CEFIC-LRI N1 Project: Final Results and Overall Conclusions on Nanomaterial Safety of ZnO and SiO_2

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OBJECTIVES

- To provide information whether existing OECD guidelines can characterise the toxicity of nanostructured materials adequately
- To analyse candidates for an expanded endpoint pattern to be included in test guidelines
- To use risk-related exposure scenarios → Dry dispersion/agglomerates

MATERIALS AND METHODS

Zinc Oxide

- **Z-COTE® HP1** **(nano-ZnO; coated with triethoxycaprylylsilane; BASF)** → **Cosmetics sector**
- **Z-COTE®** **(nano-ZnO; uncoated; BASF)**
- **ZnO 205532** **(µ-ZnO; Sigma-Aldrich)**

Table 1 Test Programme for Zinc Oxide

Part of study	Exposure path	Zinc Oxide
n vitro work		Biocompatible formulation of test and reference items – Genotoxicity tests
		Dermal corrosion test in human skin model
		Dermal penetration test ^{65}ZnO (skipped)
		Chromosomal aberration
		Mouse lymphoma assay
in vivo work	Inhalative	14-day nose-only test + 14-day rec herein: MN test in vivo; Comet assay
		90-day nose-only test + 28-day rec
	Dermal	Acute toxicity test
		Dermal penetration ^{65}ZnO

Synthetic Amorphous Silica (SAS)

NM-200 **(nanostructured, precipitated SAS; JRC Ispra material)** → **Food sector**

Table 2 Test Programme for Amorphous SiO_2

Part of study	Exposure path	Synthetic Amorphous Silica – NM-200
in vitro work		Biocompatible formulation of test and reference items – Genotoxicity tests
		Chromosomal aberration
		Mouse lymphoma assay
		Comet assay
in vivo work	Inhalative	14-day nose-only test + 14-day rec herein: MN test in vivo herein: Comet assay
		90-day nose-only test + 30-day/ + 90-day rec
	Oral	28-day test

SEM Photographs

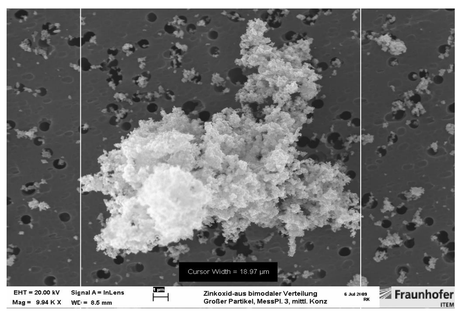


Figure 1 Zinc oxide Z-COTE® HP1

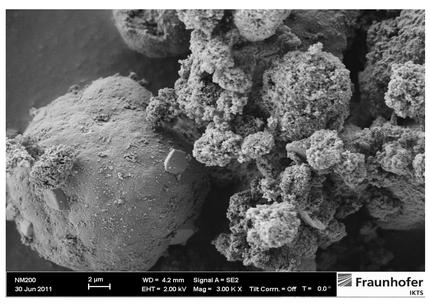


Figure 2 SAS NM-200

Table 3 ZnO Solubility in Physiological Fluids

test item	Matrix	pH	Solubility (%)
blank	Gambles S	4.5	< 0,01
		7.4	< 0,01
	Artificial LF	4.5	< 0,01
	Artificial AF	7.4	< 0,01
Z-COTE HP1	Gambles S	4.5	< 20
		7.4	< 0,05
	Artificial LF	4.5	> 90
	Artificial AF	7.4	< 0,05
Z-COTE	Gambles S	4.5	< 10
		7.4	< 0,05
	Artificial LF	4.5	> 90
	Artificial AF	7.4	< 0,05
Micro-scaled ZnO	Gambles S	4.5	< 20
		7.4	< 0,05
	Artificial LF	4.5	> 90
	Artificial AF	7.4	< 0,05

RESULTS

ZnO – DERMAL ABSORPTION

Table 4 *In vivo* Absorption - Experimental design according to OECD 427
^{65}Zn label by neutron activation

Not absorbed fraction (%) Swabs, O-rings, gauze and plasters	Skin total (%) Tesa strippings, skin at application site	Absorbed fraction %) Urine/feces; carcass	Reco-very 100 (%)
74.4	25.5	0.01	100.0

CONCLUSIONS

Nanostructured ZnO

INHALATIVE

- **14-day study: No systemic but local acute effects on olfactory epithelium and in lungs in high dose groups**
  → LOAEL: 8 mg/m³ - NOAEL: 2 mg/m³
- **90-day study:**
  → LOAEL: 4.5 mg/m³ - NOAEL: 1.5 mg/m³
- **Adverse effects in high doses only → reversible**
- **Effects independent of ZnO particle size**

