ENPRA Risk Assessment of Engineered NanoParticles

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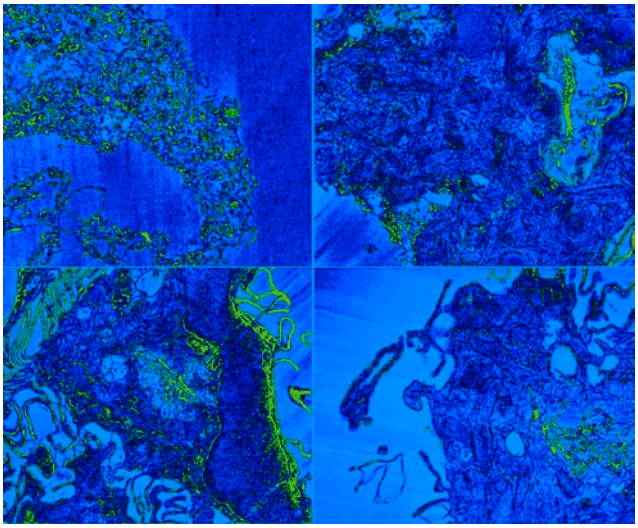

Environmental, Health and Safety Impacts of Nanoparticles

European Observatory on NanoSafety

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Transmission Electron Microscopy images of (top left) a necrotic cell, (top right) phagocytosis of single wall carbon nanotubes (SWCNTs) coloured in green, and SWCNTs observed within (bottom left) lysosomes and (bottom right) cytoplasm. Reprinting with permission from ACS Nano 3(6), 1485 (2009). Copyright 2009 American Chemical Society.
To see also: "Imperial College of London: Characterization of carbon nanotubes in cells using HAADF-STEM and EELS mapping" on page 5.

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Introduction

*The present document is **an excerpt** of the first bi-annual report from the European Observatory on NanoSafety (EONS), a collective initiative supported by the European project "Risk Assessment of Engineered NanoParticles" (ENPRA).*
Gathering over 20 key experts in nanotechnology environment and safety research from the ENPRA consortium and from the Observatory for Micro & Nanotechnologies (OMNT), EONS features comments and reviews from the most recent trends and research progresses in the field of Nanosafety. From toxicology to legal aspects, a wide range of areas potentially impacted by nanoparticle safety issues are addressed by EONS.

The first EONS report summarizes the expert analyses and discussions developed during the 1st ENPRA Expert Panel Meeting, which has been held in Paris on November 26th, 2009. The present excerpt compiles 3 key information highlighted during the panel discussion.

Epidemiology & Occupational Health

Capital Univ. of Medical Sciences: Severe pulmonary complications possibly due to occupational exposure to nanoparticles

W. De Jong

A first clinical report on serious lung damages possibly related to nanoparticle exposure

While commercial applications using nanotechnologies are in constant progress, potential risks to workers, consumers and environment arising from nanomaterial exposure remain mostly unknown. So far, there is no clinical report in humans following long-term exposure to nanoparticles.

Published in the European Respiratory Journal, this study from the **Capital University of Medical Sciences of Beijing** reports the case of several Chinese women who developed severe respiratory complications possibly in relation to occupational exposure to nanoparticles. The women were working in an area with limited to no ventilation and they developed pulmonary symptoms after 5 to 13 months exposure. Seven workers were hospitalised with dyspnoea and two of them died of respiratory failure. Clinical evaluation showed shortness of breath and pleural effusions. In the pleural effusions and lung tissue 30 nm nanoparticles were observed by transmission electron microscopy (TEM). Immunological tests, bacteriology, virology and tumour markers were evaluated, and found to be mainly negative.

An evaluation of the actual working conditions was performed. Work comprised the use of a paste in a machine to air spray materials, heat and dry boards. The ventilation unit of the machine (a gas exhauster) broke down 5 months prior to the occurrence of the reported symptoms. Dust particles obtained from the exhauster were analysed and TEM evaluation of both paste and dust identified nanoparticles of 30 nm.

The present study is the first clinical report on severe respiratory toxicity possibly due to occupational exposure to locally generated nanoparticles. What was remarkable was the unconventional severity of the respiratory symptoms eventually leading to death of two persons. Such a severe lung injury has never been seen with an exposure to conventional nanoparticles only. The exposure occurred under a very low quality of occupational hygiene at the working place without any specific safety measures.

