SAFETY INVESTIGATION OF NANOMATERIALS: ANALYSIS OF GENOTOXIC EFFECTS IN A BRONCHIAL EPITHELIAL CELL LINE

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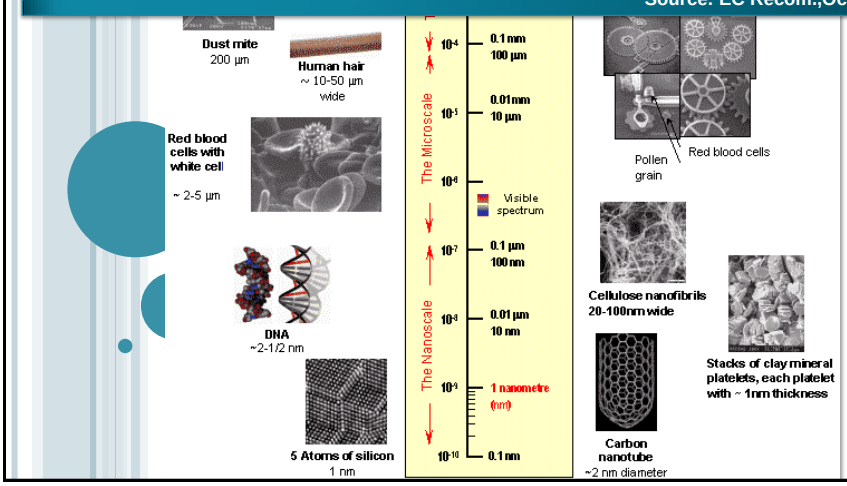
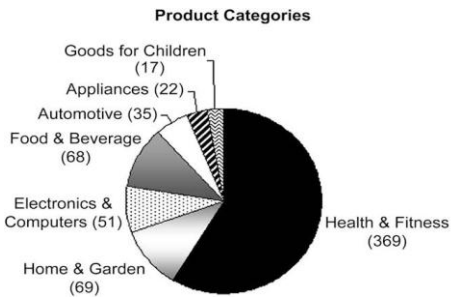
BACKGROUND

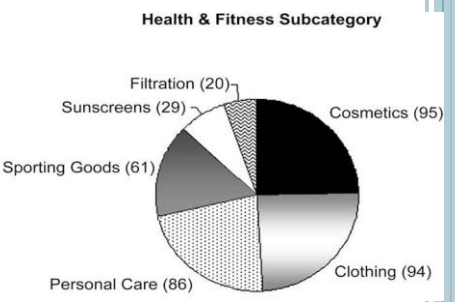
« NANOMATERIAL - a natural, incidental or manufactured material containing particles….where…one or more external dimensions is in the size range 1 nm-100 nm. »

Source: EC Recom.,October 2011



Products containing nanomaterials (NMs) are increasingly being used in a wide range of applications in science, industry and biomedicine.

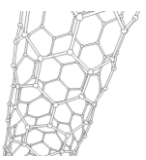


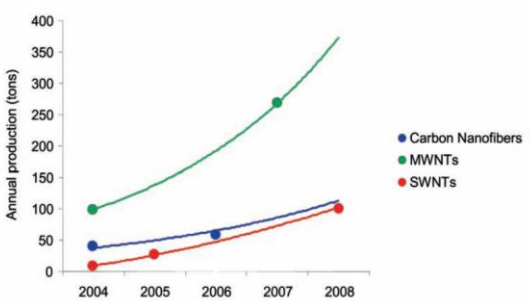


(Singh *et al.*, 2009)

• Small size, higher surface area per mass and other new/modified physico-chemical properties render NMs more reactive than larger-sized particles of similar chemistry.

CARBON NANOTUBES (CNT)





Multi-walled carbon nanotubes (MWCNT):

- structural composites
- energy appliances
- electronics

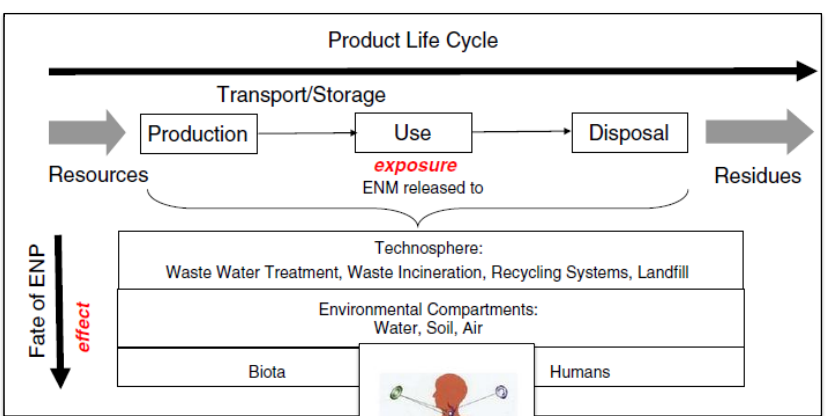
Source: The Royal Society & The Royal Academy of Engineering, 2004

Estimated future global production of carbon nanotubes:

- **Titanium dioxide (TiO_2)**
- **Synthetic amorphous silica (SAS)**
- **Zinc Oxide (ZnO)**

- Food
- Cosmetics
  - Skin care products
  - Sunscreen products
- Pharmaceuticals
- Inks
- ….

