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

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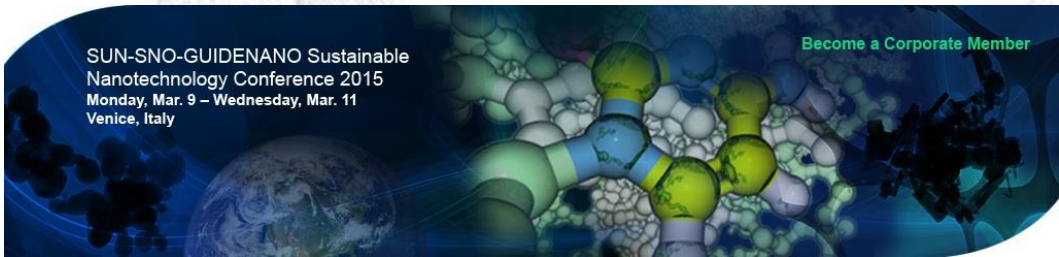


# Presentation Overview

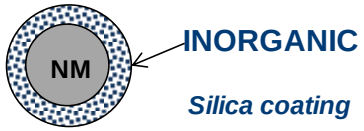
1. Introduction to the Sanowork Project
2. The “Sanowork Approach” on how to derive human threshold hazard values using *in vitro* toxicity data
3. Proof of Concept on correlation between *in vitro* and *in vivo* data
4. Risk Assessment Strategy
5. Example of *in vitro* toxicity assay evaluating hazard 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

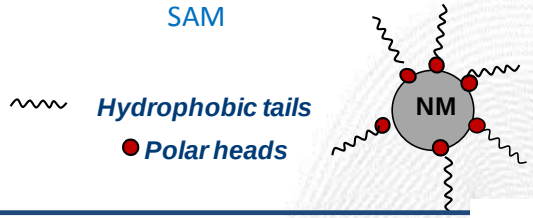
Inorganic coating with inert materials by heterocoagulation or chemical synthesis




