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NANOGENOTOX

Safety evaluation of manufactured nanomaterials: comparison of genotoxic effects of multi-walled carbon nanotubes in two human cell lines

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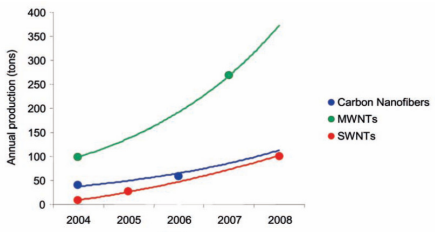
Background

Nanotechnologies are developing very rapidly and presently nanomaterials are increasingly used in a wide range of applications in science, industry and biomedicine.

CARBON NANOTUBES (CNT)

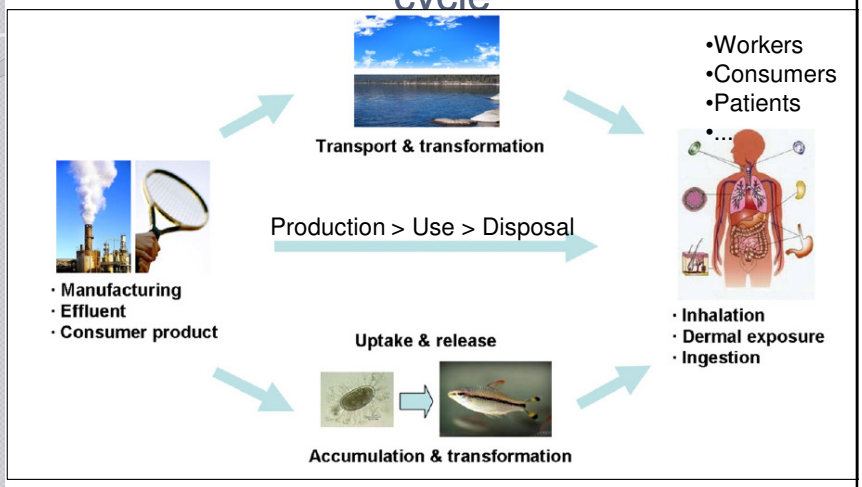


Source: The Royal Society & The Royal Academy of Engineering, 2004

Multi-walled carbon nanotubes (MWCNT) have been widely applied in structural composites, energy appliances and electronics.

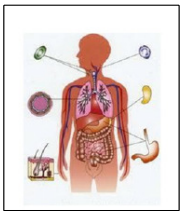
Estimated future global production of carbon nanotubes:

Carbon nanotubes release to human beings and environment during its life cycle



Zhao & Liu, 2012

Although human exposure is also growing very fast…



- Solid information about hazard is lacking for the vast majority of nanomaterials, including CNTs, especially related to chronic exposure to low doses, that are likely to occur through consumers products.
- The genotoxic effects of CNTs, which may be linked to carcinogenic effects, are of special concern because cancer has a long latency period and thereby these effects can be less obvious and more difficult to predict than the acute effects.

Toxicology of CNTs

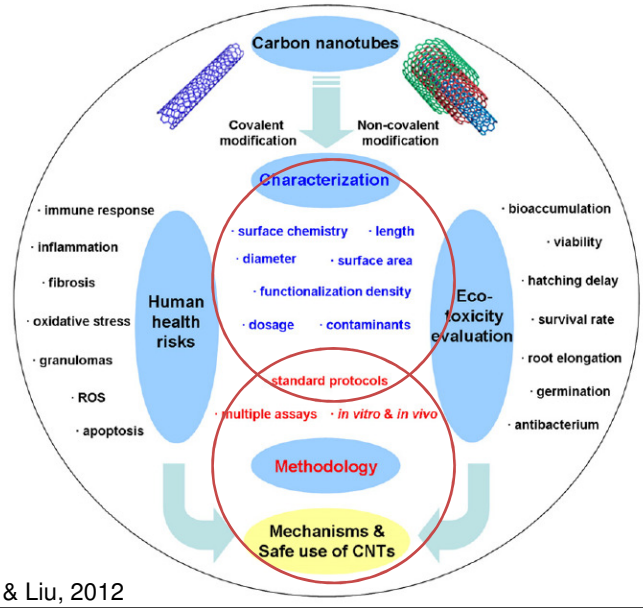
- The particular physicochemical properties that have rendered CNTs attractive for many applications -surface area, shape, stability, rigidness, coating and electrical charge - might also underlie relevant biological effects