The wide apllicability of NMs > increased risk of human exposure and environmental dissemination during their life cycle

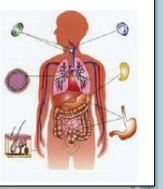


- Workers
- Consumers
- …

- Inhalation
- Dermal exposure
- Ingestion

Zhao & Liu, 2012
Som et al., 2011

ALTHOUGH HUMAN EXPOSURE IS ALSO GROWING VERY FAST…

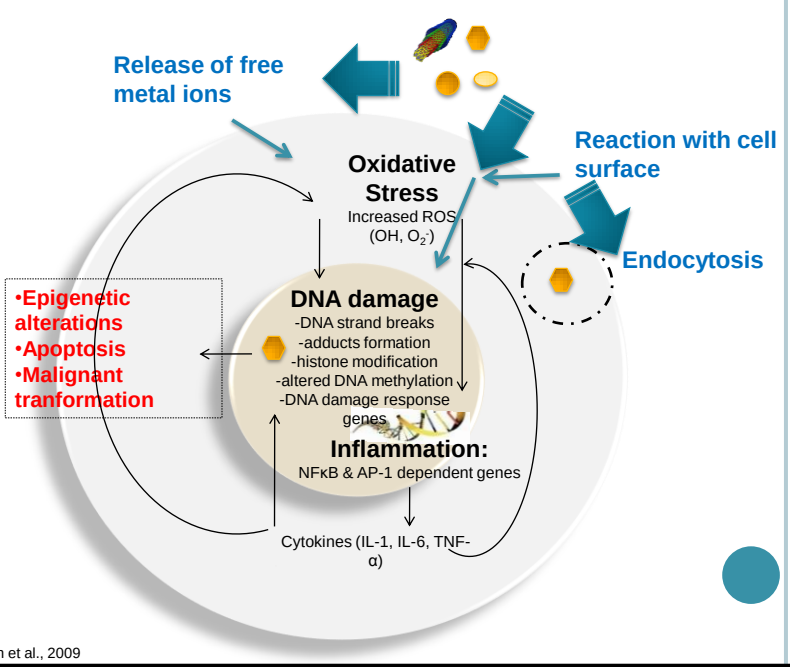


- Solid information about hazard is lacking for the vast majority of NMs, especially related to chronic exposure to low doses, that are likely to occur through consumers products.

SAFETY ?

- The genotoxic effects of NMs, 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 eventual acute effects.

MECHANISMS OF NMS TOXICITY



Adapetd from Singh et al., 2009

Nanotoxicology studies > conflicting results

NANOMATERIALS

- Size
- Surface area
- Shape
- Stability
- Coating
- Electric charge
- Agglomeration/aggregation

Physico-chemical characterization

EXPERIMENTAL SYSTEMS

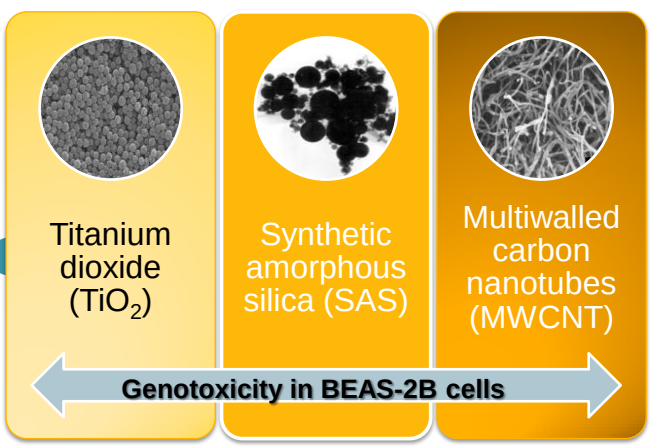
- Means of dispersion in aqueous media
- Agglomeration/aggregation state
- Cell systems
- Exposure conditions
- Genotoxicity tests

