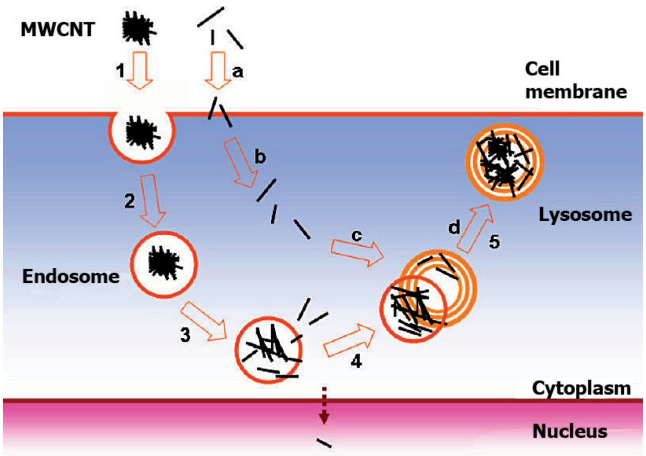
ENPRA Risk Assessment of Engineered NanoParticles



Environmental, Health and Safety Impacts of Nanoparticles

European Observatory on NanoSafety

N°2
June 2010

Environmental, Health and Safety Impacts of Nanoparticles n°2
June 2010
Edition:
Observatoire des Micro et NanoTechnologies
CEA-CNRS - UMS 2920
Minatec® - MMNT, 3 Parvis Louis Néel
38 054 Grenoble cedex 09
Tél. : +33 (0)4.38.78.20.70
Fax : +33 (0)4.38.78.24.21
E-mail : OMNT@cea.fr
http://www.omnt.fr

Publishing Director:
Stéphane Fontanell

Managing Editor:
Marie-Claire Toufektsian

Publishing Assistant:
Interligne

Printing:
PressVercors
France

N°ISSN en cours
Dépôt légal 2ème trimestre 2010

Cover Illustration:
Proposed mechanisms of multiwall carbon nanotube (MWCNT) cell uptake and trafficking. Reprinted with permission from Nano Letters 9, 4370 (2009). Copyright 2009 American Chemical Society.
To see also: "Shandong Univ.: Carbon nanotube uptake and trafficking in cells " on page 6.

Upstream processes and devices
- Molecular Electronics
- Nanoconstruction
- Bio-inspired Nanotechnologies
- NEMS

ICT
- Organic Electronics (*)
- Materials and components for optics
- Spintronics
- Nano-electronics
- Nanocomponents

Biology & Health
- Micro & Nanosystems for biology (*)
- Nanomedicine (*)
- Environmental, Health and Safety Impacts of Nanoparticles (*)

Energy
- Batteries and fuel cells, Photovoltaïc, Thermo-electricity, Power MEMS and energy harvesting

(*) Periodic reports written in English. Other reports are written in French

Environmental, Health and Safety Impacts of Nanoparticles

N°2
June 2010

Introduction

Experts' Analysis

Toxicology

Environmental impacts

Risk Assessment & Risk Management

State-of-the-art report

Environmental Impacts of Nanotechnologies

Index

Introduction

*The present report is **an excerpt** of the second issue from the European Observatory on NanoSafety (EONS), a collective initiative from the European consortium ENPRA (Risk Assessment of Engineered NanoParticles) and the Observatory for Micro & NanoTechnologies (OMNT).*

Launched in November 2009, EONS aims at highlighting the latest trends and research progresses on environmental, health and safety issues related to nanotechnology. State-of-the-art reviews and experts' analyses of selected publications are presented during bi-annual meetings by European key scientists in NanoSafety, including partners of the ENPRA project, OMNT experts and invited personalities.

The second EONS report summarizes the discussions of the expert panel meeting held at the Joint Research Centre in Ispra on April 13th, 2010. The present excerpt compiles the 3 key information highlighted in the table of contents.

Toxicology

In vitro studies

Nat. Inst. for Occupational Safety and Health & West Virginia Univ.: An *in vitro* model for studying cellular mechanisms of SWCNT-induced fibrogenesis

D. Lison

In vitro **evidence of an atypical fibrogenic potential of SWCNT**

Several experimental studies in mice or rats have shown that when carbon nanotubes reach the lung they induce several toxic manifestations, including inflammation (alveolitis), fibrosis and genotoxic damage. The nature, amplitude and localisation of these toxic responses may, however, vary with the type of preparation examined, especially with the degree of dispersion of the nanotubes. Using a preparation of well-dispersed single wall carbon nanotubes (SWCNT), investigators have recorded a strikingly rapid (7 days) and intense fibrogenic response in the lung of mice exposed via pharyngeal aspiration or inhalation, in the apparent absence of inflammatory reaction.