Similarities with asbestos: *fiber-like* paradigm

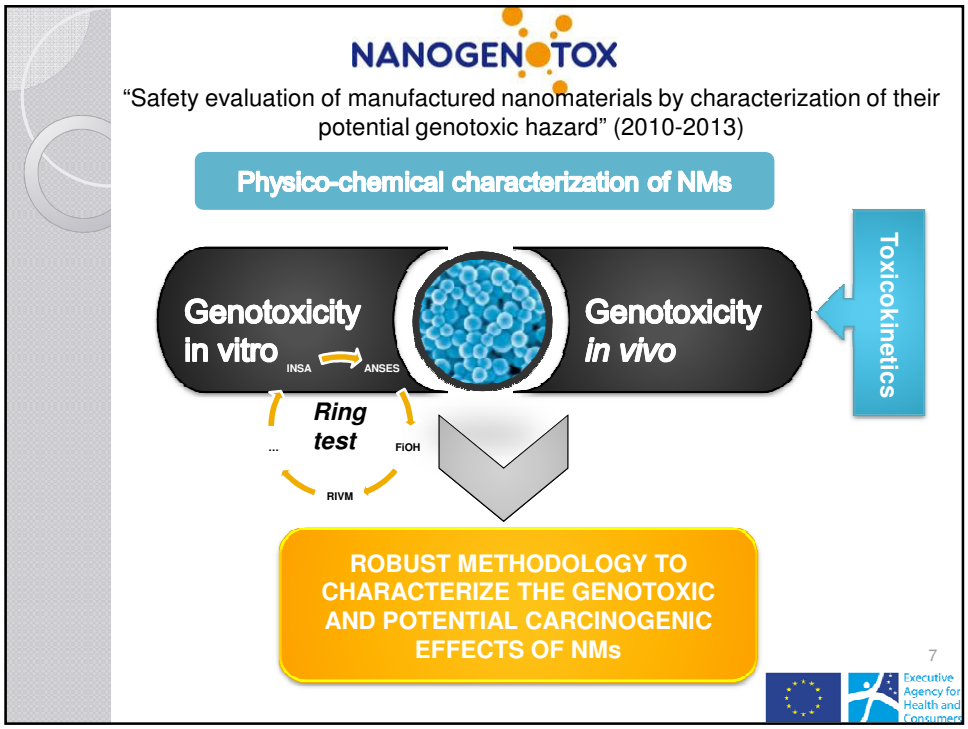
•Takagi et al. 2008- mesothelioma induction in p53+/- mice i.p. 3 mg multiwalled CNT (MWCNT)

•Muller et al. 2009- no carcinogenicity in rats exposed by i.p. to MWCNT

Toxicology of CNTs



Zhao & Liu, 2012



Objective

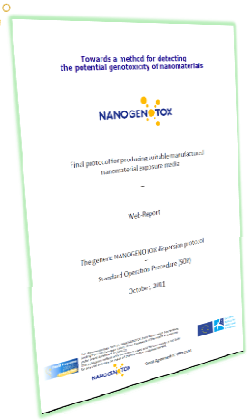
- The objective of the present work was to compare the potential genotoxic effects of two thin MWCNT with different length (NM-402 and NM-403; JRC repository) in a human type-II alveolar epithelial cell line (A549 cells) and in primary human lymphocytes.

