

NANOREG

A common European approach to the regulatory testing of nanomaterials

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Genotoxicity testing towards a knowledge-based regulation of nanomaterials

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IPQ, 3 April 2014**



1st NATIONAL MEETING "Nanot



regul:

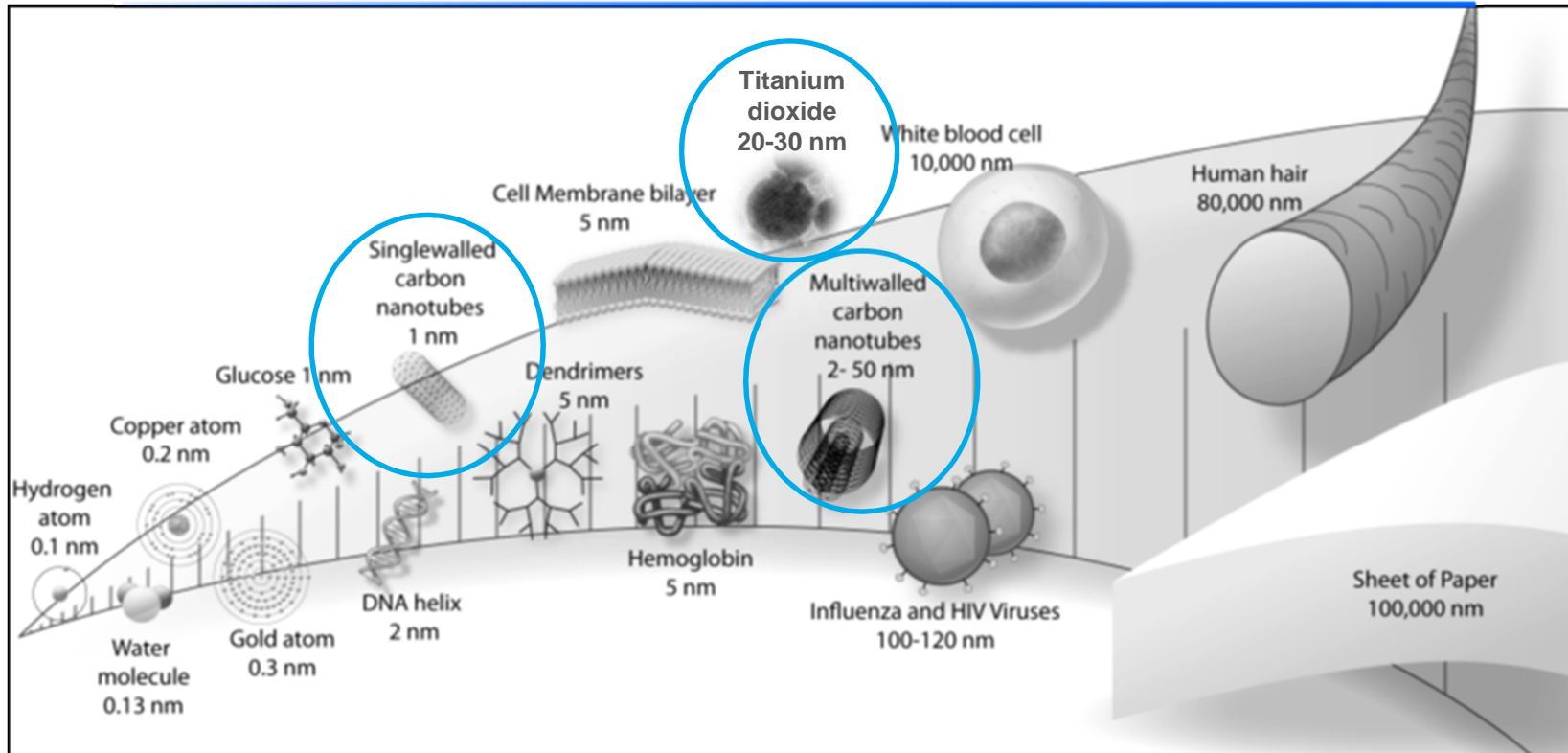


ete", IPQ, Caparica 3 april 2014

The «nanoscale»

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Yokel & MacPhail, 2011



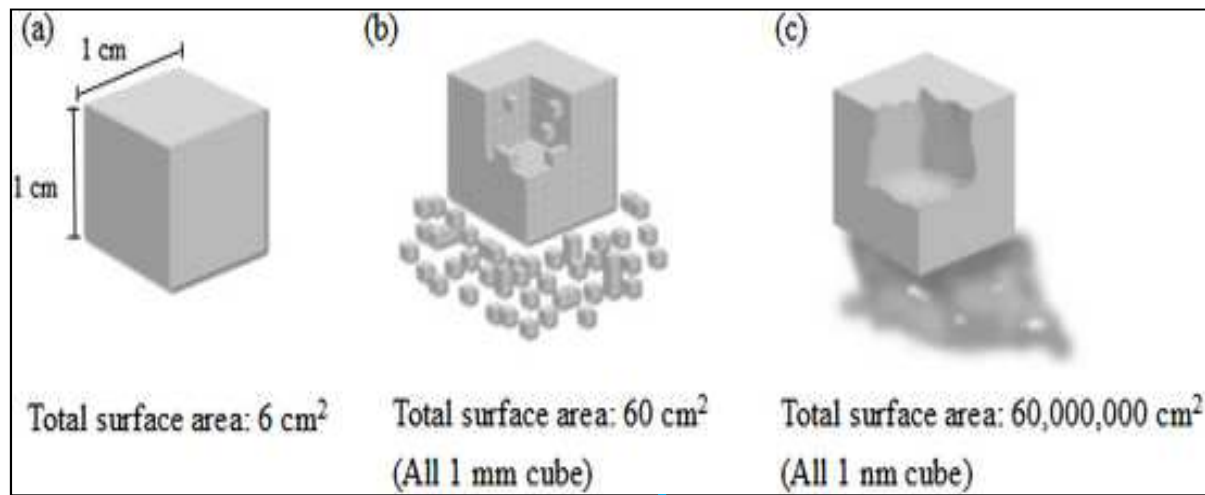
«NANOMATERIAL (NM) – means a natural, incidental or manufactured material containing particles, in an unbound state or as an aggregate or as an agglomerate and where, for 50 % or more of the particles in the number size distribution, one or more external dimensions is in the size range 1 nm - 100 nm»

