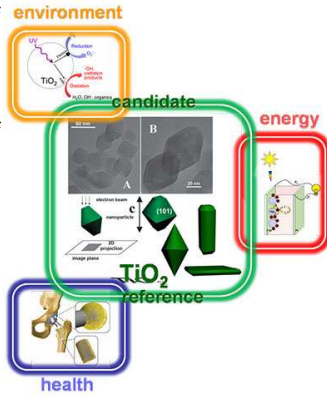


Introduction

The European seventh framework project SETNanoMetro explores the highly-defined development and production of titanium dioxide (TiO₂) nanoparticles (NPs) in terms of homogeneous bulk structure, size, shape and surface structure. The outcomes of the project are anticipated to benefit hallmark applications in the specific areas of environment (i.e. photocatalytic treatment of polluted air and water), energy (i.e. dye sensitized solar cells) and health (i.e. nanostructured coatings in prostheses). The nanosafety aspects in SetNanoMetro are being addressed using *in vitro* toxicological testing of the highly-defined TiO₂ nanoparticles in respiratory and gastrointestinal tract cell lines (A549 human lung epithelial cells and Caco-2 human colon epithelial cells), as inhalation and ingestion are considered to represent the most relevant uptake routes of nanoparticles. To determine the pro-inflammatory potential of TiO₂ nanoparticles also lung alveolar macrophages (NR8383 rat macrophages) are being tested due to their important role as regulator of the early immune response. Cells are being evaluated amongst others for changes in the cytotoxicity, expression of pro-inflammatory cytokines interleukin(IL)-1β and IL-8 and the oxidative stress markers heme oxygenase (HO-1) and inducible nitric oxide synthase (iNOS).

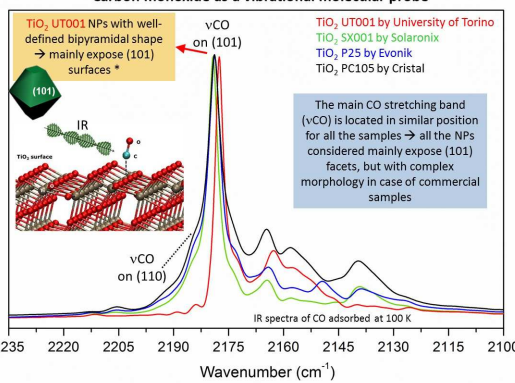


AIM: Toxicological characterisation of shape-engineered TiO₂ nanoparticles in lung and gastrointestinal tract cells.

Results

Characterisation of TiO₂ nanoparticles

TiO ₂ Nanoparticle	Code	Description
P25 (by Evonik)	SNM1	Fumed TiO ₂ NPs obtained by flame pyrolysis of TiCl ₄ mixture of anatase and rutile
PC105 (by Cristal)	SNM2	TiO ₂ NPs obtained through hydrolysis of titanyl sulfate and unspecified thermal treatment, anatase
SX001 (by Solaronix)	SNM3	TiO ₂ NPs obtained through hydrothermal process, anatase
UT001 (by University of Torino)	SNM4	Anatase TiO ₂ NPs truncated bipyramids, obtained by hydrolysis of an aqueous solution of the Ti(TEOA) ₄ complex (TEOA = triethanolamine)