The present study (**National Institute for Occupational Safety and Health** and **West Virginia University**) used a fibroblastic cell line (CRL-1490) to examine whether SWCNT may have direct effects on these cells. Increased proliferation and collagen synthesis was observed in cells exposed *in vitro* to increasing doses (0.06-0.200 µg/cm²) of well-dispersed SWCNT. An increased production of MMP-9, a pro-fibrotic mediator, was also recorded both in the same cell line exposed *in vitro* and *in vivo* in the lung of animals treated with SWCNT (10 µg/mouse).

This is the first experimental indication of a direct effect of SWCNT on mesenchymal cells, which may contribute to explain the strong fibrotic effect of this material in the lung. These effects were recorded in the apparent absence of inflammation, consistent with a direct effect of SWCNT on fibroblasts, uncoupled from an inflammatory reaction. This observation should be confirmed in primary fibroblasts, possibly after optimisation of the experimental conditions (e.g. robust proliferation assays, quantitative measurement of extracellular collagen deposition).

These results suggest a strong and atypical fibrogenic activity of SWCNT. This endpoint might need to be systematically included in the toxicological assessment of nanoparticles.

"Direct fibrogenic effects of dispersed single-walled carbon nanotubes on human lung fibroblasts" ; Wang et al. : ***Journal of Toxicology and Environmental Health Part A*** 73,410 (2010).

EONS06-10-1

Environmental impacts

Univ. of South Carolina: Behavior of gold nanoparticles within estuarine ecosystems

M. Auffan, J. Rose, J.Y. Bottero

The fate of gold nanoparticles in a complex ecosystem

Research teams from the **University of South Carolina** (Department of Chemistry and Biochemistry, National Center for Coastal Ocean Science, and Department of Environmental Health Sciences) studied the fate of gold nanoparticles in a complex ecosystem containing sediments, biofilms, primary producers, filter feeders, grazers and omnivores. They built three replicate estuarine mesocosms already used for estimating the coastal impact of several other contaminants (*e.g.* atrazine, fipronil, endosulfan and nutrients). The mesocosms (Figure 5a) were made up with natural sea water (salinity adjusted to 20‰), a periodically submerged intertidal sediment (from a reference site in South Carolina), and *Spartina alterniflora* (grass). Three month after establishment (sea water, sediment and *Spartina*), the mesocosms were exposed to the fauna, and 5-10 days after the fauna was exposed to nanoparticles.

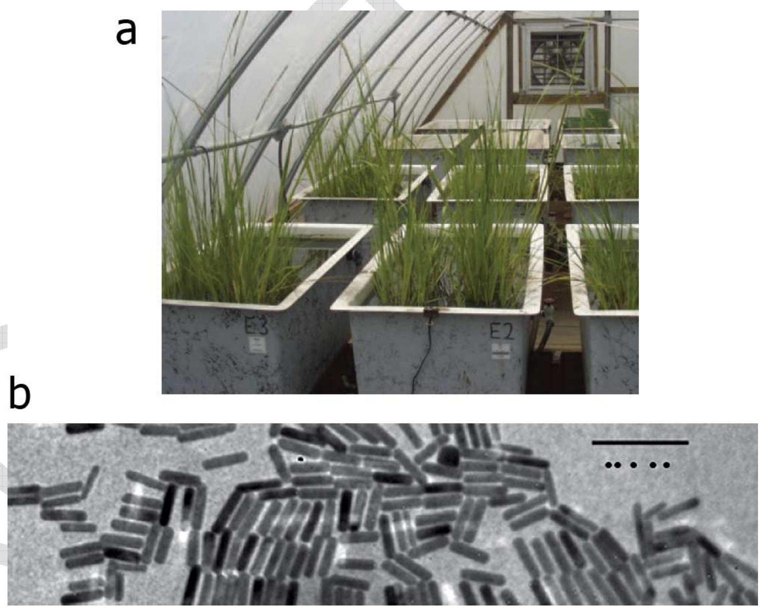


Figure 5: (a) Estuarine mesocosms. (b) Transmission electron microscopy image of the gold nanoparticles.
Reprinted with permission from Nature Nanotechnology 4, 441 (2009). Copyright 2009 Macmillian Publishers Ltd.

Gold nanorods (Figure 5b) (65 nm x 15 nm) used were coated with CTAB (cetyltrimethylammonium bromide) conferring a net positive charge. Previous work has shown that elemental gold is stable against dissolution in aerated sea water. Nanorod solutions were injected (in less than 1 minute) by means of gravity feed through a Teflon capillary into the water feed line of the mesocosms during the rising tide cycle to ensure the best possible mixing. The final theoretical particle loading in the 366 L tank was 7.08×10^8 particles.mL^{-1}, which is of the same order of magnitude particle loading for combined bacterial, planktonic and viral particles expected in estuarine waters.