Source: EC Recom., October 2011

Unique phy-chem properties of NMs

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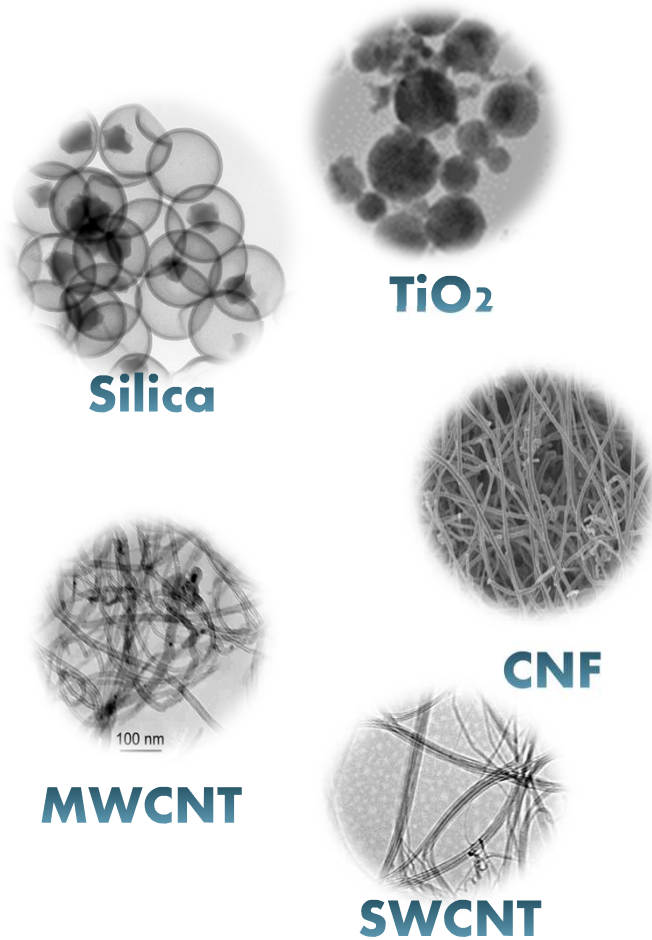
- Reduced size
- High surface area-to-volume ratio
- Higher chemical reactivity than bulk material
- Modified /improved mechanical, optical, magnetic and electric properties



Novel applications for industry, medicine and consumer goods

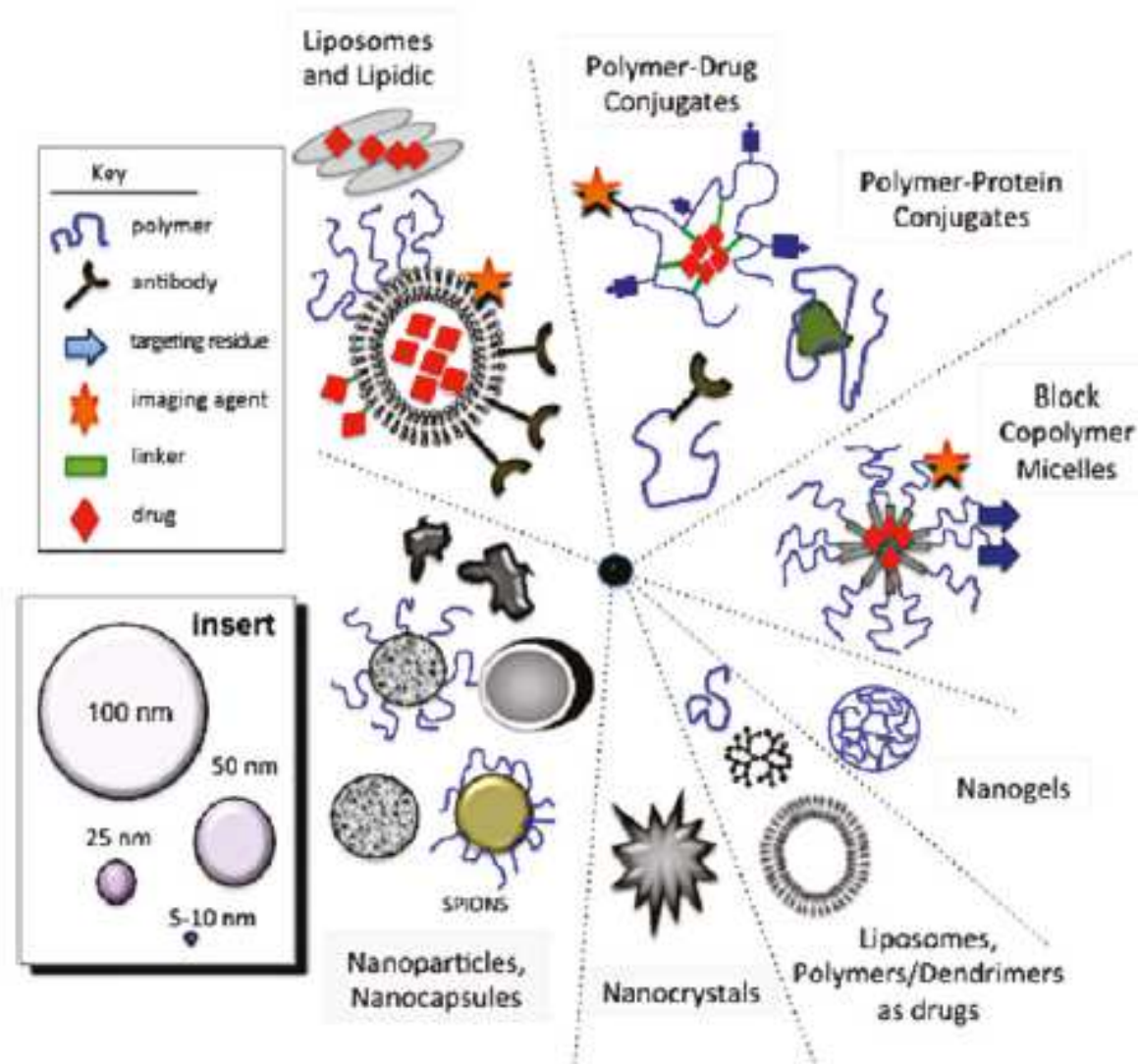
NMs in the construction sector

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New Jubilee Church (Rome, Italy) made of nano photocatalytic concrete

