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

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OBJECTIVES

- To test the suitability of existing OECD testing guidelines for nanoscaled ZnO.
- To supplement OECD guidelines with modifications addressing nanoparticle-specific features of toxicity testing
- To include promising *in vitro* correlates in the test programme
- To evaluate the potential need for modification of standard test methods
- To contribute substantially to a reliable database for defining suitable test strategies
  - For nanomaterial hazard assessment
  - For nanomaterial toxicity screening and bridging effects to fine particle references

INTRODUCTION

OECD's Working Party on Manufactured Nanomaterials (WPMN) is currently developing a test programme that can provide crucial information relevant for hazard assessment of nanomaterials. A set of toxicological endpoints has been agreed on. This project is aiming at contributing to and refining the outcome of this OECD programme. The main objective is to work out the potential needs for a modified nanoparticle test approach as compared to the traditional one used for the corresponding fine fraction dusts. Thus, the first step is to generate a dataset based on the standard test programme for fine fraction dusts. In addition, endpoints such as toxicokinetics [transmission electron microscope (TEM), chemical analysis] and genotoxicity assays will expand the toxicological pattern with regard to nanomaterials.

METHODS

Test item and reference items: Test item was a coated nano-ZnO: Z-COTE® HP1. Two reference items were included in the 14-day test, an uncoated nano-ZnO: Z-COTE® and a micro-ZnO (see Table 1).
Animals and inhalation study design: Male Wistar (WU) rats, approx. 8 weeks of age and 250 g of weight were exposed by nose-only inhalation (6 hrs/day) to clean air and the test/reference items. A 5-day dose range finding (DRF) study was followed by a 14-day and a 90-day study (see **Table 1**).
Aerosol generation: For exposure the ZnO powders were dispersed by a dry aerosol dispersion technique using pressurised air. The aerosolisation resulted in respirable aerodynamic particle diameters (mass median aerodynamic diameter – MMAD < 3 µm).
Micronucleus test: This *in vivo* genotoxicity assay was integrated into the 14-day test and was conducted according to OECD guideline 474 (including both sexes).
Additional nanospecific endpoints:
Bronchoalveolar lavage (BAL): Lungs were lavaged with 2 x 5 ml saline without massage. Lavage fluid was analysed for cytologic and biochemical/immunological endpoints.
Cell proliferation analysis was done after preceding administration of bromodeoxyuridine (BrdU) by osmotic minipumps (90-day test only).
For **TEM analysis** of lungs and selected remote organs the rats were sacrificed using a whole-body fixation technique.
Toxikokinetics was done on tissues and excretions using inorganic zinc analysis.

Table 1 Nanoscaled Test Item and Microscaled Reference Items (14-day study)

Zinc Oxide Materials Supplier	Properties	Aerosol Target Concen-trations (mg/m³)	Solubility (%) in: Artificial alveolar (pH 7.4) and lysosomal fluid (pH 4.5)	
Z-Cote® HP1 BASF	ZnO, content w/w: 98%, coated on its surface with triethoxycaprylylsilan	0.5 2 8	pH 7.4 pH 4.5	< 0.05% > 90%
Z-COTE® BASF	no coating of surface	8	pH 7.4 pH 4.5	< 0.05% > 90%
Zinc Oxide 205532, Micron Sized Powder Aldrich	no coating of surface	8	pH 7.4 pH 4.5	< 0.05% > 90%