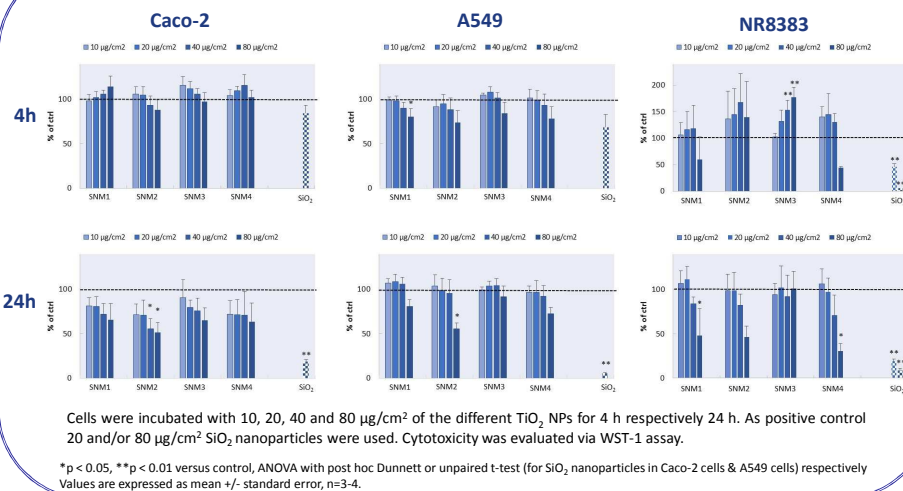
DLS measurements

MATERIAL	Sample	P25	Conc. mg L ⁻¹	DLS Hydrodynamic Radius, nm				Remarks
				Cumulants, 2nd Order	PDI	Number, nm	Mass, nm	
P25	10	72	0.225	18.7-70	18.8-76	Bimodal		
	100	70.5	0.188	14.5-62	14.7-75	Bimodal		
	100	75	0.138	11.3-62	11.3-80	Bimodal		
PC105	30	315	0.276	12-269	12-288	Bimodal		
	SX001	30	47	0.238	12.5-37.4	12.8-46.5	Bimodal	
		30	48	0.203	11.3-38.1	11.3-48.8	Bimodal	
30		48	0.185	15.5-49.3	13.9-52.0	Bimodal		
UT001	30	22	0.102	16.2	19.2	Monomodal		
	30	23	0.146	16.5	19.5	Monomodal		

P25: shows a number distribution dominated by a NP mode at 12-18 nm and a mass distribution dominated by a mode centered at 75-80 nm (agglomerates).
 PC105: contains stable aggregates (ca. 300 nm) of primary crystalline anatase NP (ca. 10 nm).
 SX001: shows a number distribution dominated by a NP mode at 12-15 nm and a mass distribution dominated by a mode centered at 47-50 nm (agglomerates).
 UT001: size distribution is monomodal, truncated bipyramids with a mean number distribution at 16-17 nm and a mean mass distribution at 19-20 nm.

* C. Deiana, M. Minella, G. Tabacchi, V. Maurino, E. Fols, G. Martra, *Phys. Chem. Chem. Phys.*, 15 (2013) 307

Cytotoxicity (WST-1 assay)

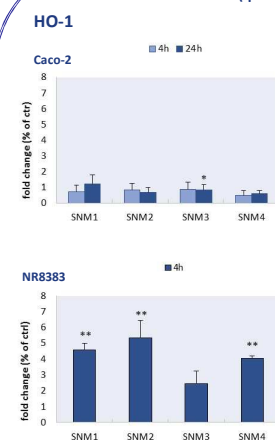


Summary

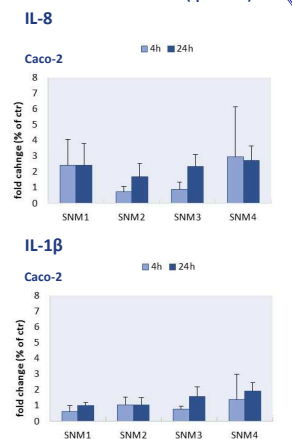
- SNM1 is an anatase/rutile mixture, SNM2, SNM3 & SNM4 are pure anatase powders
- The main surface facets are type (101), but also facets type (110) are present except on SNM4
- TiO₂ NPs induce low cytotoxicity compared to SiO₂ reference material in concentration-, time- & cell type-dependency
- TiO₂ NPs induce oxidative stress in NR8383 alveolar macrophages:
 - significant increase of HO-1 mRNA expression for all samples except SNM3
 - significant increase of iNOS mRNA expression for SNM2 & SNM3
- TiO₂ do not affect mRNA expression of pro-inflammatory cytokines IL-8 & IL-1β in Caco-2 cells

Material & Methods

Oxidative stress (qRT-PCR)



Inflammation (qRT-PCR)



Cells were incubated with 20 μg/cm² of the different TiO₂ NPs for 4 h or 24 h, respectively. Via qRT-PCR the mRNA expression of oxidative stress markers HO-1 and iNOS, pro-inflammatory cytokines IL-8 and IL-1β was analysed.

*p < 0.05, **p < 0.01 versus control, ANOVA with post hoc Dunnett. Values are expressed as mean +/- standard error, n=2-4.

Acknowledgment

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