Nanomedicines in clinical trial or in routine clinical use

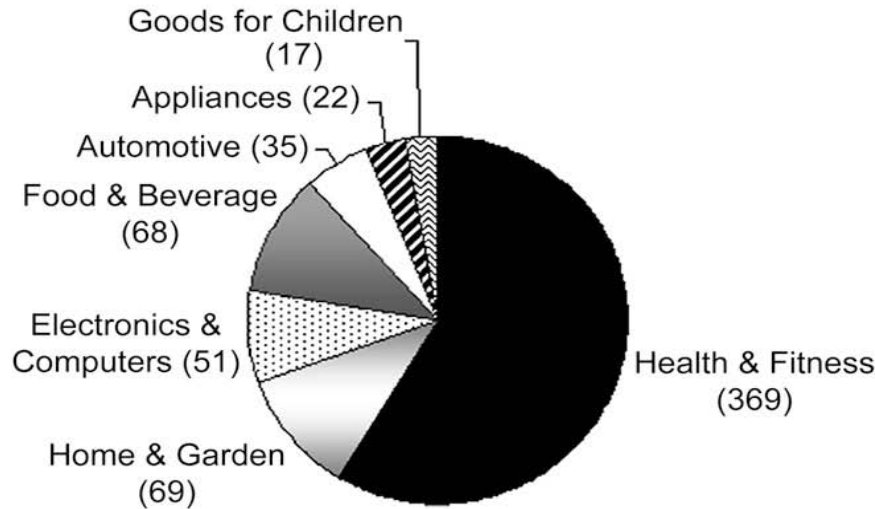


Duncan & Gaspar, Molecular Pharmaceutics, 2011

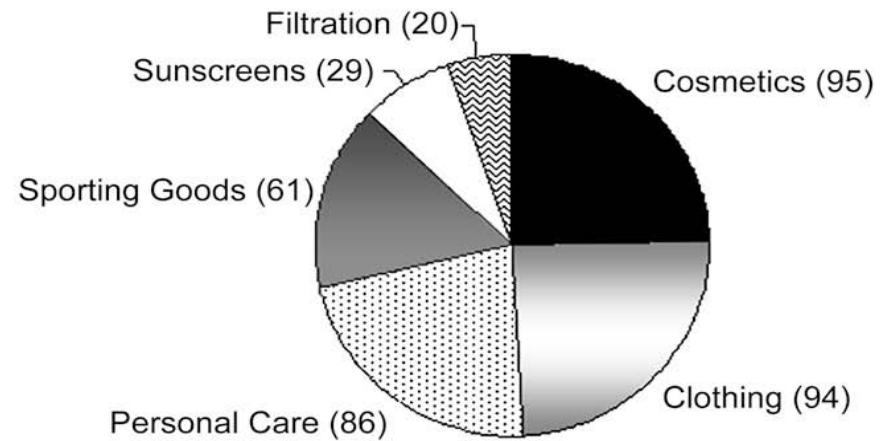
NMs in Consumers goods

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Product Categories



Health & Fitness Subcategory



Singh et al., 2009



Electronics and batteries



Vehicles



Food additives



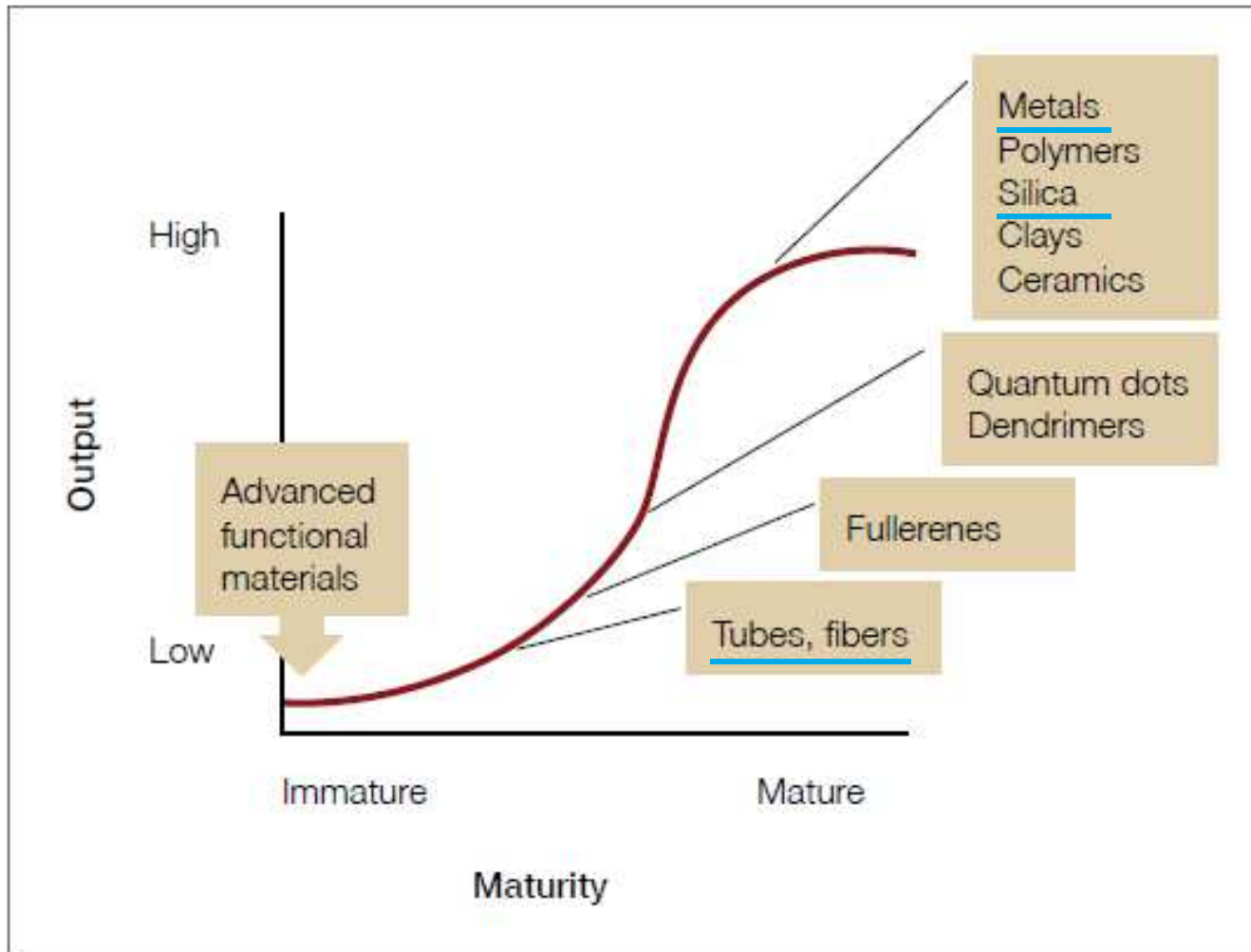
Cosmetics



Stress free Khakis

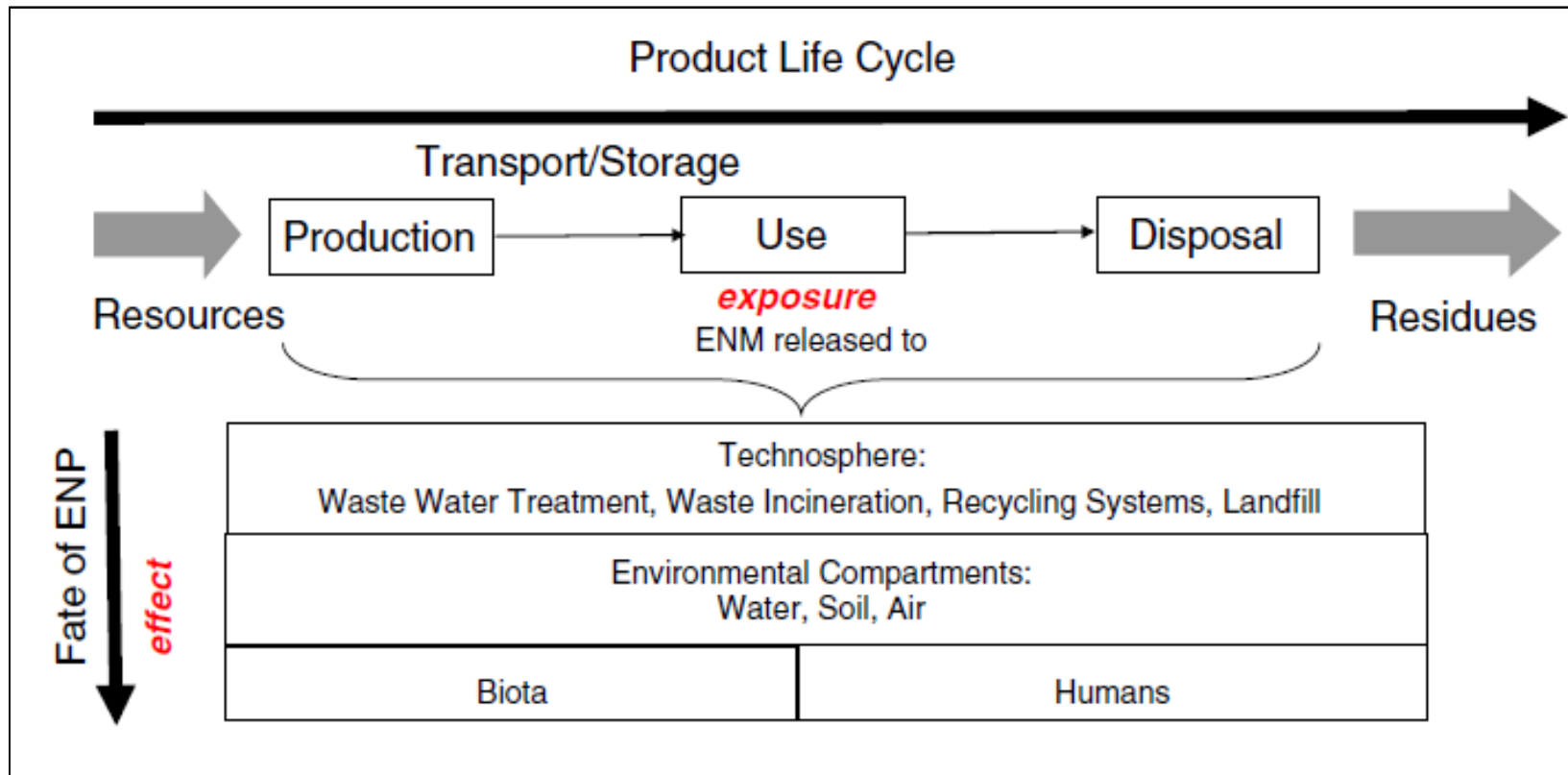
Manufacturing output by NM class

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Note: "Immature" materials are those that are still at the research and development stage, whereas "mature" materials are already being produced and commercialized.