This report should be considered as a case report for possible induction of pulmonary disease by nanoparticles. It is possible that the symptoms in the patients were caused by the exposure to polyacrylate nanoparticles in view of the fact that nanoparticles were identified in the pathological lesions. Whether the particles themselves were responsible or a contributing factor is not known, as the authors did not publish a definitive characterization and comparison of both the nanoparticles identified in tissues and in the workplace. Additional (animal) studies would be needed for demonstration of such a cause–effect relationship.

Although the authors claimed that the cause of the respiratory symptoms and toxicity was due to the exposure of nanoparticles, this was not definitively demonstrated. However, it cannot be excluded either. But even when the exposure to the particulates in the air would be the cause for the respiratory symptoms, it has to be realised that the occupational conditions were very uncharacteristic and of very low hygienic quality. The observations itself warrant specific hygienic measures and conditions when working with paints and spray coating. However, the presence of such low quality occupational settings without any health or safety measures is exceptional and not common practice.

"Exposure to nanoparticles is related to pleural effusion, pulmonary fibrosis and granuloma" ; Y. Song, X. Li, X. Du : ***European Respiratory Journal*** **34**, 559 (2009).

EONS02-10-2

Toxicology

Bristol Southmead Hospital: CoCr particles can induce distant DNA damage *via* an ATP-dependant mechanism

S. Loft

Nanoparticles may cause DNA damage from a distance

Internal exposure to cobalt-chromium (CoCr) nanoparticles is likely in humans following wear mechanisms of artificial orthopaedic joints. CoCr particles and ions are known to display cytotoxic and genotoxic effects. Remote effects of genotoxins have been previously described in the context of ionizing radiations.

In the present study [1] performed by the **Bristol Southmead Hospital** in collaboration with the **University Hospitals Bristol NHS Foundation Trust**, the **Cardiff University**, the **University of Bristol** and the **Imperial College of London**, the authors show that CoCr nanoparticles can cause genotoxicity across a cellular barrier consisting in a tight layer of BeWo chorioncarcinoma cells, an *in vitro* model frequently used for studying the blood placenta barrier (Figure 1). Human fibroblasts were used as target cells and genotoxicity was assessed by measurements of DNA single and double strand breaks as well as chromosomal aberrations. Toxic effects were observed in fibroblast cells while nanoparticles were not found to cross the BeWo barrier.

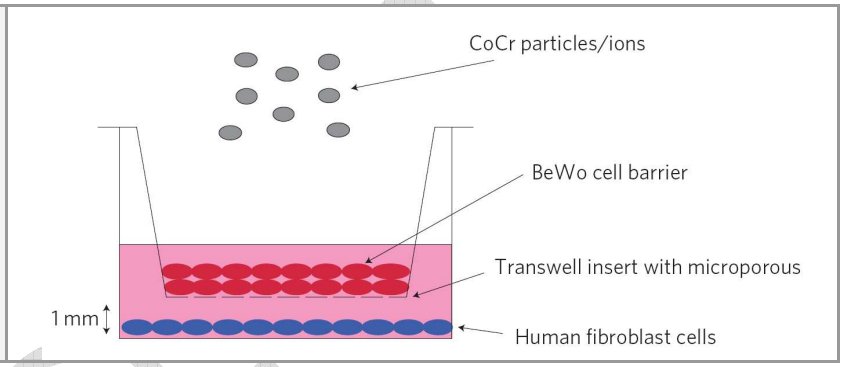

Figure 1: Experimental exposure setup (BeWo cell barrier).
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As for the intracellular mechanisms of toxicity, the authors suggest that nanoparticles and/or Co^{2+} ions may affect mitochondrial metabolic activity and mimic hypoxic conditions in the top layer of the BeWo barrier (Figure 8). This would subsequently lead to intracellular release of ATP which may pass through connexin and pannexin hemichannels to act on P2Y receptors in the second layer. Alternatively, ATP may cross connexin gap junctions (CxGJ) to reach the underneath cell layer. Both pathways would result in intracellular Ca^{2+} rise in the second layer and subsequent ATP secretion, again via connexin and pannexin hemichannels. ATP may then cause DNA alteration to human fibroblasts beneath the barrier (via P2 receptors).

