Feasibility of using *in vitro* toxicity studies for Human Risk Assessment of nanomaterials

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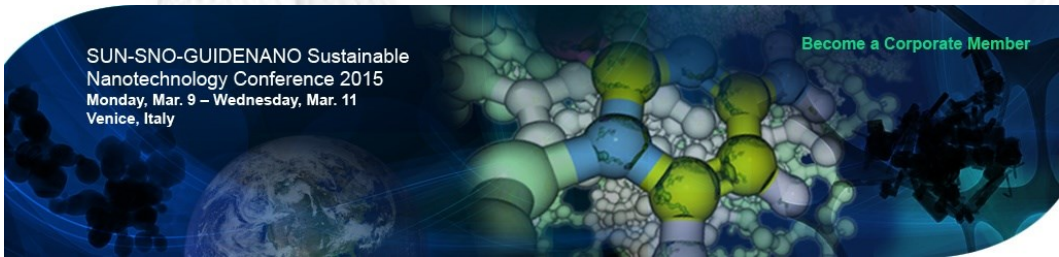


Presentation Overview

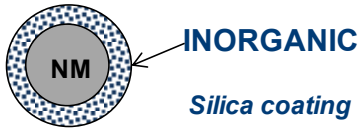
1. Introduction to the Sanowork Project
2. The “Sanowork Approach” on how to derive human threshold hazard values using *in vitro* toxicity data
3. Proof of Concept on correlation between *in vitro* and *in vivo* data
4. Risk Assessment Strategy
5. Example of *in vitro* toxicity assay evaluating hazard on AgNPs
6. Risk assessment on ZrO_2 nanomaterials in a spraying exposure scenario.
7. Conclusions

The Sanowork Project

«SAFER BY DESIGN» Risk Remediation Strategies to manage Occupational Risk

1 Inorganic coating

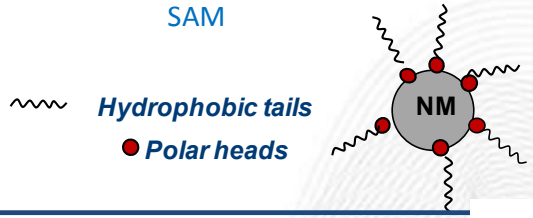
Inorganic coating with inert materials by heterocoagulation or chemical synthesis



HEALTH HAZARD ADDRESSED

Organic coating

with organic antioxidant molecules by SAM



2 Micronization

Keeping nanoscale reactive structures in a microstructure

Spray granulation *Forced aggregation*

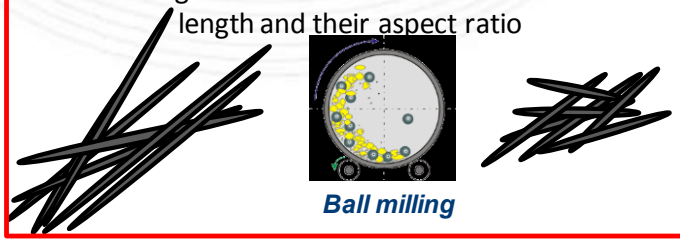
«NANO IN MICRO» EXPOSURE ADDRESSED

Immobilization 3

Embedding *Gelation*

4 Size control

Wet milling of nanofibers in order to reduce their length and their aspect ratio

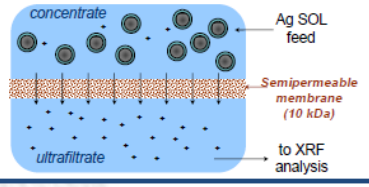


Ball milling

Purification 5

Ultrafiltration treatment applied to remove the unreacted cations from pristine

Ultrafiltration



OBJECTIVE: develop and implement "**Design Options**" based on **Risk Remediation Strategies** mainly Surface Engineering, as **Primary Prevention Control Measure** to manage the potential occupational risk of nanomaterials

SANOWORK APPROACH on how to derive human threshold hazard values by using *in vitro* data