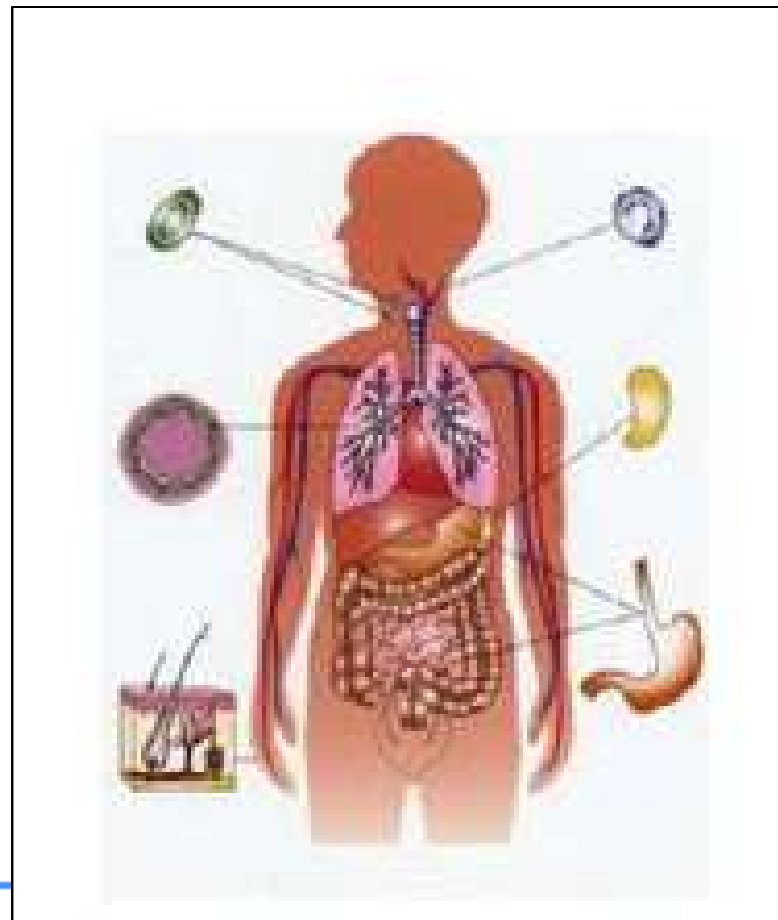
The life cycle of the nanomaterial defines the exposure, the fate and hazard scenarios



Som et al., 2011

The wide applicability of NMs > increases the risk of human exposure during their life cycle

- Occupational settings (workers)
- Environmental settings (Consumers)
- Clinical s. (Patients)
- ...



- Inhalation
- Transdermal
- Oral route
- Intravenous route

Human exposure to NMs is growing very fast but...

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§ 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, e.g., through consumers products.