The study develops a new concept of distant effects induced by nanoparticles. The proposed cellular mechanism of genotoxicity involving ATP signalling through P2Y receptors or CxGJ (Figure 2) is close to the one described for the radiation-induced bystander effect [2]. This kind of remote (clastogenic) effect has been previously suggested following exposure to asbestos; it may however involve distinct cellular mechanisms [3]. DNA alterations were not specifically related to the "nano" size of the particles; micron sized particles had similar effects as described in the initial part of the study. However, the intracellular effects of micron sized particles were not fully investigated. On another hand, Co^{2+} can bind to proteins and may therefore have fewer effects in its free (ionic) form as compared with its (nano)particular form. The genotoxicity in the BeWo cell barrier and the time course of responses were not addressed. Moreover, it has been shown that particulate matter can impair gap junction communications [4].

In this experimental model, high doses of nanoparticles were used and such toxic effects may not occur in case of more realistic exposures (in terms of nanoparticle concentrations). At this step of the researches, the outcome of the present findings should not be rashly generalized to other situations (placenta or blood brain barriers) which are not relevant to potential nanoparticle exposure following joint replacement. Moreover, precautions should be taken with the concept of remote DNA damages since cellular mechanisms described in this model may have limited impact in vivo in terms of distance scale.

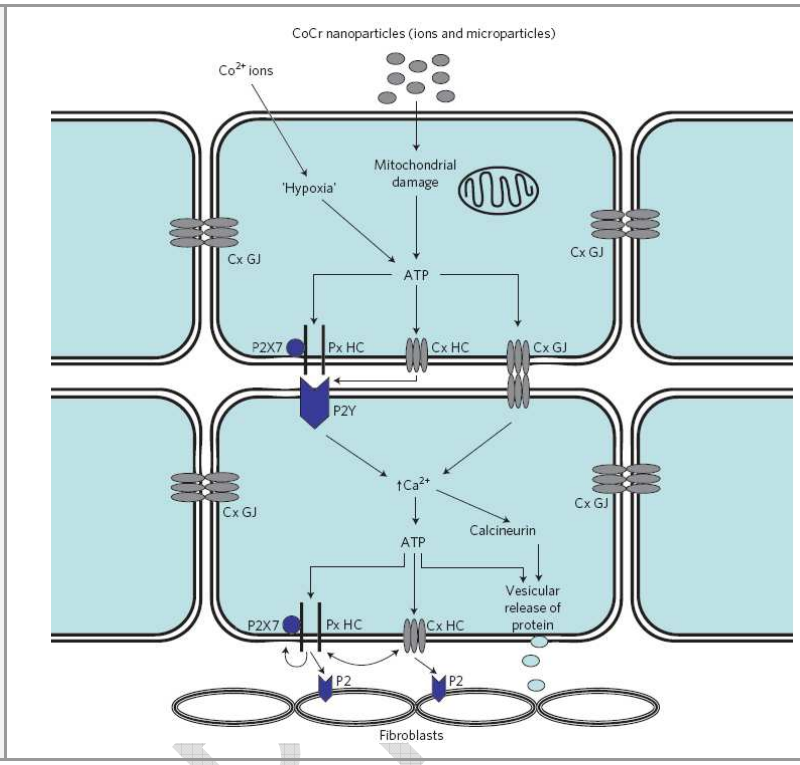

Figure 2: Schematic of the proposed intracellular signalling pathway by wich CoCr nanoparticles induce distant DNA damage.
Reprinted with permission from Nature Nanotechnology 4(12), 876 (2009). Copyright 2009 Macmillan Publishers Ltd.