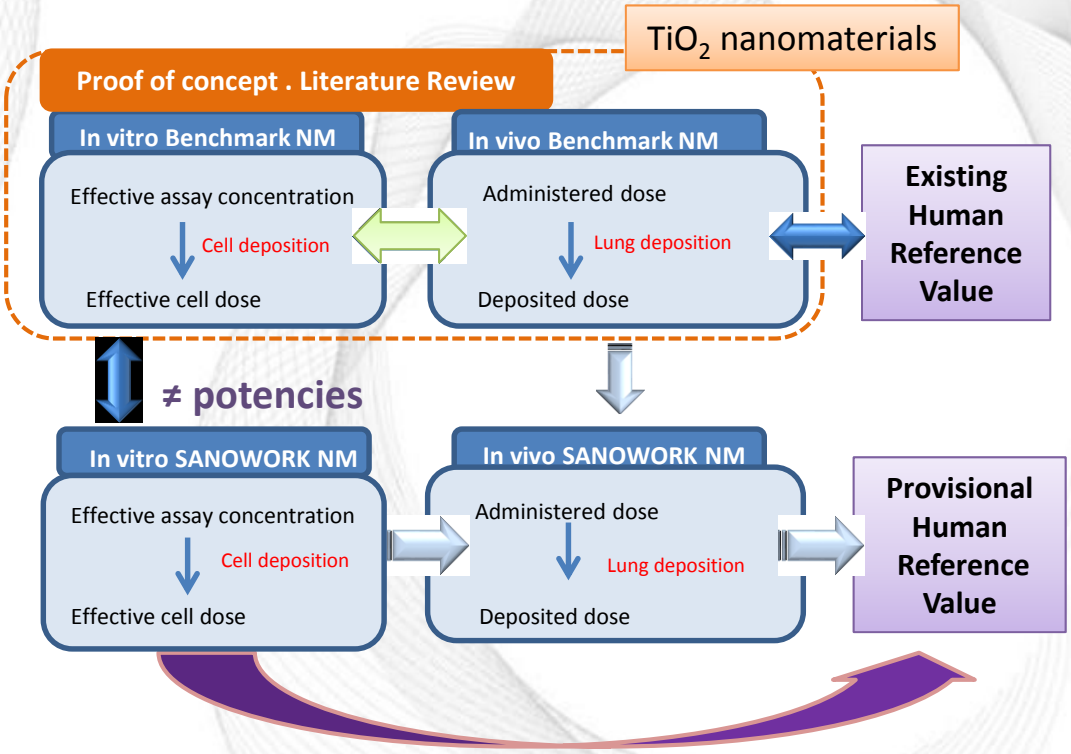
1. Grouping of NMs expected to share mechanisms of toxicity

Group	Type of Nanomaterial	Sanowork Nanomaterials	Main mechanism of toxicity	Parameter modulating toxicity	Benchmark Nanomaterials	*In vitro* relevant endpoint
1	Low solubility, low toxicity	ZrO_2, TiO_2 (NP and nanosols)	Sustained inflammation due to accumulation in lungs	Surface reactivity	**AEROXIDE® TiO_2 P25**	Oxidative stress / Inflammation response
2	Low solubility, high aspect ratio/fibrous	MWCNT, polyamide nanofibers, TiO_2 nanofibers	Sustained inflammation due to physical cell damage and frustrated phagocytosis	Morphology	**UICC Crocidolite Asbestos**	Oxidative stress / Inflammation response
3	High ion release rate (solubility)	Ag nanosols	Silver ion toxicity	Ion release rate	**Silver salt**	Cell viability

2. Generate experimental *in vitro* data (relevant endpoints) for Sanowork NMs and Benchmark NMs

3. Gather relevant human reference values for Benchmark NMs (with relevant *in vivo* data available from the literature)

4. By considering differences in potency *in vitro* and dosimetry, estimate *in vivo* and approximated human reference values for Sanowork NMs.



PROOF OF CONCEPT

(Correlation *in vitro* and *in vivo* data)

