CEFIC – LRI N1 Project :

Approach on Nanomaterial Safety of ZnO and SiO_2

Final Results and Overall Conclusions

Dr. Otto Creutzenberg

Fraunhofer Institute for Toxicology and Experimental Medicine, Hannover, Germany

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CEFIC-funded Project

Nanoscaled ZnO and Amorphous SiO_2

Project Period M1-M24 (June 2009 – October 2012)

Sponsor
CEFIC
The European Chemical Industry Council

Sponsor's Study Monitors
Dr. Karin Wiench, BASF (ZnO)
Dr. Monika Maier, Evonik Industries (SiO_2)

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Test/Reference Items - Exposure

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

- **Amorphous SiO_2** (nano-SiO_2; JRC Ispra; precipitated material/Rhodia) → **Food sector**

- **Risk-related exposure scenarios** → **Dry dispersion/agglomerates**

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N1: Tiered Approach to Testing and Assessment of Nanomaterial Safety to Human Health

ZnO

Part of study	Exposure path	Task to be done within the CEFIC-Fraunhofer N1 project
in 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 herein: Comet assay
		90-day nose-only test + 28-day rec
	Dermal	Acute toxicity test
		Dermal penetration ^{65}ZnO

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Solubility of Test Items in Various Media

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
Microscaled ZnO	Gambles S	4.5	< 20
		7.4	< 0,05
	Artificial LF	4.5	> 90
	Artificial AF	7.4	< 0,05

INHALATIVE EXPOSURE PATH

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14-Day Nose-only Inhalation Test + 14 days rec

→ **Aerosol concentrations:**

Z-COTE® HP1	**0 – 0.5 – 2.0 –**	**8 mg/m³**
Z-COTE®		**8 mg/m3**
Microscaled ZnO		**8 mg/m³**

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14-Day Study: Summary ZnO

Bronchoalveolar Lavage

Significant acute effects were detected in the high dose groups of Z-COTE® HP1, Z-COTE® and microscaled ZnO 1 day after exposure

Histopathology

No systemic effects detected
Local effects in the olfactory epithelium at day 1

Reversibility within 14-day post-exposure period

14-Day Study: Summary ZnO

Toxicokinetics

Zn chemical analysis: Detectable only at day 1 post-exposure

ZnO particles not detectable (TEM)

Genotoxicity

In vivo: Negative

In vitro: Negative

90-Day Nose-only Inhalation Test + 28-day rec

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
↓ statistically significant decrease
- no statistically significant change as compared to concurrent controls

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DERMAL EXPOSURE PATH

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Dermal Toxicity/Absorption

In vivo/in vitro

Acute Dermal toxicity test (OECD 402) → Not classified

Skin corrosion test according to OECD 431 → Not corrosive

In vivo: AbsorptionExperimental design according to OECD 427

^{65}Zn label by neutron activation

Not absorbed fraction (%) Swabs, O-rings, spacers, gauze and plasters	**Skin total (%)** Tesa strippings, skin at application size	**Absorbed fraction (%)** Urine/feces; carcass	**Recovery (%)**
70.3	24.1	0.01	94.5

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Conclusions

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 restricted to high doses → 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

Amorphous SiO_2

Part of study	Exposure path	Task to be done within the CEFIC-Fraunhofer N1 project
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

INHALATIVE EXPOSURE PATH

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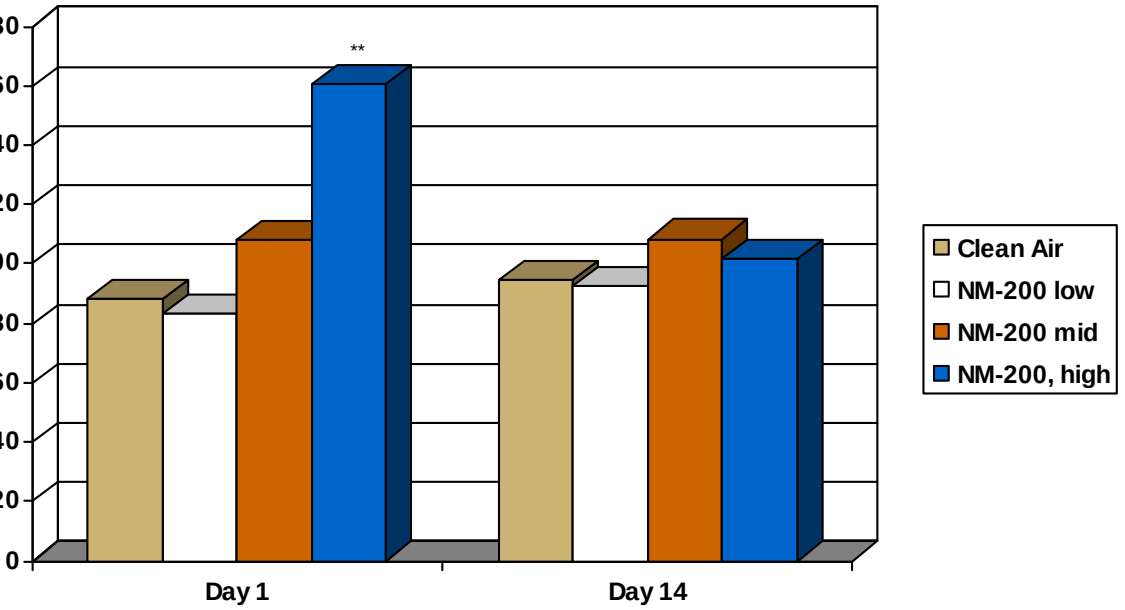
14-Day Nose-only Inhalation Test + 14 days rec

