CEFIC-LRI N1 Project: INHALATION TOXICITY OF A SYNTHETIC AMORPHOUS SILICA (SAS) IN RATS

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Background

The translocation of nanoparticles to remote organs upon inhalative uptake in lungs is often discussed as an additional, nanospecific toxic potential. However, in inhalation tests with occupational exposure scenarios regularly agglomerates of nanostructured particles are deposited in lungs. There exists an ongoing debate whether considerable amounts of individual nanoparticles could be formed by disintegration.

Objectives

- To investigate the toxic potential of a precipitated synthetic amorphous silica (NM-200) upon inhalation
- To conduct a 14-day (+ 14-day recovery) and a 90-day inhalation test (+ 90-day recovery)
- To expand the endpoint pattern requested by OECD guidelines 412 and 413
  - by proper characterisation of the dissolution behaviour
  - by a toxicokinetic analysis (chemical analysis; TEM analysis)

Methods

- Animals: Wistar rats [strain: Crl: WI(Han)], approx. 9 weeks old at study start
- Test item: NM-200, a precipitated synthetic amorphous silica used in the food sector and delivered by the EU JRC repository of nanomaterials
- Properties
  Nanostructured → nanoscaled primary particles; sintered to aggregates in the µm range
  Water solubility → approx. 5% (in water up to 14 days at 5 g/L; RPMI; DMEM)
  Size distribution → Strong aggregates; bad dispersability; DLS: $x_{50,3} \approx 10$ µm
  Raw density (gas pycnometry) → 2.19 g/cm³
  Specific surface → 199 m²/g
  Porosity → Adsorption average pore width (4V/A by BET): 17 nm
  Skeletal and pour density → Bulk: 0.12 g/cm³; tap: 0.16 g/cm³
  Thermogravimetry → 110 °C – 7%; 800 °C – 12% (water only)
- Aerosol generation: Dry dispersion technique; pressurised air
  MMAD: approx. 2.4 µm (SEM)

Results

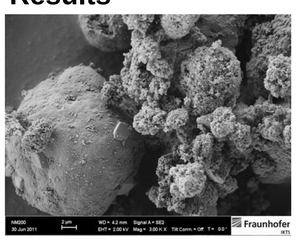


Figure 1: NM-200 bulk - 3K magnification

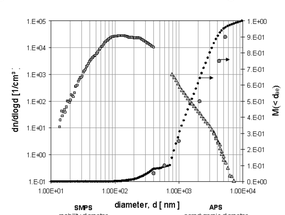
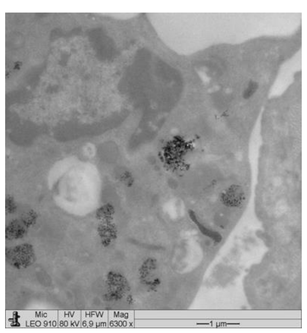
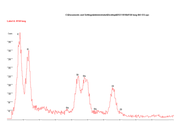


Figure 2: Number size in aerosol





EDX-Analysis: Si

Figure 3: High dose; day 91 of recovery → SiO_2 particles in lung intraalveolar macrophages

Results

14-Day Test

Table 1 14-Day Test: Statistically Significant Effects/NOAEL - LOAEL

Endpoint	NM-200 Low 1 mg/m³	NM-200 Mid 5 mg/m³	NM-200 High 25 mg/m³
Body weights	-	-	-
Food consumption	-	-	-
Organ weights Lungs Day 1	-	- ↑	- ↑
Haematology, clinical chemistry	-	-	-
BAL PMN Day 1	-	↑	↑
BAL Lymph. Day 1	-	↑	↑
BAL LDH Day 1	-	-	↑
BAL β-Glu Day 1	-	↑	↑
BAL Protein Day 1	-	-	↑
Histopathology nose (day 1): Mucous cell hyperplasia; epithelial inflammatory cell infiltration	-	↑	↑
Histopathology lungs (day 1): Granulocyte/mononuclear cell infiltration	-	-	↑
OECD Guideline 412	NOAEL		
Toxicokinetics	Chemical analysis: Clearance half-time = approx. 10 days		
TEM	TEM: NM-200 particles detectable in cytoplasm of alveolar macrophages up to 14 days of recovery (no quantification done)		
Recovery after 14 days		yes	yes

90-Day Test

Table 2 90-Day Test: Statistically Significant Effects/NOAEL - LOAEL

Endpoint	NM-200 Low 1 mg/m³	NM-200 Mid 2.5 mg/m³	NM-200 High 5 mg/m³
Body weights	-	-	-
Food consumption	-	-	-
Organ weights Lungs Day 1	-	- ↑	- ↑
Haematology, clinical chemistry	-	-	-
BAL PMN Day 1	-	↑	↑
BAL Lymph. Day 1	-	↑	↑
BAL LDH Day 1	-	↑	↑
BAL β-Glu Day 1	-	↑	↑
BAL Protein Day 1	-	↑	↑
Histopathology nasal cavities (day 1): Mucous cell hyperplasia; epithelial inflammatory cell infiltration	↑	↑	↑
Histopathology lungs (day 1): Bronchiolo-alveolar hyperplasia	↑	↑	↑
Histopathology lungs (day 1): Granulocyte/mononuclear cell infiltration	-	↑	↑
OECD Guideline 413	LOAEL		
Cell proliferation	-	-	-
Toxicokinetics (non-GLP)	Chemical analysis: Clearance half-time = approx. 30 days		
TEM (non-GLP)	TEM: NM-200 particles detectable in cytoplasm of alveolar macrophages up to 90 days of recovery (no quantification done)		
OECD 413 + additional parameters	LOAEL		

Results

Table 3 90-day test: Retention of NM-200 in lungs (µg/lung)

Retention µg/lung	90 + 1 day	90 + 30 days	90 + 90 days	$t_{1/2}$ (days)
NM-200 low	91	35	12	**32**
NM-200 mid	172	79	21	**31**
NM-200 high	307	150	34	**28**
Controls: <5µg/lung				

True density excluding voids: 2.19 g/cm³ Bulk density: 0.12 g/cm³
Tap density: 0.16 g/cm³ Agglomerate density: approx. 0.5 - 1 g/cm³

No overload in the high dose group at day 1

Conclusions

14-Day Test

→ **NOAEL: 1 mg/m³**

90-Day Test

→ **Evident dissolution in lungs, in addition to the physiological clearance: $t_{1/2}$ = approx. 30 days in 90-day test**

→ **Si analysis: Chemically detectable only in lungs – Day 1, 29 and 91 post-exposure**

→ **By TEM, SiO2 particles detectable in lungs /LALN up to 91 days post-exposure - not detectable in remote organs**

→ **LOAEL = 1 mg/m³** (mucous cell hyperplasia in nasal cavities)
NOAEL < 1 mg/m³; BMCL = 0.6 mg/m³ (benchmark approach; PMN)

Overall: Translocation of NM-200 beyond target organ not detectable

Acknowledgement

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Fraunhofer ITEM