Genotoxicity testing towards a knowledge-based regulation of nanomaterials

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The «nanoscale»

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

- 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
NM\textsuperscript{s} in the construction sector

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

Silica  TiO\textsubscript{2}  CNF  MWCNT  SWCNT

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Nanomedicines in clinical trial or in routine clinical use

Duncan & Gaspar, Molecular Pharmaceutics, 2011
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NMds in Consumers goods

Product Categories
- Goods for Children (17)
- Appliances (22)
- Automotive (35)
- Food & Beverage (68)
- Electronics & Computers (51)
- Home & Garden (69)

Health & Fitness Subcategory
- Filtration (20)
- Sunscreens (29)
- Sporting Goods (61)
- Personal Care (86)
- Cosmetics (95)
- Clothing (94)

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

- Occupational settings (workers)
- Environmental settings (Consumers)
- Clinical settings (Patients)
- Inhalation
- Transdermal
- Oral route
- Intravenous route

Zhao & Liu, 2012
Som et al., 2011
Human exposure to NMs is growing very fast but...

- 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.

- 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.

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Potential genotoxicity of NMs

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Shukla et al., 2011;

Pro-inflammatory effects?
Genotoxicity testing of NMS

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 \textit{in vitro} or \textit{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

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

Source: The Royal Society & The Royal Academy of Engineering, 2004
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
A concentration-effect relationship was observed for A549 cells after exposure to both NMs (exponential model $R^2 = 0.909$ & $0.931$).

$IC_{50}$ NM402 = 19.03 µg/cm²

$IC_{50}$ NM403 = 24.03 µg/cm²
MWCNT – genotoxicity in alveolar cells

Dispersion of NMs using a standardized protocol

Micronucleus assay

MN formation

NPB formation

MWCNT – genotoxicity in alveolar cells

Micronucleus assay

- Concentration-effect relationship for NM402 (quadratic model $R^2 = 0.861$)
- No concentration-effect relationship for NM403

* Significantly different from vehicle controls
(P<0.02, 2-tailed Fisher’s exact test)
### Comparison NM402 and NM403

<table>
<thead>
<tr>
<th>Property</th>
<th>NM402 vs NM403</th>
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<tbody>
<tr>
<td>Length (nm)</td>
<td>NM402 = 3x NM403</td>
</tr>
<tr>
<td>Width (nm)</td>
<td>NM402 = NM403</td>
</tr>
<tr>
<td>TEM-analysis</td>
<td>Highly bend MWCNT</td>
</tr>
<tr>
<td>Diameter</td>
<td>Low-Diameter MWCNT</td>
</tr>
<tr>
<td>Purity (wt %)</td>
<td>NM402 &lt;&lt; NM403</td>
</tr>
<tr>
<td>Impurities (%)</td>
<td>Important concentrations of inorganic impurities</td>
</tr>
<tr>
<td>Elements detected</td>
<td>Different elements</td>
</tr>
</tbody>
</table>
NM402 and NM403 are closely related NMs... but 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.
### NANoREG – in vitro toxicity

(WP5)

Advancement of Regulatory Risk assessment and testing

**SOME QUESTIONS TO BE ADDRESSED:**

<table>
<thead>
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<tr>
<td>Which metrics should be used for NMs regulatory toxicology?</td>
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<td>Guidance for possibilities of read-across, categorisation and grouping?</td>
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<tr>
<td>What are the physical and chemical pp driving toxicity of NMs along their life cycle?</td>
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<tr>
<td>Which methods should be used to assess the human and environmental toxicity?</td>
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<tr>
<td>What is the applicability of conventional testing methods for NMs?</td>
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<tr>
<td>How should human and environmental exposure to NMs be assessed?</td>
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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
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Dispersion of NMs using a standardized protocol

MWCNT (NM 400, 401, 402)

Human bronchial cells

Comet assay

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
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"Nanothechnology" by Murray Robertson