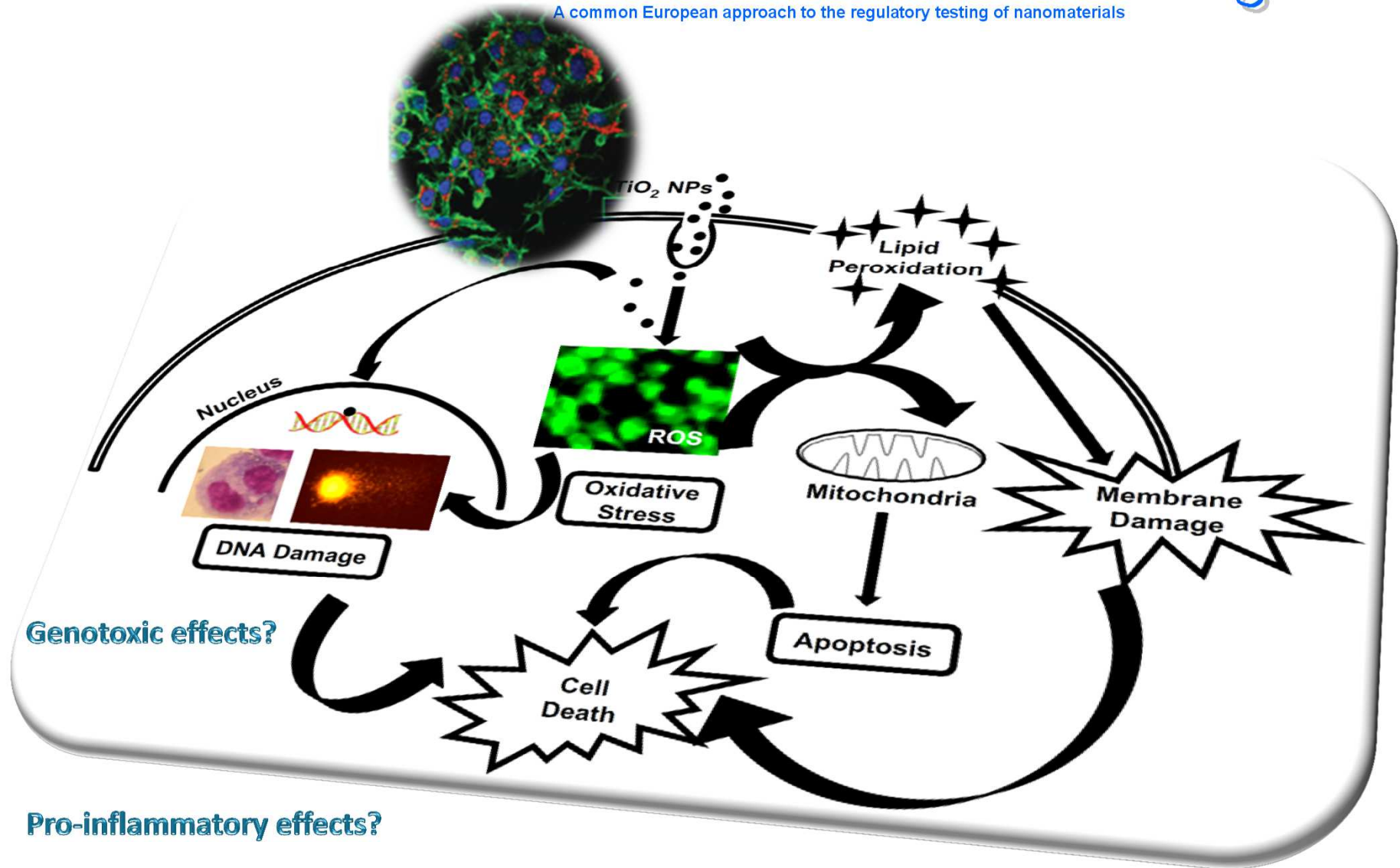
NANOTOXICOLOGY

§ The genotoxic effects of NMs, which may be linked to carcinogenic effects, are of special concern

- cancer has a long latency period and thereby these effects can be less obvious and more difficult to predict than eventual acute effects.

Potential genotoxicity of NMs

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Shukla et al., 2011;

Factors interfering with genotoxicity tests interpretation and comparability

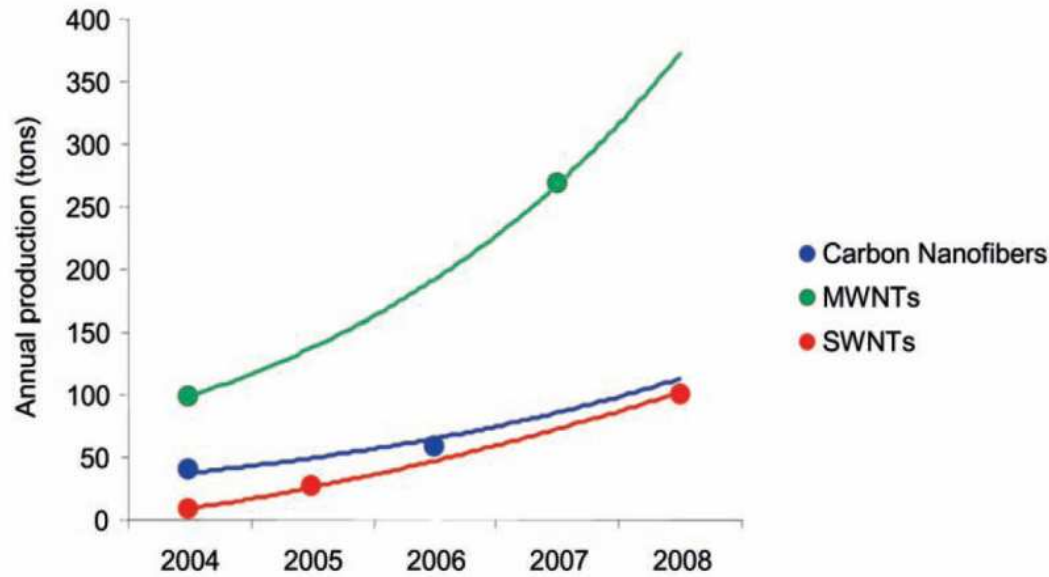
- § Incomplete description of the NMs physicochemical characteristics
- § Dynamic behavior of NMs (formation of aggregates and agglomerates, and the kinetics dependent of the medium conditions)
- § Dosing - difficult to picture a real exposure scenario in *in vitro* or *in vivo* assays (lack of human exposure data)
- § The dose-metrics (e.g., mass, particle number or surface area)
- § Differences in the means of dispersion of insoluble NMs for cells or animals exposure

Factors interfering with genotoxicity tests interpretation and comparability

- § Interference with colorimetric assays (e.g., cytotoxicity assays)
- § Corona formation and composition
- § Incomplete knowledge of the uptake capacity of the different cell lines towards the variety of NMs available
- § Inexistence of SOPs and validated methods
- § Lack of positive controls at the nanoscale

Carbon nanotubes

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.