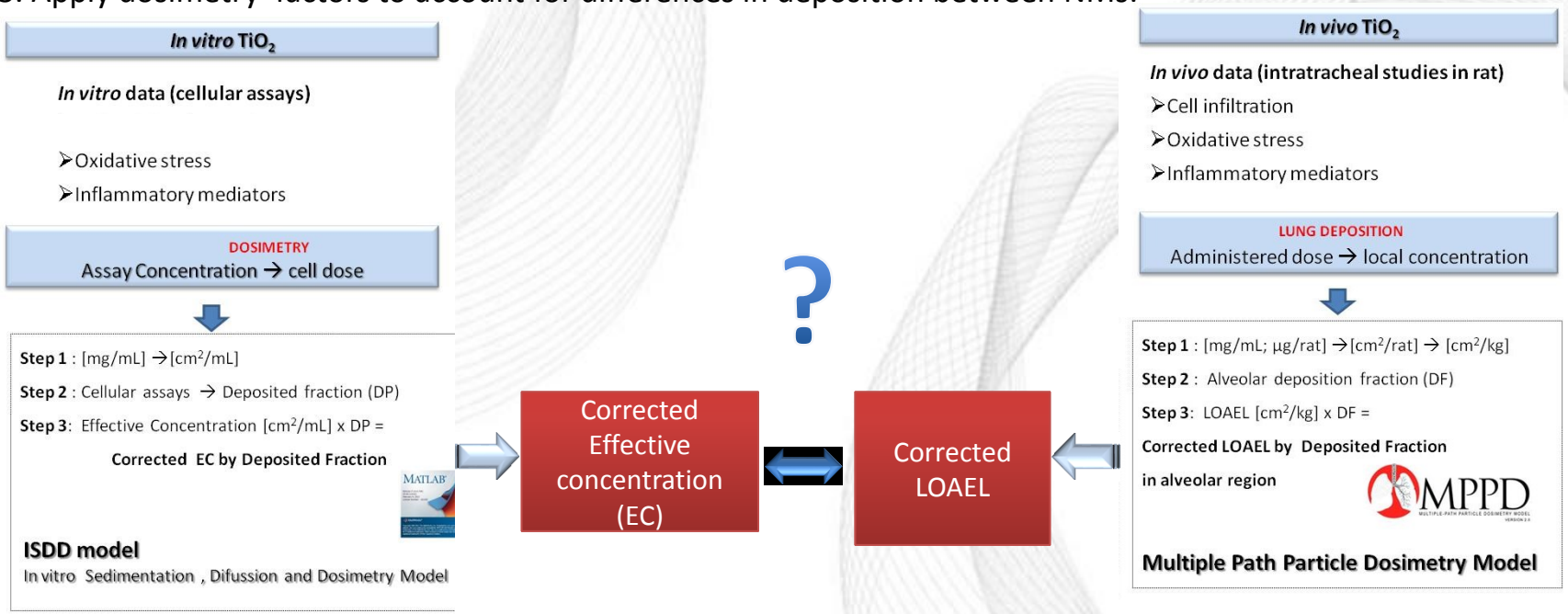
1. Gather *in vitro* and *in vivo* *(inhalation route)* data for several of TiO_2 NMs (7 publications)

References **1**:Lu S. et al. Environ. Health Perspect. 2009 Feb;117(2):241-7; **2**: Xu J et al. Carcinogenesis. 2010 May;31(5):927-35; **3**: Rushon et al. J Toxicol Environ Health A. 2010;73(5):445-61 **4a**: Han X et al. Toxicology. 2012 Jul 16;297 (1-3):1-; **4b**: Jiang J et al. Nanotoxicology. 2008 Mar;2(1):33-42. **5**: Park et al. Arch Toxicol. 2013 Jul;87(7):1219-30 ; **6**: Park et al. J Appl Toxicol. 2014 Apr;34(4):357-66; **7**: Numano et al. Asian Pac J Cancer Prev. 2014;15(2):929-35.

2. Identify comparable **endpoints** and derive **lowest effective concentration/doses**

in vitro: oxidative stress & inflammation ***in vivo***: Inflammation (PMN↑in BAL, cytokine ↑ in BAL, lung histopathology)