While water and sediments represent 99% of the total mass of the system and are a sink for nanoparticles (35% of the total mass of recovered particles), on a per mass basis the filter feeders and biofilms appear to be the most effective sink. The *Spartina alterniflora* had the lowest concentration factor: only 0.2% of the total recovered amount. The estimated mass of photosynthetic biofilms was less than 0.5% of the mass of the complete system, but roughly 60% of recovered nanoparticles are entrained in biofilms. This high affinity is related to the negative surface charge of the marine biofilm with the positively charged CTAB-stabilized gold nanorods. These results are interesting because biofilms offer (i) a route into the food web through grazing by detritovores and (ii) a route for mineralization through biofilm calcification. The filter feeder (*Mercenaria mercenaria*) took up 5%

of the total nanoparticle added even though they account for less than 0.01% of the total mass of the system.

These results indicate that knowledge of the ratio of filter feeder mass to biofilm mass may be critical to predicting the direct uptake of nanomaterials into the estuarine food web. Moreover, adult *Mercenaria* are commercially important shellfish for human consumption and can be an important route into the human food chain.

"Transfer of gold nanoparticles from the water column to the estuarine food web" ; J.L. Ferry, P. Craig, C. Hexel, P. Sisco, R. Frey, P.L. Pennington, M.H. Fulton, I.G. Scott, A.W. Decho, S. Kashiwada, C.J. Murphy, T.J. Shaw : ***Nature Nanotechnology*** **4**, 441 (2009).

EONS06-10-6

Risk Assessment & Risk Management

NIOSH:
Nanomaterials in the workplace. The Nanoparticle Emission Assessment Technique (NEAT)

D. Bloch

A simplified strategy for assessing nanoparticle emission sources in the workplace

Assessment of workers' exposure to nanomaterials remains a crucial issue for risk management strategy and the safe development of nanotechnology. They are currently no Occupational Exposure Limits (OEL) specific for nanomaterials, nor any scientific consensus on what would be the most relevant metrics for quantifying an exposure dose for workers. Several techniques are currently available to characterize airborne nanomaterials with respect to parameters as mass concentration, size distribution, number concentrations or surface area. However, besides that they cannot discriminate engineered nanoparticles for ambient nanoparticules, these techniques are expensive, not easily transportable and difficult to use on a routine basis.

The **National Institute for Occupational Safety and Health (NIOSH**) proposes a simple, pragmatic and easy to use strategy for identifying potential nanoparticle emission sources in the workplaces (NEAT) [1].

The NEAT is based on the combination of direct measurements of airborne nanomaterials with handheld instruments, supplemented by filter-based samples analysis for determination of shape and/or chemical nature of nanoparticles.

The general principle of the technique (Figure 7) is to firstly quantify the average level of background nanoparticle number concentration, by measurements with nanoparticle counters in the vicinity of the processes. A thorough examination of processes, tasks being performed and their likelihood to release nanoparticles in the workplaces allow the identification of the potential nanoparticle emissions sources. Nanoparticles counters are located near the suspected sources in order to increase the probability of capturing nanoparticles emissions.

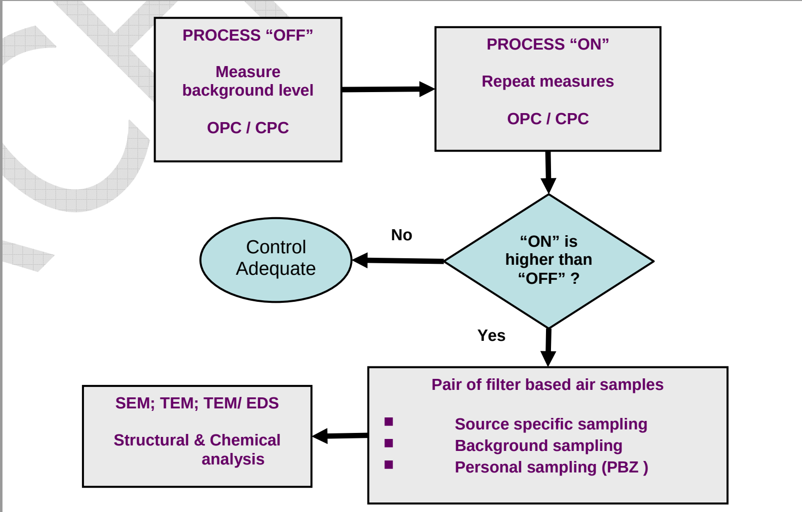


Figure 7: Scheme summarising the steps of the nanoparticle emission technique (NEAT).
OPC : optical particle counter;
CPC: condensation particle counter;
SEM: scanning electron microscopy;
TEM: transmission electron microscopy;
EDS: energy dispersing X-ray spectroscopy.