***HEALTH HAZARD ADDRESSED***

### Organic coating

with organic antioxidant molecules by SAM



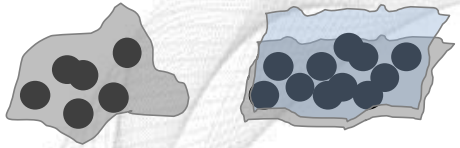

### 2 Micronization

Keeping nanoscale reactive structures in a microstructure

*Spray granulation* *Forced aggregation*

*«NANO IN MICRO» **EXPOSURE** ADDRESSED*

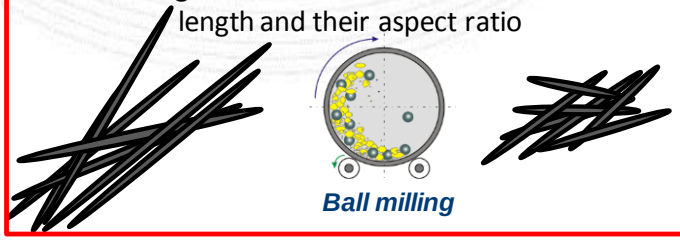
### Immobilization 3



*Embedding* *Gelation*

### 4 Size control

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

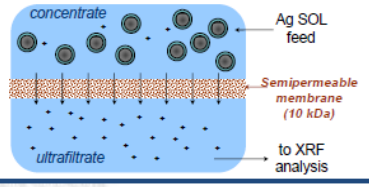


*Ball milling*

### 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

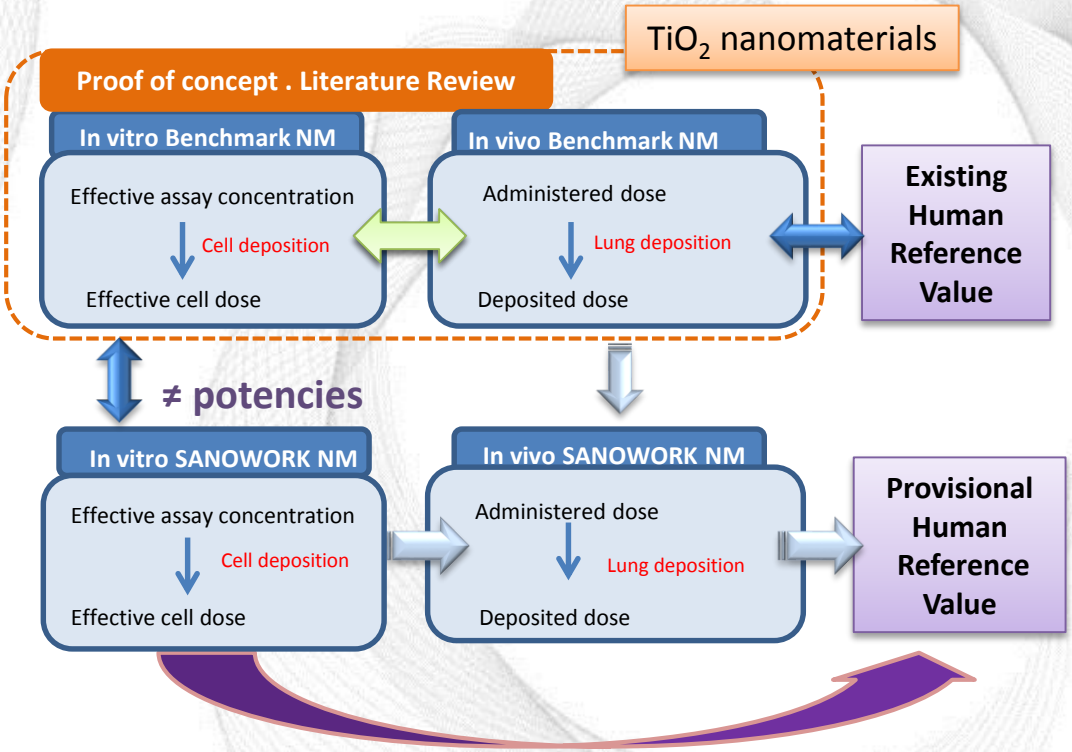
1. Grouping of NMs expected to share mechanisms of toxicity

| 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:

***In vitro* $TiO_2$**

***In vitro* data (cellular assays)**

- Oxidative stress
- Inflammatory mediators

DOSIMETRY
Assay Concentration → cell dose

**Step 1** : [mg/mL] →[$cm^2$/mL]
**Step 2** : Cellular assays → Deposited fraction (DP)
**Step 3**: Effective Concentration [$cm^2$/mL] x DP =
**Corrected EC by Deposited Fraction**

**ISDD model**
In vitro Sedimentation , Difussion and Dosimetry Model

Corrected Effective concentration (EC)

?

Corrected LOAEL

***In vivo* $TiO_2$**

***In vivo* data (intratracheal studies in rat)**

- Cell infiltration
- Oxidative stress
- Inflammatory mediators

LUNG DEPOSITION
Administered dose → local concentration

**Step 1** : [mg/mL; µg/rat] →[$cm^2$/rat] → [$cm^2$/kg]
**Step 2** : Alveolar deposition fraction (DF)
**Step 3**: LOAEL [$cm^2$/kg] x DF =
**Corrected LOAEL by Deposited Fraction in alveolar region**