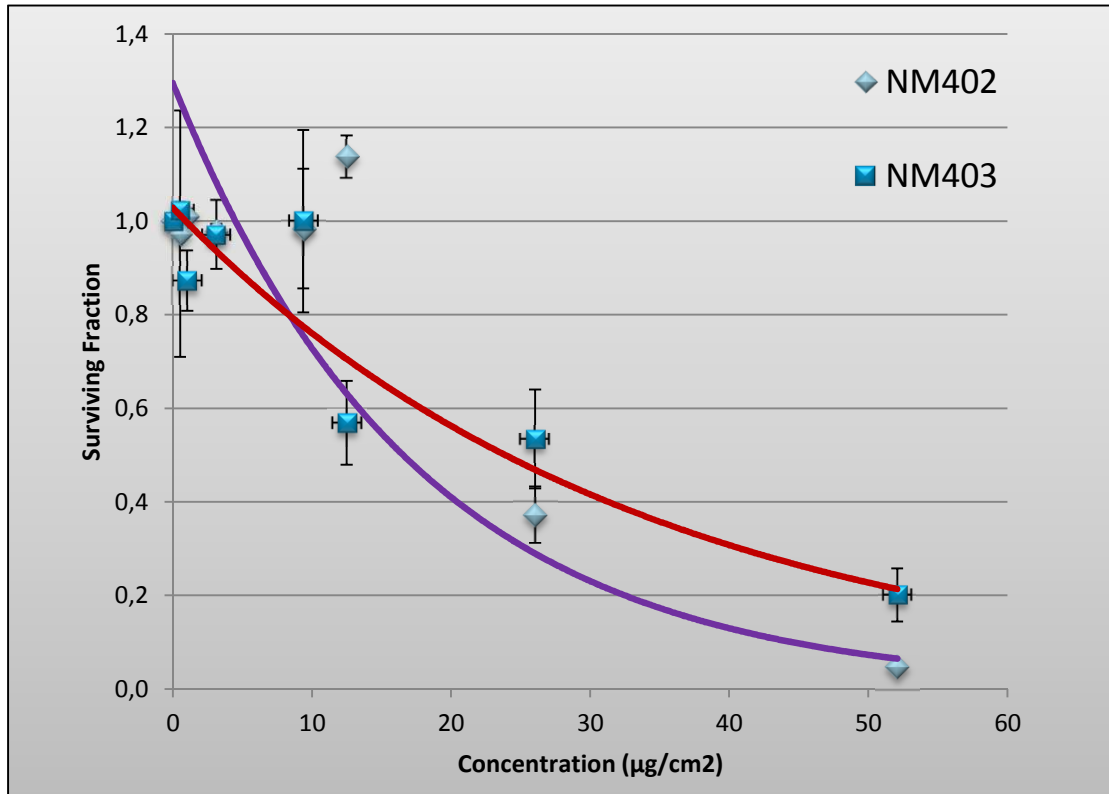
MWCNT – Risk?

§ The particular physicochemical properties that have rendered CNTs attractive for a wide range of applications 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

MWCNT – cytotoxicity in alveolar cells



Clonogenic assay



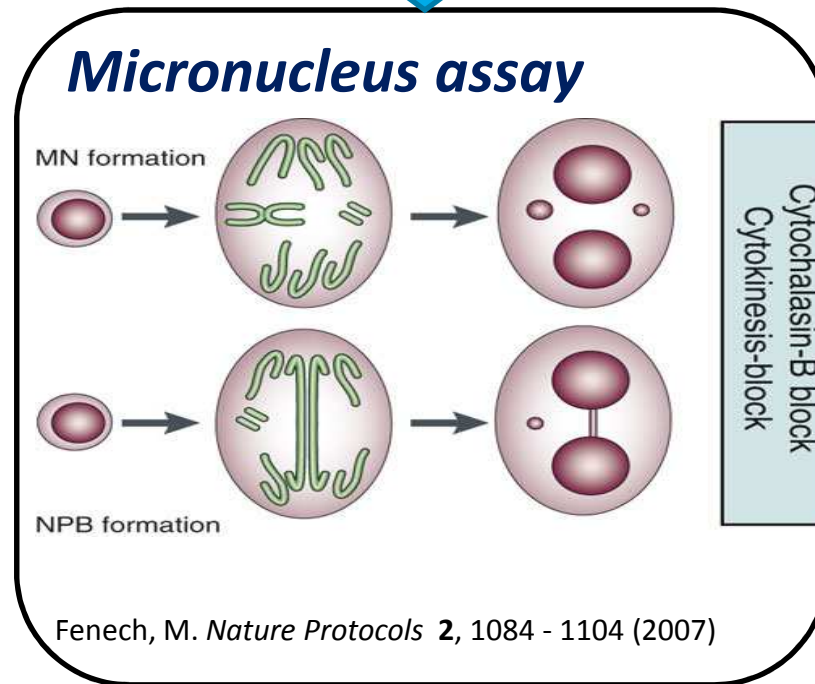
IC_{50} NM402 = 19.03 µg/cm²

IC_{50} NM403 = 24.03 µg/cm²

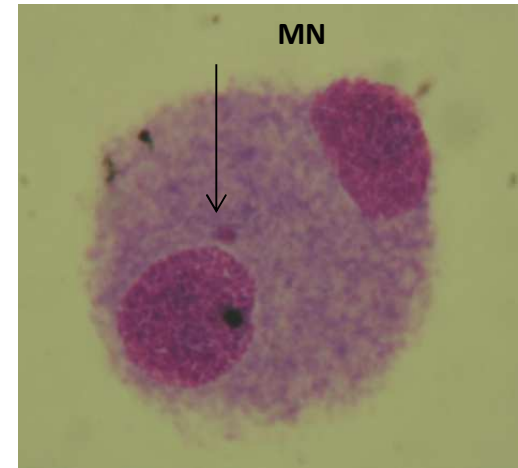
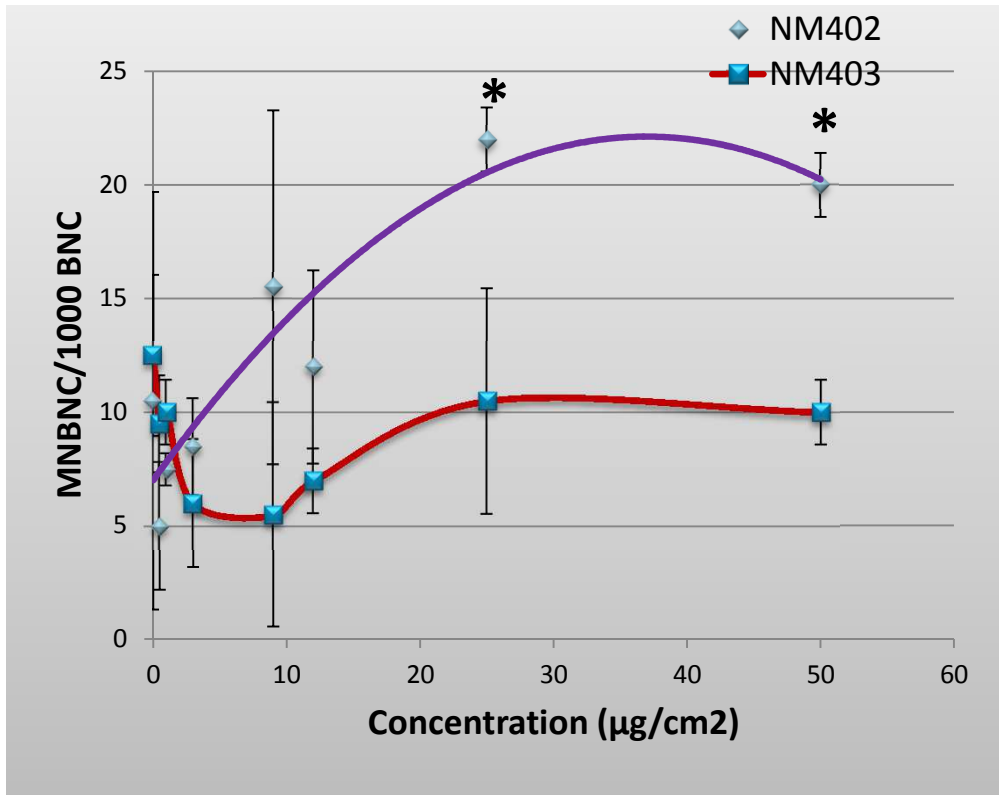
A concentration-effect relationship was observed for A549 cells after exposure to both NMs (exponential model $R^2 = 0,909$ & $0,931$).