3. Apply dosimetry factors to account for differences in deposition between NMs:



4. Evaluate correlation between *in vitro* and *in vivo* equipotent concentration/doses.

RESULTS

CORRECTED EFFECTIVE DOSES/CONCENTRATIONS *IN VITRO* & *IN VIVO*

Ref.	Size (nm)*	*In vitro* Endpoint	Corrected EC (cm^2/mL)		*In vivo* Endpoint	Corrected LOAEL (cm^2/kg)	
1	35^R	Electron Parametric Ressonance (cell free)	>	3000	PMN number in BAL	>	796
		DCFH (cell free)	>	1500			
		LDH Release	>	52,6			
	5^A	Electron Parametric Ressonance	>	3000		>	255
		DCFH assay	>	1500			
		LDH Release	>	63,3			
2	20^R	Cell proliferation assay	>	5,66	Oxidative stress markers, inflammatory mediators and histopathology evaluation	=	2854
						=	3993
3	250^A	Electron Spin Ressonance (cell free)	>	800	Increase neutrophils & PMN concentration in BAL.	>	9
		Electron Spin Ressonance	>	80			
		Lucifer Reporter (ROS release assessment)	>	0,91			
	20^A	Electron Spin Ressonance (cell free)	>	8600		>	276
		Electron Spin Ressonance	>	860			
		Lucifer Reporter (ROS release assessment)	>	1,42			
	$25^{A/R}$	Electron Spin Ressonance (cell free)	>	5700		>	187
		Electron Spin Ressonance	>	570			
		Lucifer Reporter (ROS release assessment)	>	1,04			
4	30^A	Cell free ROS assay	≤	26,3	PMN number in BAL	=	428
	50^A			15,8			225
	7^A			104,8			447
	16^A			47,9			365
5	30^A	Cell ROS assay	=	7,02	Inflammatory cell infiltration (NK & T cells) and Cytokine	=	1309
	50^B		=	3,9		=	438
6	$30,5^R$	IL-8 expression	=	17,1	Inflammatory cell infiltration in BAL	>	488
		IL-1b expression	=	17,1			
		TNFa expression	=	51,3			
7	20^A	Expresion & level of MIP1α in PAM	=	1,54	Numer of macrophages, MIPα expresion & 8-OHdG levels in lung tissue	=	3720
	25^R		>	1,64		=	4553

EC: *In vitro* Effective Concentration
LOAEL: *In vivo* Lowest Observed Adverse Effect Level (Intratracheal studies in rat)
*** Crystalline form:** R: Rutile A: Anatase B: Brookite
PMN: Polymorphonuclear cells
BAL: Bronchoalveolar lavage

NO COLOR	**NEGATIVE RESULT** (No effects at highest concentration tested)
GREEN	**POSITIVE RESULT**