MPPD

**Multiple Path Particle Dosimetry Model**

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** |

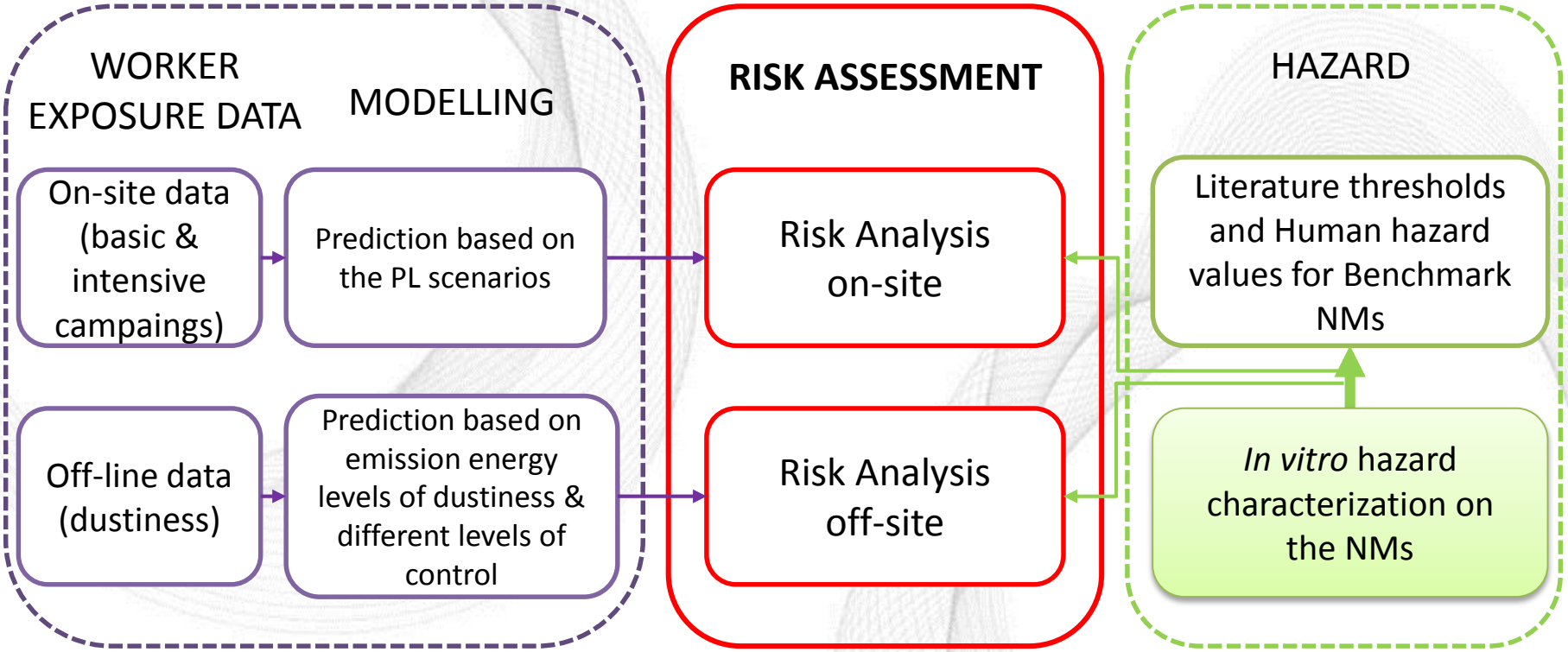
## DRAWBACKS

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

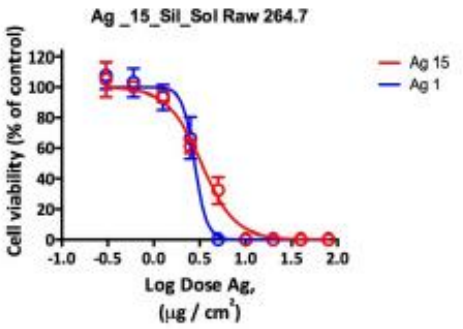
## CONCLUSIONS

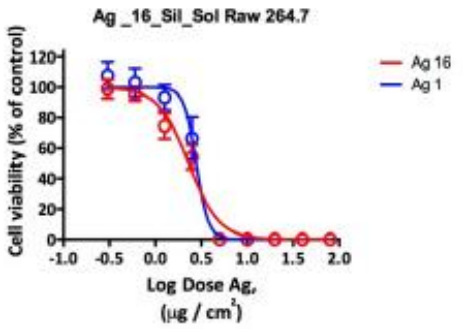
- **NO CORRELATION COULD BE DEMONSTRATED** BETWEEN *IN VITRO* AND *IN VIVO* EFFECTIVE CONCENTRACIONS/DOSES
- **FURTHER** STUDIES WIDER DOSES REACHING EFFECTIVE LEVELS COMPARABLE ENDPOINTS
- USE OF THE "SANOWORK APPROACH" WAS **DISCARDED**