[1] "Nanoparticles can cause DNA damage across a cellular barrier" ; G. Bhabra, A. Sood, B. Fisher, L. Cartwright, M. Saunders, W. H. Evans, A. Surprenant, G. Lopez-Castejon, S. Mann, S. A. Davis, L A. Hails, E. Ingham, P. Verkade, J. Lane, K. Heesom, R. Newson, C.P. Case : ***Nature Nanotechnology*** **4**(12), 876 (2009).
[2] "Multiple bystander effect of irradiated megacolonies of melanoma cells on non-irradiated neighbours" ; W M. Przybyszewski, M. Widel, A. Szurko, B. Lubecka, L. Matulewicz, Z. Maniakowski, R. Polaniak, E. Birkner, J. Rzeszowska-Wolny : ***Cancer Lett***. **214**(1), 91 (2004).
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EONS02-10-6

Catholic Univ. of Leuven: How can experimental conditions influence effects of nanomaterials *in vitro*?

P. Hoet

Recently, the number of scientific publications in the field of nanotoxicology has increased exponentially. However, reproducibility of published results is often difficult.

Nanotoxicological tests are highly influenced by experimental conditions

In this article from the **Catholic University of Leuven**, the influence of several steps of the experimental design on the outcome was investigated. The role of cell density and particle dispersion method were studied. The authors show that the cell density used in the cytotoxicity study has an impact on the results. Using A549-cells the viability varied from 10% to 55% with increasing cell density for the same concentration of Min-U-Sil (silica benchmark particles). As shown in Figure 3, addition of foetal calf serum (FCS 10%) in the culture medium attenuated the cytotoxicity of Min-U-Sil; however this effect was not observed for carbon nanotubes (CNT). When Tween 80 was used to disperse the particular test agent (CNT), there was an apparent interaction between the material and the dispersant.

As standard cytotoxicity assays for chemicals can generate conflicting results when applied to nanomaterials, it was already advised not to rely upon a single

cytotoxicity assay when assessing carbon nanomaterial toxicity. It has been previously reported that nanomaterials can interference with the assay constituents.

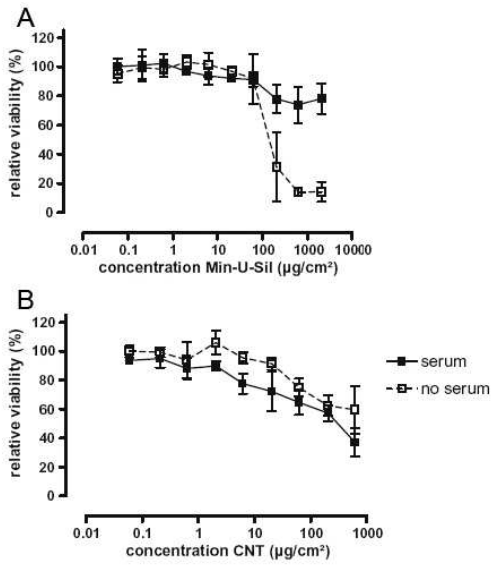

Figure 3: Influence of serum on the cytotoxicity of particles. Min-U-Sil (A) or CNT (B) were suspended and diluted in medium containing or not 10% FCS and antibiotics. Viability was assessed after 24 h using the MTT assay.
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Moreover, to form a reasonably stable suspension, different approaches have been already proposed in the general literature. Besides a detailed characterization of the materials, the authors of the present paper call for a better standardization of basic experimental conditions. They list and discuss items that should be included in the description of nanotoxicology experiments including:

- a detailed description of the experimental design;
- metrics used for particle exposure should include volume and growing area (surface area) of the wells (to allow comparison among studies);
- to determine whether the cell type used consists in actively dividing (e.g. A549 cells) or fully differentiated cells (e.g. stimulated THP-1 cells);
- the use of several methods for assessing cytotoxicity as well as appropriate controls (including for additives).

This research paper explored an interesting facet of nanotoxicity testing, and the authors tried to point out the gaps in performing hazard evaluation. Unfortunately, they did not show all these aspects from their own experiments; in fact they only presented a limited set of experiments and linked those to a broad set of guidelines. In order to prove all statements made in the discussion, a full project should be started on assay conditions in nanotoxicology. Therefore, future experiments will have to validate the strength of this methodological/opinion paper.

"Assay conditions can influence the outcome of cytotoxicity tests of nanomaterials: Better assay characterization is needed to compare studies" ; J. Geys, B. Nemery, P.H. Hoet : ***Toxicology In Vitro*** in press (2009). DOI: 10.1016/j.tiv.2009.10.007.

EONS02-10-7

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