DERMAL

- **Z-COTE® HP1 was not absorbed *in vivo***
- **Acute test → no effects**

GENOTOXICITY

in vitro/in vivo **→ negative**

ZnO – 90-Day Inhalation Study

Table 5 Summary of Effects after 90 Days of Exposure

Summary 90-Day Inhalation with ZnO Endpoint	Z-COTE® HP1 Low 0.3 mg/m³	Z-COTE® HP1 Mid 1.5 mg/m³	Z-COTE® HP1 High 4.5 mg/m³	Microscaled ZnO 4.5 mg/m³
Body weights	-	-	-	-
Food consumption	-	-	-	-
Organ weights Lungs	-	-	-	↑
Haematology, clinical chemistry	-	-	-	-
BAL PMN Day 1	-	-	-	↑
BAL Lymph. Day 1	-	-	-	↑
BAL LDH Day 1	-	-	↑	↑
BAL Protein Day 1	-	-	-	↑
Histopathology nasal cavities	-	-	-	↑
Histopathology lungs: bronch.-alveolar hyperplasia	-	-	↑	↑
Histopathology lungs: mononuclear cell infiltration	-	-	↑	↑
Cell proliferation	n.d.	n.d.	↓	↓
Toxicokinetics	Practically complete dissolution of the retained test item; no translocation			
TEM	No distinct ZnO particles detectable at any time-point			
		NOAEL	**LOAEL**	

↑ **statistically significant increase;** ↓ **decrease;** **- no statistically significant change as compared to concurrent controls**

Nanostructured SiO_2

INHALATIVE

- **14-day study: No systemic but local acute effects in nose/lungs → rapid reversibility**
  → LOAEL: 5 mg/m³ - NOAEL: 1 mg/m³
- **90-day study: Adverse effects restricted to high dose → reversible**
  → LOAEL: 1 mg/m³; BMCL: 0.6 mg/m³

ORAL

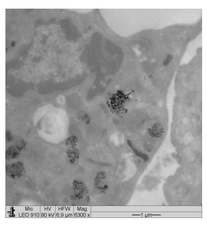
- **No adverse effects detected *in vivo* *(28-day repeated toxicity test)***

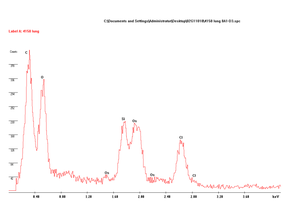
GENOTOXICITY

in vitro/in vivo **→ negative**

TOXICOKINETICS

- **Si chemical analysis: $t_{1/2}$ = approx. 30 days remote organs (TEM)**
- **SiO_2 detectable in intraalveolar MΦ**





SiO_2 – 90-Day Inhalation Study

Table 6 Summary of Effects after 90 Days of Exposure

Summary 90-Day Inhalation with Amorphous SiO_2 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	-	-	-
Haematology, clinical chemistry	-	-	-
BAL PMN Day 1	↑	↑	↑
BAL Lymph. Day 1	-	-	-
BAL LDH/β-Glu/Protein Day 1	-	↑	↑
Histopathology nasal cavities (day 9): Mucous cell hyperplasia	↑	↑	↑
Histopathology lungs (day 9): bronchiolo-alveolar hyperplasia	-	-	↑
Histopathology lungs (day 9): mononuclear cell infiltration	-	↑	↑
Cell proliferation	-	-	-
Toxicokinetics/TEM	Chemical analysis: Mean clearance half-time: 11.8 days TEM: NM-200 particles detectable in cytoplasm of alveolar macrophages up to 90 days of recovery (no quantification)		
	LOAEL		

↑ **statistically significant increase;** ↓ **decrease;** **- no statistically significant change as compared to concurrent controls**

Recommendations for nano-specific additional endpoints

In vitro

- **To generate sufficient phys.-chem. data for a test item**
- **To make high efforts to achieve an optimal formulation of the test items**

In vivo

- **Analysis of solubility: In (mimicked) physiological fluids at various pH**
- **Toxicokinetics: a. Chemical analysis b. TEM**
- **Immunohistochemistry (8-OH-dG) → oxidative damage on epithelial cells**
- **Genotoxicity tests (micronucleus, mouse lymphoma assay)**

In vitro/in vivo **tests complement each other, i.e concept of N1 was confirmed**

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