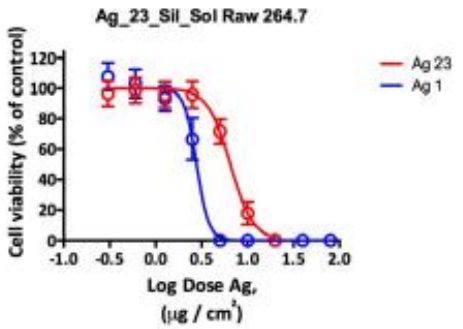
# FINAL RISK ASSESSMENT STRATEGY

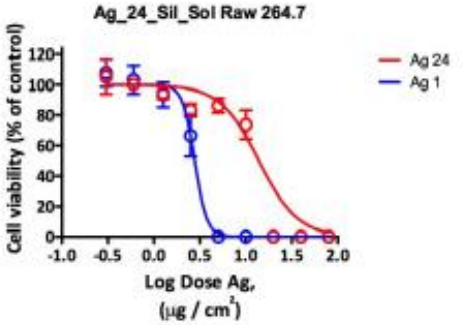


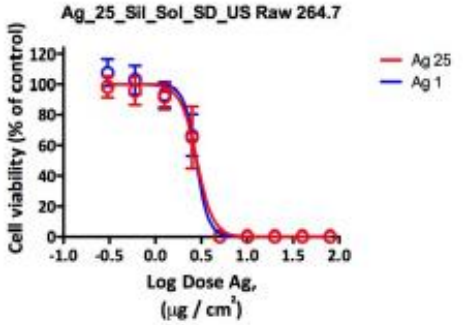
# IN VITRO HAZARD CHARACTERIZATION

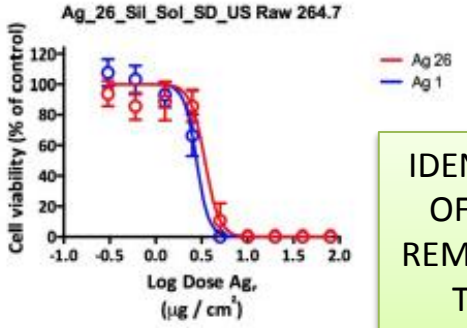


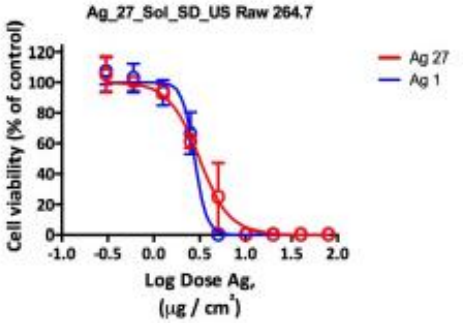



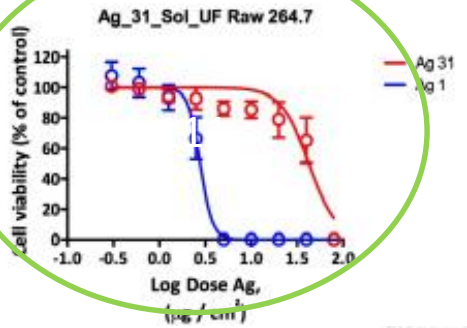



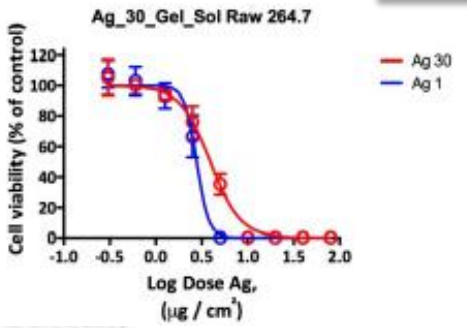




IDENTIFICATION OF EFFICIENT REMEDIATION IN TERMS OF HAZARD

# *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