Physico-chemical properties of carbon nanotubes

Carbon nanotubes	Specific surface area (m^2/g)[a]	Thickness (nm)	Length (nm)	Aspect ratio	Morphology	Purity
NM-402 Multi-walled	250	~11	~1100	~107	Flexible; Highly bended	Inorganic impurities present
NM-403 Multi-walled	-	~11	~ 400	~36	Flexible; Highly bended	Lower level of impurities

[a] Information provided by the Joint Research Center (http://ihcp.jrc.ec.europa.eu/our_activities/nanotechnology/nanomaterials-repository/list_materials_JRC_rep_oct_2011.pdf) and by de Temmerman et al., personnal communication.

Methods

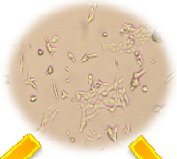


http://www.nanogenotox.eu/

Concurrent control cultures were also analysed: vehicle control, positive chemical control (mitomycin C, MMC) and a nanosized tentative control (ZnO, NM-110)

Methods

Exposure of cells to NM402 or NM403



8-days exposure

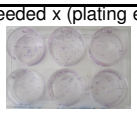
48h - exposure to NM

Cytotoxicity - clonogenic Assay

Colony : > 50 cells.

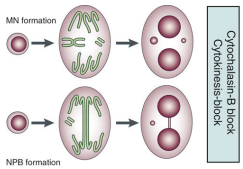
Surviving fraction:

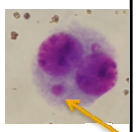
No. of colonies formed after treatment
No. of cells seeded x (plating efficiency/100)

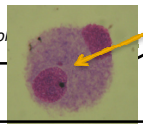


Herzog et al. (2007) Toxicology Letters,174, 49-60.

Genotoxicity – Micronucleus assay







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

In summary…

RESULTS

MWCNT	Cells	Genotoxicity (MN assay)	Cytotoxicity
NM-402	Lung epithelial cell line	Positive, with a dose-response relationship	Positive (clonogenic) No cell cycle disturbance (CBPI)
	Primary lymphocytes	Equivocal	No cell cycle disturbance (CBPI)
NM-403	Lung epithelial cell line	Negative	Positive (clonogenic) No cell cycle disturbance (CBPI)
	Primary lymphocytes	Positive, with a dose-response relationship at low dose-range	No cell cycle disturbance (CBPI)

Both chemical and nanosized (ZnO) positive controls yielded positive results in both cell lines.

Discussion

A differential response was observed for closely related MWCNT in two human cell lines, a primary and a permanent cell line, which might be explained by:

- Different physicochemical properties of MWCNT:
  - Aspect ratio
  - Surface reactivity
  - Transition metals present as impurities

- Differences in the uptake capacity or sensitivity of the two cell lines

Implications for nanomaterials safety investigation

NM402 and NM403 are closely related NMs…

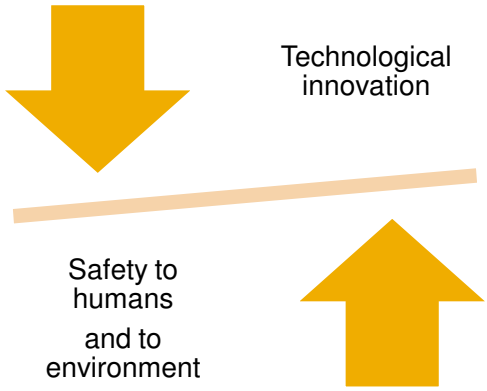
BUT

…present physicochemical differences that result in different genotoxic activities.

- **This study illustrates the difficulty of implementing hazard-grouping strategies based on similarity of chemical composition or some physicochemical properties of the NMs and their hypothesized mode of action.**
- **It is important to:**
  - Investigate the toxic potential of each NM individually
  - uncover the main characteristics that determine NMs genotoxicity, allowing a “safe-by-design” approach

"Realising the benefits of nanotechnologies requires a willingness to accept some risk because without risk there can be no progress"

International risk governance council, 2007



15

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