of the parameters studied. Particle retention after 24 h was 102% (SD ± 4.7). In blood samples, 80 min after exposure, 1.1% (±0.4) of initially deposited activity was detected and 91% of the activity was not bound to particles. Lung retention was 30% after 1 h in the subject exposed to the leaching aerosol (*n*= 1). In conclusion, there was no significant quantitatively important translocation of 100-nm nor 35-nm particles to the systemic circulation from the lungs. More research is needed to establish if the low percentage cleared activity originates from leached activity or insoluble translocated particles, and whether a few percent of translocated particles is sufficient to cause harmful effects.

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Pulmonary Toxicity of Nanomaterials/Single-Walled Carbon Nanotubes

Anna A. Shvedova

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Nanotechnology is a fast growing field with emerging development of modern materials for electronics and information technology industries, cosmetics, health and medicine and in a variety of manufactured goods. The toxic effects of nanoscale materials have not been fully characterized, and the limited studies conducted thus far indicate the urgent necessity for toxicological assessments. Single-Walled Carbon Nanotubes (SWCNT) with unique physico-chemical, electronic and mechanical properties are novel materials of technological importance. Therefore, they may exhibit unusual interactions with cells and tissues, thus causing adverse health effects. Pulmonary exposure to SWCNT studied in mice revealed respiratory toxicity within the range of doses relevant to exposure limits established by regulatory agencies for fine size carbon particles. This toxicity was characterized by transient inflammatory and progressive fibrogenic responses which were found to correlate with the progression of oxidative stress and altered pulmonary functions. Realistic exposures to SWCNT are likely to occur in conjunction with other pathogenic influences, e.g., microbial infections. Therefore, it is of interest that exposure to SWCNT decreased bacterial clearance in mice experimentally infected with *Listeria monocytogenes*.

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Disclaimer: The findings and conclusions in this report are those of the authors and do not necessarily represent the views of the National Institute for Occupational Safety and Health.

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Single-Walled Carbon Nanotubes: Recognition by Macrophages, Inflammatory Response, Oxidative Stress/Signaling

Valerian E. Kagan

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Single-Walled Carbon Nanotubes (SWCNT) are new materials of emerging technological importance. Because SWCNT have unique physico-chemical, electronic and mechanical properties, they may exhibit unusual interactions with cells and tissues, thus necessitating studies of their toxicity and health effects. Functioning of macrophages as "pathogen sensors" is essential for the regulation of adaptive immunity and the inflammatory response. SWCNT – if recognized by macrophages – may compete and affect the immune functions. Further, uptake of SWCNT by macrophages may be essential for the particle tissue distribution and spread into systemic circulation. We established that non-functionalized SWCNT are poorly recognized by macrophages both in vitro and in vivo. However, coating of SWCNT with a recognition signal, phosphatidylserine (PS), activates interactions of SWCNT with macrophages and promotes their engulfment. The effect is specific to PS as phosphatidylcholine-coated SWCNT remain unrecognized by macrophages. Similar to macrophages, microglial cells respond to PS-coating of SWCNT and engulf the modified (but not non-modified) particles. The importance of the SWCNT modification by PS will be discussed in relation to toxicity and targeted drug delivery of specific cargoes to phagocytozing cells.

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Nanotoxicology Research and Risk Assessment

Lang Tran[1] and Ken Donaldson[2]

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With the rapid development of nanotechnology comes the increasing likelihood of human and environmental exposure. For a proper risk assessment, we need to have a good understanding of the physico-chemical properties of nanoparticles (NP) and relate them to their potential toxicological effects. Yet, the behaviour and interactions of NP with the human body are unpredictable and cannot be easily extrapolated from knowledge based on larger scale materials. Oxidative stress is becoming the dominant paradigm for the action of nanoparticles at the cellular level. Nanoparticles can initiate oxidative stress, via cellular pathways, leading to inflammation. Because of the many industrial and consumer uses of nanoparticles, they can enter the body through different routes of exposure: inhalation, ingestion and dermal. Once inside the body, nanoparticles can be translocated to different target organs. Thus, it is important to determine *the nature* of the internal dose-response relationship and *the most influential attributes* of the internal dose for each target organ. To assess the risks pose by nanoparticles, a combination of *in vitro/in vivo* toxicology models, together with mathematical models to describe the chain of events, from exposure to responses, is needed. The *in vivo* models are aimed at determining the dosimetry issues relating to the translocation of nanoparticles in the body. *In vitro* studies, with well characterized nanoparticles, can help in relating the various physico-chemical attributes to the cellular responses. Pharmaco-kinetics and dynamics models can be used to describe the exposure-dose-response relationship then extrapolate to different exposure scenarios as well as extrapolation of the animal-based results to humans. Currently, there is also interest in developing a QSAR approach to screening nanoparticles for their potential hazard. In this presentation, we will demonstrate an approach to nanoparticle risk assessment using methods above and the data available.

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Nanotoxicology – Regulatory Aspects, Looking to the Future

Eva Hellsten

Scientific Advisor

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Through nanotechnology it is possible to produce molecular structures and materials with new properties and behaviors. The step from research to industrial applications has been short, and the marketing of a broad range of nanotechnology-based consumer products is expected to rapidly increase. In May 2004 the European Commission adopted the communication *"Towards a European Strategy for Nanotechnology"* in which a safe, integrated and responsible strategy was proposed. The communication aims to reinforce R&D and innovation whilst addressing environmental, health, safety and societal concerns upfront. In the "*Nanosciences and Nanotechnologies: Action Plan for Europe 2005-09"* from June 2005, the Commission proposes a set of concrete actions covering a broad range of areas, including examination of relevant EU regulations and measures to fill knowledge gaps. Nanotoxicology is a main element of risk and safety assessment of nanomaterials and thus needed in order to provide adequate information for regulations and risk management measures. A close dialogue between researchers and legislators is needed to identify central issues that need to be tackled from both a scientific and a regulatory perspective.

Further reading

The Commission Action Plan and other relevant documents can be found on

http://cordis.europa.eu/nanotechnology/

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Closing remarks

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