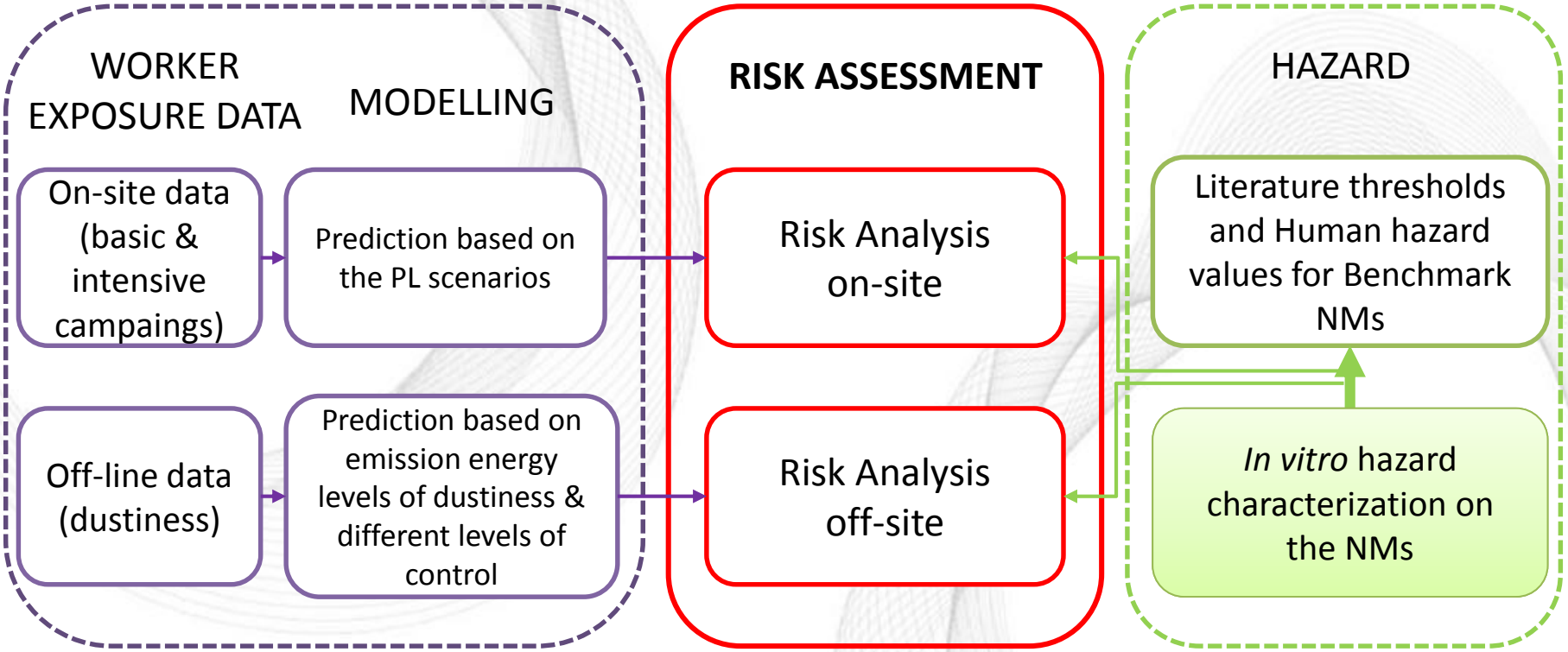
DRAWBACKS

- NO ADVERSE EFFECTS IN SEVERAL STUDIES
- DIFFERENT ENDPOINTS
- LIMITED INFORMATION FOR DOSIMETRY

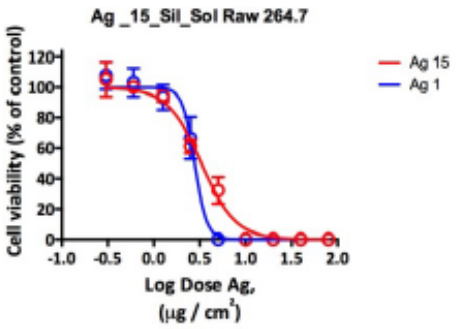
CONCLUSIONS

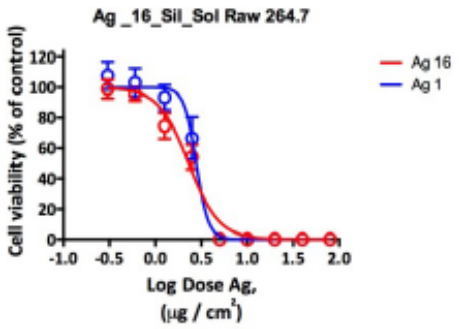
- **NO CORRELATION COULD BE DEMONSTRATED** BETWEEN *IN VITRO* AND *IN VIVO* EFFECTIVE CONCENTRACIONS/DOSES
- **FURTHER** STUDIES WIDER DOSES REACHING EFFECTIVE LEVELS COMPARABLE ENDPOINTS
- USE OF THE "SANOWORK APPROACH" WAS **DISCARDED**