MWCNT – genotoxicity in alveolar cells

Dispersion of NMs using a standardized protocol



Micronucleus assay



- Concentration-effect relationship for NM402 (quadratic model $R^2= 0.861$)

* Significantly different from vehicle controls (P<0.02, 2-tailed Fisher's exact test)

- No concentration-effect relationship for NM403

MWCNT – Phys-Chem pp.

Comparison NM402 and NM403	
Length (nm)	NM402 = 3x NM403
Width (nm)	NM402 = NM403
TEM-analysis	Highly bend MWCNT
Diameter	Low-Diameter MWCNT
Purity (wt %)	NM402 << NM403
Impurities (%)	Important concentrations of inorganic impurities
Elements detected	Different elements

MWCNT – implications for safety assessment

NM402 and NM403 are
Closely related NMs...



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

For safety investigation of NMs , we must be caution when generalizing the mechanisms responsible for toxicity.

Further investigation should focus on the properties responsible for the different genotoxic effects observed.

Advancement of Regulatory Risk assessment and testing

SOME QUESTIONS TO BE ADDRESSED:

- § **Which metrics should be used for NMs regulatory toxicology?**
- § **Guidance for possibilities of read-across, categorisation and grouping?**
- § **What are the physical and chemical pp driving toxicity of NMs along their life cycle?**
- § **Which methods should be used to assess the human and environmental toxicity?**
- § **What is the applicability of conventional testing methods for NMs?**
- § **How should human and environmental exposure to NMs be assessed?**

Advancement of Regulatory Risk assessment and testing

EXPECTED OUTCOMES

- § Help filling the gaps in the safety evaluation of NMs through characterization of their potential toxicity, particularly, genotoxicity
- § Development of standards and good practice guidelines in hazard assessment
- § To provide answers for the regulatory testing of NMs and contribute to their risk assessment
- § To improve the knowledge-base regulation of NMs and nanotechnologies

NANoREG – work in progress

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Dispersion of NMs using a standardized

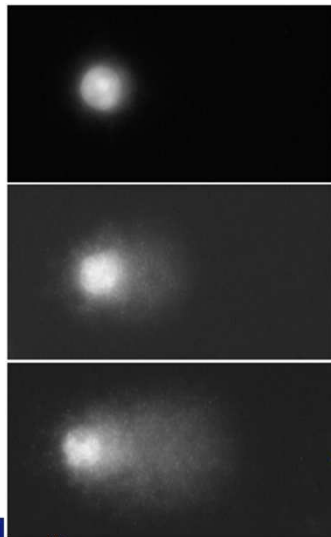


MWCNT (NM 400, 401, 402)

Human bronchial cells



Comet assay

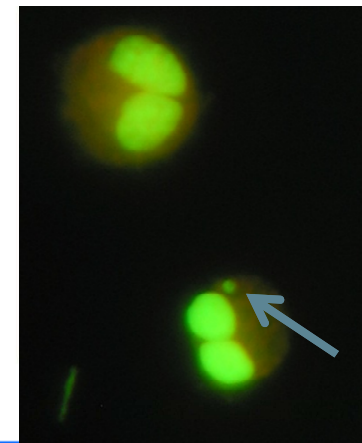
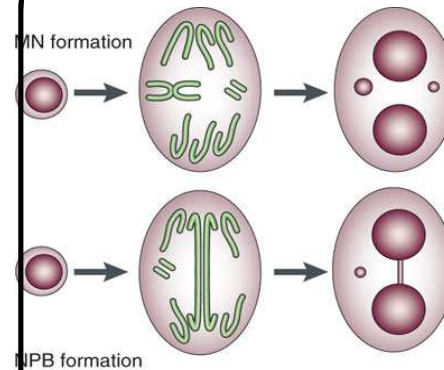