For each measurement, two different handheld nanoparticle counters are used simultaneously: a condensation particle counter (CPC; particle size range: 10 to 1000 nm) and an optical particle counter (OPC; six specific cutpoints 0.3, 0.5, 1.0, 3.0, 5.0 and 10.0 µm). Differences between results with CPC and OPC may give indications of the nanoparticle size. For example, a high particle number concentration on the CPC along with a high number concentration on the OPC in the small size range (300-500 nm) would indicate a high probability of the presence of nanoparticles. Conversely, a low number concentration on the CPC associated with a high number concentration on the upper sized ranges of the OPC would be in favour of the presence of larger particles (> 1 µm).

Measures are performed when the process is OFF (in order to determine the background level) and when the process is ON. If measures when process is ON exceed the background level, then filter based air sampling are preformed for desired analytical information. Chemical composition of particle mass concentration, elemental analysis and particle morphology can then be determined using scanning electron microscopy (SEM) or transmission electron microscopy (TEM) and energy dispersing X-ray spectroscopy (EDS).

The part B publication [2] presents field studies performed in 12 facilities including research labs, pilot scale facilities and industrial production plants. The results are only representative of the conditions in which measures have been performed and cannot be easily and safely extrapolated to others workplaces. But it demonstrates that handheld CPC/OPC direct readings are adequate for identifying emissions sources, leakages from the process and effectiveness of control measures, although their sensitivity depends highly on the background level. In addition, the TEM analysis show that nanoparticles are mainly in the form of agglomerates, clusters, nests, bundles rather than individual fibers or spherical particles.

In conclusion, NEAT is not intended to provide an individual exposure assessment to engineered nanoparticles. It is aimed at assessing the effectiveness of control measures, monitoring workplaces in the long time, e.g at each process modification or control measures implementation. Therefore, the results should NOT be interpreted as a quantitative measurement of individual workers exposure.

[1] "Nanoparticle Emission Assessment Technique (NEAT) for the Identification and Measurement of Potential Inhalation Exposure to Engineered Nanomaterials—Part A" ; M. Methner, L. Hodson, C. Geraci : ***Journal of Occupational and Environmental Hygiene*** **7**(3), 127 (2010).
[2] Nanoparticle Emission Assessment Technique (NEAT) for the Identification and Measurement of Potential Inhalation Exposure to Engineered Nanomaterials—Part B: Results from 12 Field Studies" ; M. Methner, L. Hodson, A. Dames, C. Geraci : ***Journal of Occupational and Environmental Hygiene*** **7**(3), 163 (2010).

EONS06-10-7

Companies, organisations & experts quoted in this report

List of experts involved in Environmental, Health and Safety Impacts of Nanoparticles

Rob AITKEN	IOM Edinburgh, United Kingdom	ENPRA
Karin ASCHBERGER	JRC Ispra, Italy	ENPRA
Mélanie AUFFAN	CNRS, Univ. Paul Cézanne, France	OMNT
Armelle BAEZA	Univ. Paris 7, France	OMNT - ENPRA
Daniel BLOCH	CEA Grenoble, France	OMNT
Jean - Yves BOTTERO	CNRS – Univ. Paul Cézanne, France	OMNT
Flemming CASSEE	RIVM, The Netherlands	ENPRA
Emmanuel FLAHAUT	CNRS – Univ. Paul Sabatier, France	OMNT
Stefania GOTTARDO	Univ. Venice, Italy	ENPRA
Peter HOET	Univ. Leuven, Belgium	ENPRA
Marie - Claude JAURAND	INSERM, France	OMNT
Wim de JONG	RIVM, The Netherlands	ENPRA
Jennifer McLEISH	Univ. Edinburgh, United Kingdom	ENPRA
Stéphanie LACOUR	CNRS, France	OMNT
Sophie LANONE	INSERM, France	OMNT
Dominique LISON	Univ. Louvain, Belgium	ENPRA
Steffen LOFT	Univ. Copenhagen	ENPRA
Antonio MARCOMINI	Univ. Venice, Italy	ENPRA
Juan RIEGO-SINTES	JRC Ispra, Italy	ENPRA
Jérome ROSE	CNRS, Univ. Paul Cézanne, France	OMNT
Bryony ROSS	IOM Edinburgh, United Kingdom	ENPRA
Roel SCHINS	IUF Düsseldorf, Germany	ENPRA
Lang TRAN	IOM Edinburgh, United Kingdom	ENPRA
Jacques VENDEL	IRSN, France	OMNT