→ **Aerosol concentrations:**

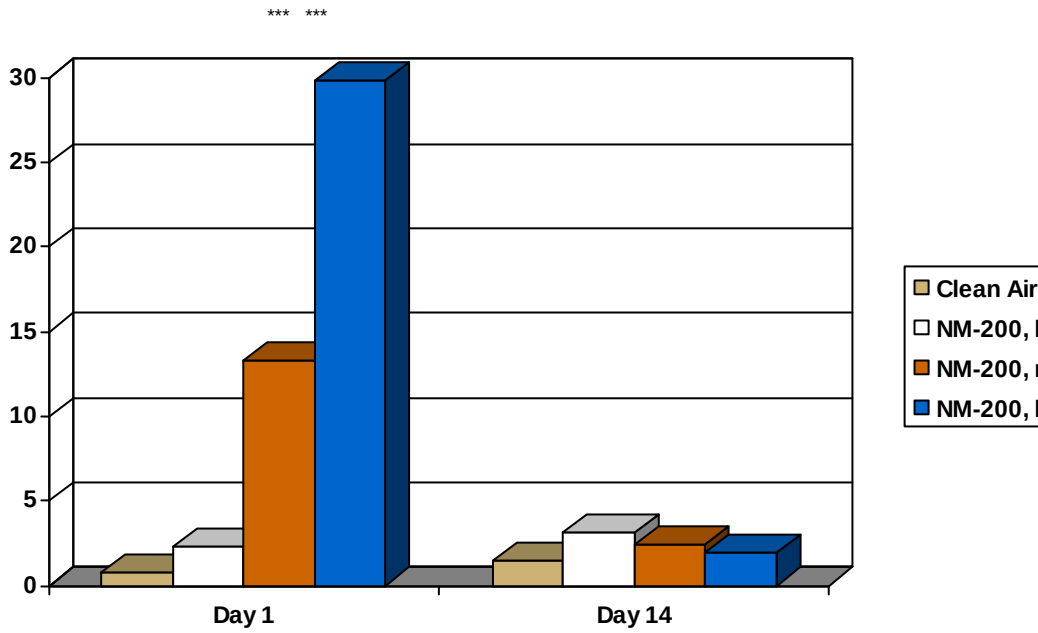
Amorphous silica/NM-200 0 – 1 – 5 – 25 mg/m^3

14-Day Study: Bronchoalveolar Lavage

Total Protein (mg/ml)



PMN (%)



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14-Day Study: Summary SiO_2

Bronchoalveolar Lavage

Significant acute effects were detected in the mid and high dose groups of NM-200 1 day after exposure

Histopathology

No systemic effects detected
Mucous cell hyperplasia/epithelial eosinophilic droplets in the nasal cavity / granulocyte infiltration in lungs at day 1 /high dose

Reversibility within 14-day post-exposure period

14-Day Study: Summary SiO_2

Toxicokinetics

Si chemical analysis: Detectable only at day 1 post-exposure

SiO_2 particles detectable in lungs/LALN up to 14 day post exposure

– not detectable in remote organs (TEM)

