Genotoxicity evaluation of three classes of manufactured nanomaterials on primary human lymphocytes

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Executive Agency for Health and Consumers

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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

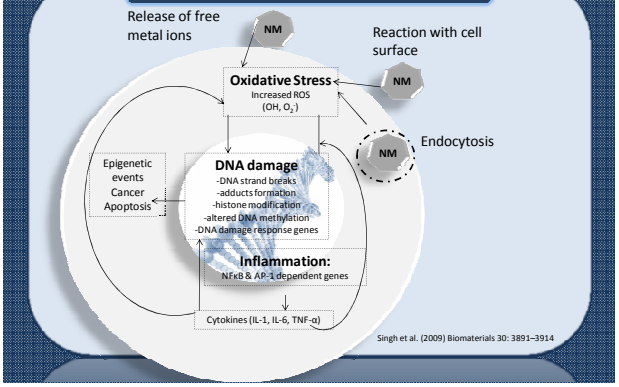
Specific properties:
-size
-high surface area/mass

Many applications include biomedicine, food additives, cosmetics, electronics and industry.

Medina et al. 2007

Due to their specific properties, do manufactured nanomaterials (MNMs) pose higher toxicity in biological systems and risks for environment and human health?

Background



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

Objectives

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

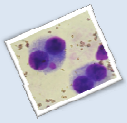
To evaluate the potential genotoxic effects of a panel of well-characterized MNMs in human lymphocytes

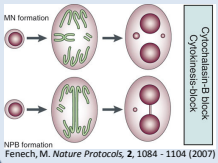
	Label	Crystal Phase	Particle Size	Purity wt (%)	Use
TiO_2	NM102	Anatase	20	95	Photocatalytic effects
	NM103	Rutile (hydrophobic)	20	89	Cosmetics (sun care, colour), pharma, food
	NM104	Rutile (hydrophilic)	20	90	
	NM105	85% anatase+15% rutile	22	-	catalysis, heat stabilizer
SiO_2	NM200		20	-	Food, processing
	NM201	Synthetic amorphous	8-15	-	Reinforcement, mechanical and optical properties and process
	NM202	Silica	8-15	<99,8	Inks, adhesives, cosmetics, reinforcement, powder process, food, pharma
	NM203		8-20	-	Food, cosmetics pharma, reinforcement
MWCNT	NM400	MWCNTs	9.5	-	Structural composite and energy applications
	NM401		10-30	-	
	NM402		-	-	
	NM403		-	-	
	NRCWE-006		-	>99.5	
	NRCWE-007		-	-	

Methods

Cultures of PBL from healthy donors
30 h- exposure to MNMs

***In vitro* Micronucleus assay (OECD guideline 487)**





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

-Micronucleated binucleated cells (MNBC) scored in 2000 binucleate cells (BC);
-Micronucleated mononucleated cells (MNMC) scored in 500 mononucleated cells (MC)

-The proportion of mono-, bi- and multinucleated cells in 1000 cells for cytokinesis-block proliferation index (CBPI)

Discussion

❑ The silicon dioxides do not show genotoxic effects in human lymphocytes.

Similar negative results for MN induction after exposure of lymphocytes to SiO_2 NMs have been previously described (Downs 2012) although positive results were observed by others (Lankoff 2012).

❑ Some doses of TiO_2 NMs and MWCNTs induce clastogenic effects.

Considering TiO_2, the results agree with several evidences showing genotoxic effects in human lymphocytes exposed in vitro (Kang 2008; Türkez 2007; Catalán 2011), while only one negative result has been published in these cells (Hackenberg 2011).

While one author reports negative results (Szendi 2008), others showed the genotoxicity of MWCNTs in lymphocytes (Cveticanin 2010; Catalán 2011), consistent with the dose-response observed for NM 403 at low dose-range. The lack of a dose-dependent effect in our micronucleus assays at higher dose levels might be explained by the increasing size of agglomerates.

Discussion

Overall, since no clear dose-response relationships could be disclosed for TiO_2 and MWCNTs in the present work, no definitive conclusions about the their genotoxicity can be drawn.

Further ongoing assays, using the same NMs in other *in vitro* and *in vivo* systems, will allow to improve the knowledge base for the evaluation of genotoxic risks associated to the emergence of nanomaterials in human environment.

It is expected that the information about physicochemical characteristics generated within Nanogenotox joint action will contribute to understand the toxicity determinants of the positive NMs, allowing a "safe-by-design" approach to these promising technologies.

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