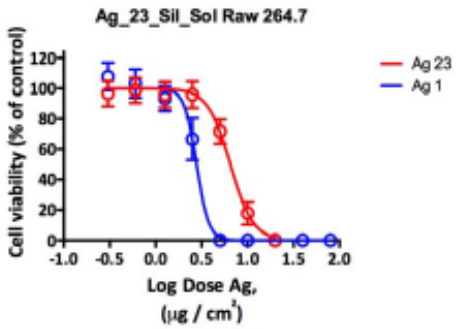
FINAL RISK ASSESSMENT STRATEGY

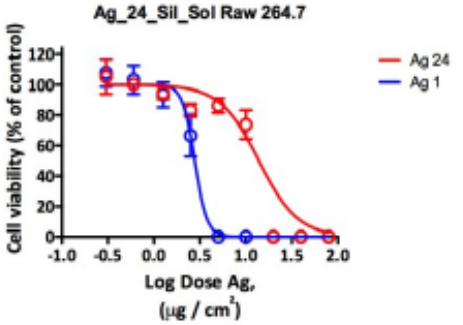


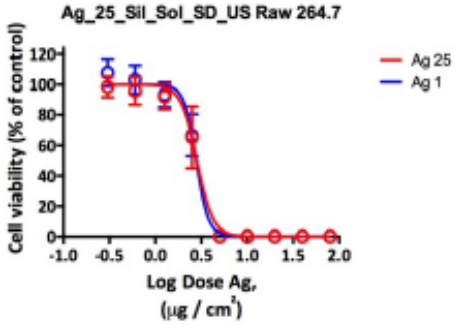
IN VITRO HAZARD CHARACTERIZATION

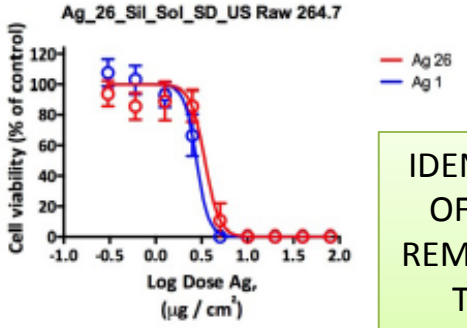


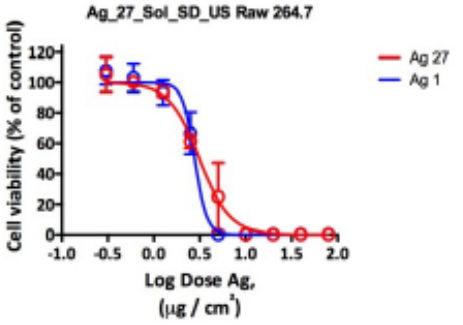


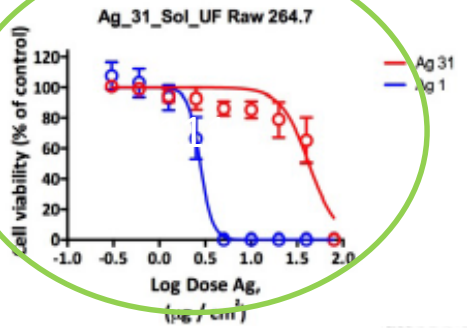


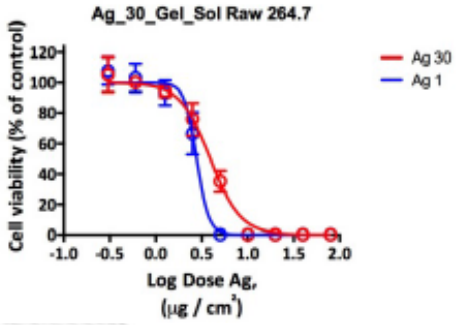












IDENTIFICATION OF EFFICIENT REMEDIATION IN TERMS OF HAZARD

In vitro hazard evidence supporting the use of Human hazard threshold values of Benchmark NM

Comparable toxicity profile among ZrO_2 materials and the benchmark material

When compared to the benchmark material (TiO_2 P25), the toxic effects observed for ZrO_2 NP at the same concentrations were in the same range in oxidative stress and inflammation assays.

In some cases even the effects were in a lower range of toxicity → conservative approach.

Human hazard threshold values used for ZrO_2 NMs

Material	Worker exposure limit	Agency proposing the threshold
[TiO_2 nanomaterial] Evonik Degussa P25 [pigment-grade TiO_2] Respirable TiO_2 Bayer AG Bayertitan T rutile-type	0,3 mg/m^3 (REL)	NIOSH (2011)
Evonik Degussa P25	0,017 mg/m^3 (DNEL)	ENRHES project (2009)
Evonik Degussa P25	0,6 mg/m^3 OEL (PL)	NEDO project (P06041; 2011)

CONSERVATIVE APPROACH

Material	Worker exposure limit	Agency proposing the threshold
Zirconium compounds (bulk)	5 mg/m^3 (TLV-TWA) + 10mg/m^3 (STEL)	ACGIH
Zirconium compounds (bulk ; zirconium tetrachloride excluded)	5 mg/m^3 (TWA- PEL)	NIOSH
Zirconium compounds (bulk; inhalable)	1 mg/m^3 (TWA)	DFG (German Research Foundation)
Metals, metal oxides and other biopersistent granular nanomaterials (1 to 100 nm; density > 6000 kg/m^3)	20.000 particles/cm^3	IFA
Non fibrous, non CMAR (carcinogenic, mutagenic, asthmagenic and reprotoxic) and insoluble nanomaterials.	20.000 particles/cm^3	BSI

RISK ASSESSMENT FOR ZrO_2
(Spraying exposure scenario)

EXPOSURE
(average worker exposure on a working day)

TWA (7.5 h) Near Field	918 (particles/cm^3) 0.00273 (mg/m^3)
TWA (7.5 h) Far Field	885 (particles/cm^3) 0.00263 (mg/m^3)

HAZARD
Worker exposure limits

Zirconium (bulk inhalable)	1 mg/m^3 (TWA)
Non fibrous, low toxicity insoluble NMs	20.000 part/cm^3
TiO_2 P25 (Benchmark)	0.017 mg/m^3 (DNEL)

Worker exposure scenario with unlikely health risk

CONCLUSIONS

- The *in vitro* toxicological characterization allowed to evaluate the efficiency of the Remediation Risk Strategies in terms of hazard.

- The similarity of the *in vitro* toxicological profile of the Benchmark materials and the project materials supported the use of already existing human reference values for the whole process of Occupational Risk Assessment.

- The risk assessment of the different NMs allowed the categorization of the Sanowork exposure scenarios into “Unlikely health risk” and “Possible health risk” groups.

Acknowledgments

THANKS FOR YOUR ATTENTION