Different genotoxic effects of multi-walled carbon nanotubes in A549 cells: implications for nanomaterials safety investigation

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NANOGENOTOX

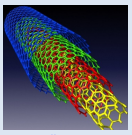
Background

« Nanomaterial means a natural, incidental or manufactured material containing particles….where…one or more external dimensions is in the size range 1 nm-100 nm. »

Source: European Commission Recommendation, 18 October 2011

Multi-walled carbon nanotubes (MWCNT)

Developed for industrial purposes: mechanically strong, flexible, good electrical conductivity and thermal conductivity.



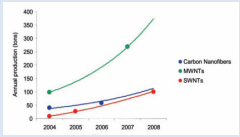
Source: http://www.turbosquid.com/

Potential applications include composites, coatings, conductive plastics, sensors, batteries and fuel cells.

The Royal Society & The Royal Academy of Engineering, 2004

Background

Estimated future global production of carbon nanotubes:

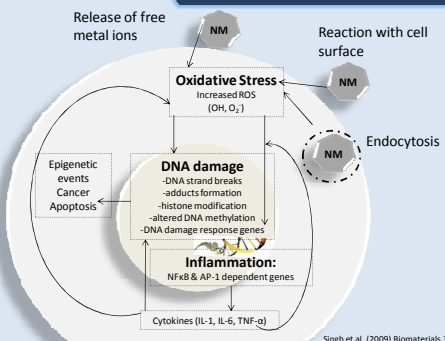


Source: The Royal Society & The Royal Academy of Engineering, 2004

Similarities with asbestos: *fiber-like* paradigm SAFETY of MWCNTs?

- Takagi et al. 2008- mesothelioma induction in p53+/- mice i.p. 3 mg MWCNTs
- Muller et al. 2009- no carcinogenicity in rats exposed by i.p. to MWCNT

Background



Singh et al. (2009) Biomaterials 30: 3891–3914

Objectives

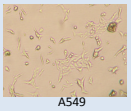
NANOGENOTOX

Safety evaluation of manufactured nanomaterials by characterization of their potential genotoxic hazard (*NANOGENOTOX Joint Action (EU)*

To characterize the potential cyto- and genotoxic effects of two multi-walled carbon nanotubes (MWCNTs) in a human type-II alveolar epithelial cell line (A549).

	NM402	NM403
Crystal Phase	MWCNT	MWCNT
Particle Size	100-10 000 nm long	100-10 000 nm long
Length (nm)	1 500	-
Diameter (nm)	11	-
Surface area (m^2/g)	250	-
Impurities	<10 wt %	-
Use	Structural composite and energy applications	Structural composite and energy applications

Source: JRC repository, 27 October 2011



A549
human lung adenocarcinoma cell line

Methods

Exposure of A549 cells to NM402 or NM403

8-days exposure

48h - exposure to NM

Clonogenic Assay

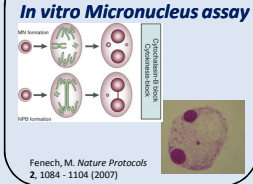
Colony : > 50 cells.

Surviving fraction:

$$\frac{\text{No. of colonies formed after treatment}}{\text{No. of cells seeded x (plating efficiency/100)}}$$

Herzog et al. (2007) Toxicology Letters,174, 49-60

In vitro Micronucleus assay



Fenech, M. *Nature Protocols* **2**, 1084 - 1104 (2007)

Results- Clonogenic Assay

A concentration-effect relation was observed in A549 cells' survival exposed to both NMs (exponencial model R^2= 0,909 & 0,931).

Results- Micronucleus Assay

Concentration-effect relation for NM402 (quadratic model R^2= 0.861).

No concentration-effect relation for NM403.

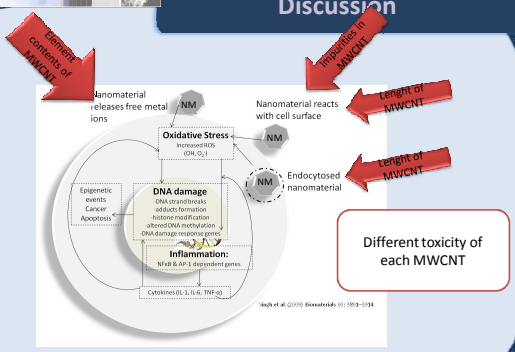
Discussion

In the present study, while both NMs were cytotoxic for A549 cells, their ability to cause DNA damage was distinct.

NM402 caused a dose-dependent genotoxic effect.

NM403 was not genotoxic.

Discussion



Different toxicity of each MWCNT

Implications for nanomaterials safety investigation

NM402 and NM403 are Closely related NMs...

BUT

...present physicochemical differences that result in different genotoxic activities.

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