No damage

Moderate damage

Severe damage

Micronucleus assay

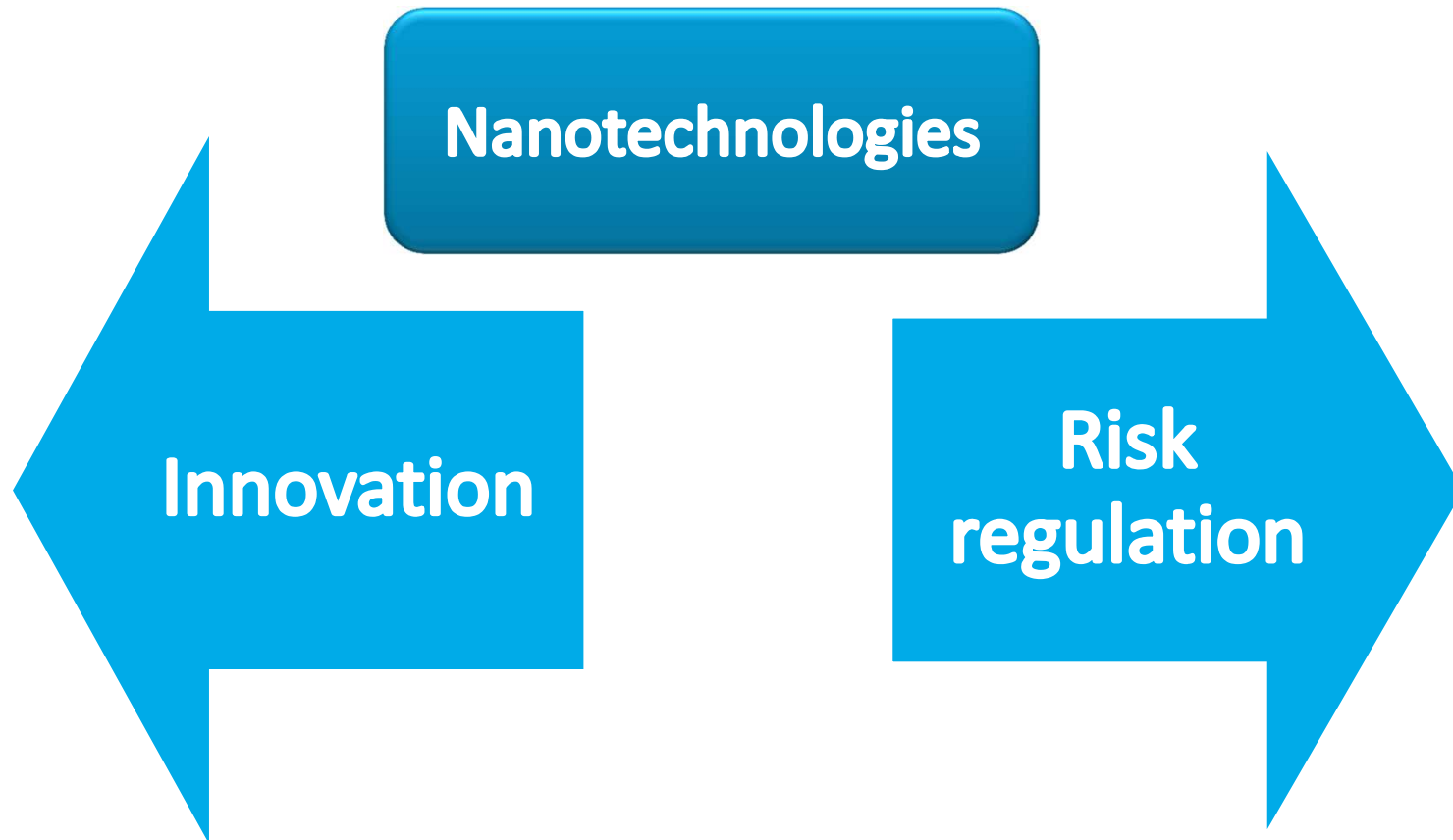


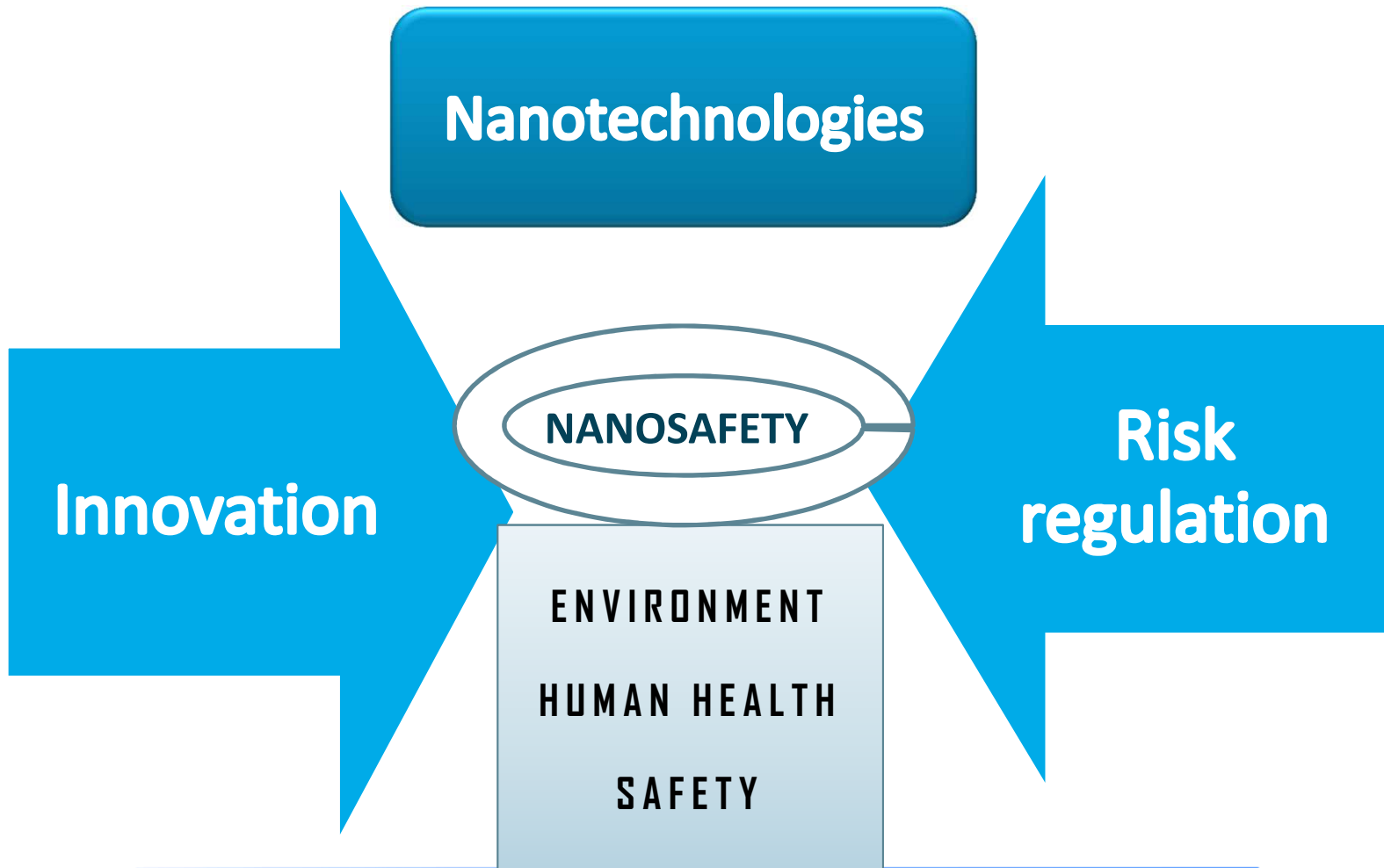
Tice et al., 2000

Fenech, 2007

«Worker safety and health is a cornerstone of responsible development of an emergent technology because workers are the first people in society to be exposed to the products of the technology and the workplace is the first opportunity to develop and implement responsible practices». NIOSH, 2013







Acknowledgments

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OBRIGADA!



“Nanotechnology” by Murray Robertson