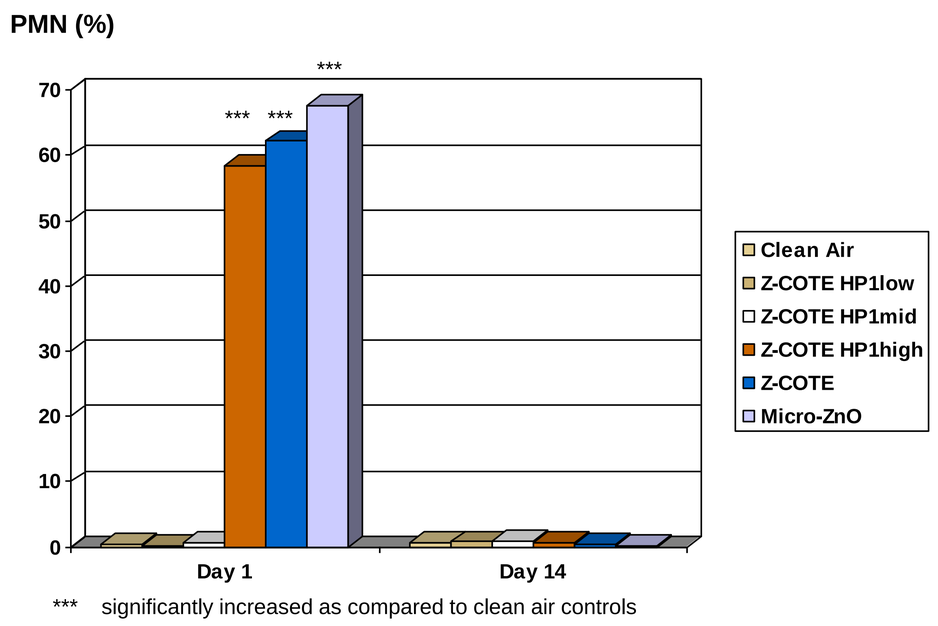
Table 2 Test Programme

Study Type	OECD Guideline	Task to be done within the CEFIC-Fraunhofer N1 project	Status/Result
in vitro work		Biocompatible formulation of test and reference items – Genotoxicity tests	Established (soy-lecithin/PBS; pH 7.4)
	431	Dermal corrosion test in human skin model	Non-corrosive
	428	Dermal penetration test	^{65}ZnO prepared; to be conducted by external CRO
	473 476	Chromosomal aberration test Mouse lymphoma assay	Negative Under evaluation
in vivo work Inhalative	412 412 + **BAL, ToxKin, REM, MN test 474**	5-day nose-only study (DRF) 14-day nose-only test herein: Micronucleus test *in vivo*	Completed Negative
	413 **+ BAL, ToxKin, REM**	90-day nose-only test	Ongoing
Dermal	402	Acute toxicity test	LD_{50} > 2000 mg/kg
	427	Dermal penetration	^{65}ZnO prepared; test to be started in Nov. 2010

RESULTS

Aerosol generation and characterization (14-day study)
- Mean target concentrations: 0.49 - 1.95 – 8.27 (Z-COTE® HP1), 8.02 (Z-COTE®) and 8.04 mg/m³ (micro-ZnO)
- The MMADs were approx. 0.7 µm in the Z-COTE® HP1, 1.8 µm in the Z-COTE® and 2.3 µm in the micro-ZnO groups.

Toxicity
- No systemic toxicity observed
- No significant changes observed in: Body weights and food consumption, clinical chemistry & hematology, urinalysis
- BAL fluid enzyme levels and differential cell count (PMN -%)
  - Significant increases in the high dose groups of all 3 ZnO varieties at 1 day after exposure
  - All these effects were reversible within 14 days post-exposure (**Figure 1**)
- Histopathology: Nasal and paranasal cavities in 8 mg/m³ Z-COTE® HP1group: (multi)focal very slight to slight degeneration of the olfactory epithelium → the only adverse effect observed in the present study
- Immunohistochemical detection of 8-OH-dG in lung tissue (oxidative damage) → Slight increase (statistically non significant) in micro-ZnO group on day 1post-exposure



*** significantly increased as compared to clean air controls

Figure 1 PMN levels determined at day 1 and day 14 after end of the 14-day exposure period

CONCLUSIONS

- **Particle-specific effects observed 1 day after end of exposure in lungs; rapid recovery of those effects within 14 days post-exposure**
- **All 3 particle types showed high solubility at lower pH (*in vitro* data: > 90 %)**
- **Translocation of particles in lungs and remote organs/tissues was not observed due to rapid dissolution of test item**
- **Toxicokinetics: Only one relevant significant increase was observed on day 1 post-exposure → in lungs**
- **Derivation of aerosol concentrations for 90-day test: 0, 0.3 – 1.5 – 4.5 mg/m³ for Z-COTE® HP1**

Overall conclusion

Nanosized particles do not show necessarily a stronger response in lungs than microsized particles of the same material.

Expansion of endpoint pattern may not be useful in case of high soluble nanoparticles

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