Standardized protocols

NANOGENOTOX

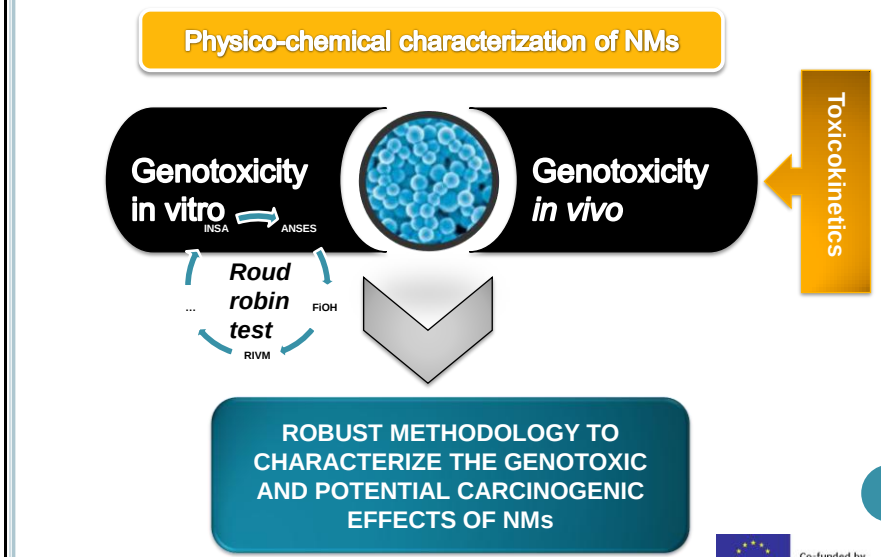
OBJECTIVES

- **To assess the potential genotoxic effects of NMs in a human bronchial epithelial cell line (BEAS-2B cells) contributing to their safety evaluation.**



NANOGENOTOX

"Safety evaluation of manufactured nanomaterials by characterization of their potential genotoxic hazard" (2010-2013)



Co-funded by the Health Programme of the European Union

Physico-chemical properties of NMs

TEM analysis	TiO_2 (NM-102)	SAS (NM-203)	MWCNT (NM-403)	ZnO (NM-110)
Phase	Anatase	Amorphous	-	-
Morphology	Polyhedral	Ellipsoidal	Flexible; Highly bended	Polyhedral
Impurities/ Coatings	-	-	Low level	Uncoated
Surface area (m^2/g)[a]	90	226	-	13
Primary particles (TEM)				
Feret Min ± SD (nm)	20.8 ± 1.6	16.0 ± 1.3	11.1 ± 1.5 (thickness)	37.1 ± 1.3
Feret Max ± SD (nm)	33.0 ± 1.5	24.0 ± 1.4	394.3 ± 1.6 (lenght)	56.3 ± 1.4
Aspect ratio ± SD	1.5 ± 1.3	1.5 ± 1.3	35.6 ± 1.8	1.4 ± 1.2
Aggregates/ agglomerates (DLS)				
Median (nm)	54	86	-	149

[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 Mast & Temmerman; Jensen, personnal communication.

METHODS

http://www.nanogenotox.eu/

Dispersion of NMs using a standardized protocol, according to Jensen, 2011.

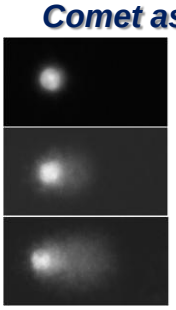
METHODS

3 and 24h exposure

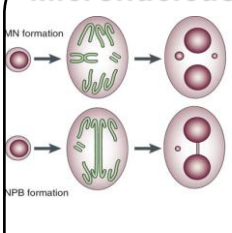
48h exposure

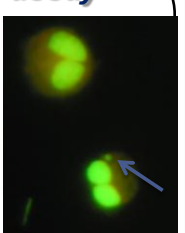
exposure of BEAS-2 B cells to NMs

Comet assay



Micronucleus assay





Cytokinesis-block proliferation index
CBPI = (MC+2BC+3MTC)/Total cells

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

CONCLUSIONS

- NMs obtained under GLP and that can serve as international benchmarks were used (JRC repository). Physico-chemical characterisation - NMs were is the nanosized range; low level of impurities
- The variability associated to experimental conditions was minimized:
  - Standard dispersion procedure for all NMs tested – stable dispersion (1h);
  - Same doses, exposure lenght and experiments performed simultaneously

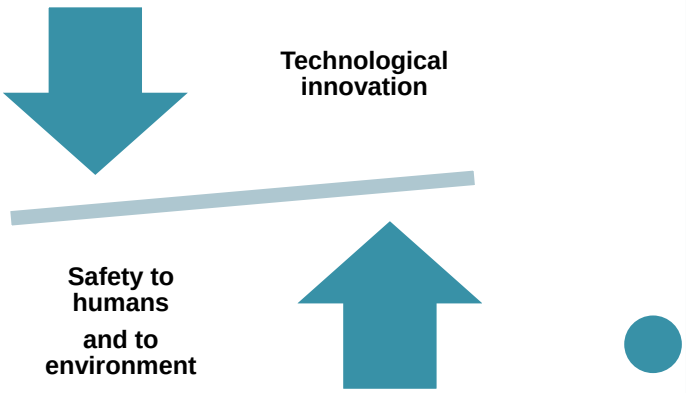
- SAS (NM-203) – NON-GENOTOXIC IN BEAS-2B CELLS
- TiO_2 (NM-102), MWCNT (NM-403), ZnO (NM-110) – INDUCED PRIMARY DNA LESIONS (COMET ASSAY) BUT NO CROMOSOME INSTABILITY (MN ASSAY)

FINAL REMARKS

- Dificulty in obtaining consistent results when testing the genotoxicity of nanomaterials
- Need to characterize the physico-chemical properties of each NM and try to associate to its biological effects
- Biological effects of low doses of NMs – more explored
- Mechanisms of NMs biological effects need to be further studied

"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



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