Genotoxicity

In vivo: Negative

In vitro: Negative

90-Day Nose-only Inhalation Test + 30/90-day rec

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)**		
	NOAEL	**LOAEL**	

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

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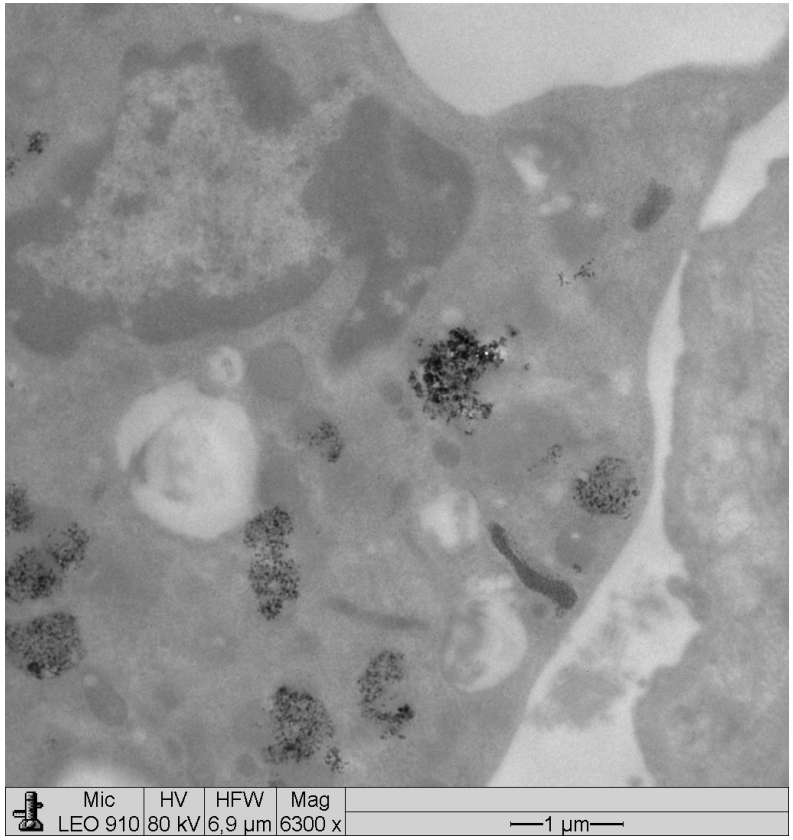
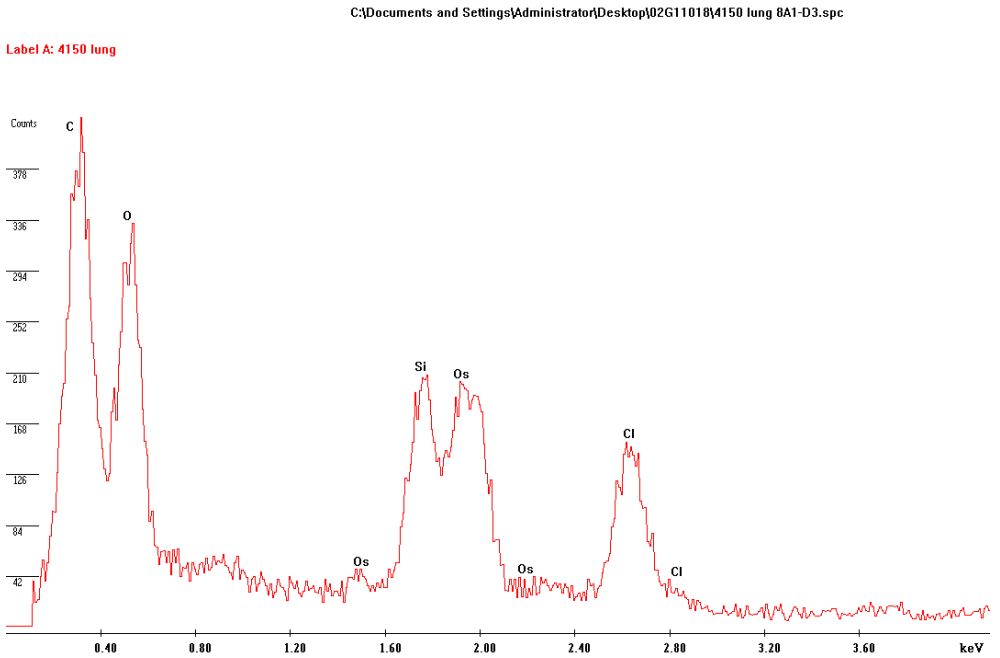
90-Day Study: TEM Analysis SiO_2

Animal 4150; high dose; day 91 of recovery





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Conclusions

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: 2.5 mg/m³ - NOAEL: 1 mg/m³**

ORAL

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

GENOTOXICITY *in vitro/in vivo* → negative

Main Recommendations for Expanded Endpoint Pattern

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**

Main Recommendations for Expanded Endpoint Pattern

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 - Other genotoxicity tests**
- **Cytokines and ROI ? Value equivocal**

Main Recommendations for Expanded Endpoint Pattern

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