Pulmonary effects and biokinetics of nanoparticles: Whole-body inhalation exposure to CeO_2 in 5-day, 28-day and 90-day rat studies

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Introduction

Cerium dioxide (CeO_2) nanoparticles are widely used for industrial applications. To assess toxicity and biokinetics of air-born nanoparticles, CeO_2 was tested in short-term inhalation studies (**5-day** [short-term, STIS] and **28-day** studies (OECD TG 412)) performed within the EU-project NanoMILE . In addition, a combined chronic and carcinogenicity long-term inhalation study (OECD TG 453) in rats is currently being performed, within the framework of the EU-project NanoREG. The cerium dioxide nanomaterial is coded as NM-212 in the list of OECD WPMN Sponsorship Programme for the Testing of Manufactered Nanomaterials and the NanoREG core selection of nanomaterials. Based on the results of the short-term studies, the long-term study was started at concentrations of 0.1 mg/m³, 0.3 mg/m³, 1 mg/m³ and 3 mg/m³ CeO_2. Interim results after 90 days of the long-term study are available. The results after 5-, 28- and 90-day of exposure are presented here.

Methods

Female Wistar rats [Crl:WI(Han)] were whole-body exposed to **0.5, 5 and 25 mg/m³ CeO_2 (NM-212)** for 6 hours per day, 5 days per week for 1 or 4 weeks in the 5-and 28-day studies, respectively. The **bronchoalveolar lavage fluid (BALF)** was examined and **histopathology of the respiratory tract** was performed**.** The **lung retention and clearance kinetics** up to a post-exposure period of 129 days in the 28-day study were analysed by ICP-MS.

Study Design

GLP, according to OECD TG 412

5-day study

Study day	1	2	3	4	5	6-7	8	9-25	26	27-28	29
Study Phase	X	X	X	X	X	R	R	R	R	R	R
Examination					OB H		L		OB H		L

28-day study

Study day	1-28	29	30	31	32-36	37	38-61	62	63	64-92	93	94-156	157
Study Phase	X	R	R	R	R	R	R	R	R	R	R	R	R
Examination		OB L	H OB	OB		OB		H	OB L		OB		OB

90-day interim examination

Study day	1-94	
Study phase	X	
Examination	OB	L

X: Whole body exposure to aerosol for 6 hours per day, 5 days per week
R: Post-exposure period (24 days or 129 days)
OB: **Organ burden**
L: Examination **bronchoalveolar lavage fluid**
H: **Histopathology** of selected organs

Aerosol characterization

MMAD[1] < 1.6 µm
GSD[2] < 2.5

1 Mass median aerodynamic diameter;
2 Geometric standard deviation

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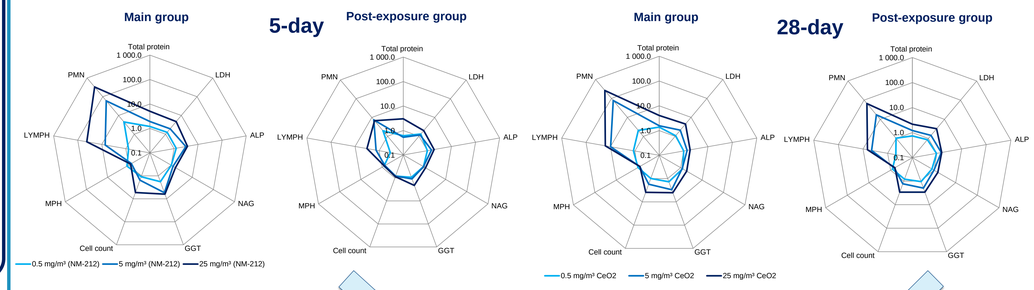
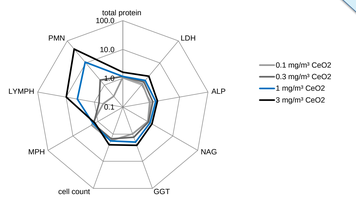
Bronchoalveolar Lavage of 5-day, 28-day and 90-day studies

Comparison of changes in BALF parameters* after exposure to CeO_2

*Changes are shown as x-fold differences compared to controls using a logarithmic scaling.



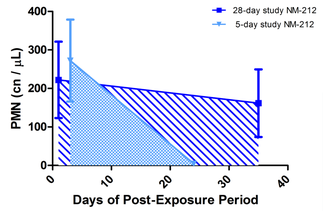
90-day



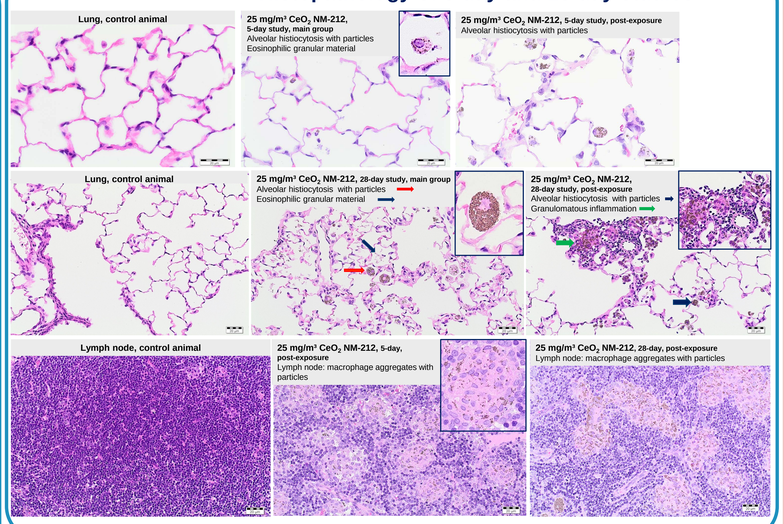
Cells:
MPH = Macrophage
PMN = Polymorphonulcear neutrophilic granulocyte
LYMPH = lymphocyte

Enzymes:
LDH = Lactate dehydrogenase
ALP = Alkaline phosphatase
NAG = N-Acetylaminglucosaminidase
GGT = γ-Glutamyltransferase

Differences in inflammatory response (PMN in BALF)
after exposure to 25 mg/m³ CeO_2
in the **5-day study** (light blue) and in the **28-day study** (dark blue) over 24 or 35 days of post-exposure

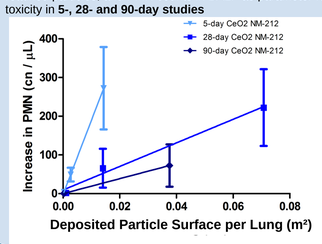


Histopathology of 5-day and 28-day studies



Dose-response curves of CeO_2

Relation between internal dose, expressed as surface area of retained particles, and PMN counts in BALF as parameter for toxicity in **5-, 28- and 90-day studies**



Retention and Clearance Kinetics of 5-day, 28-day and 90-day studies

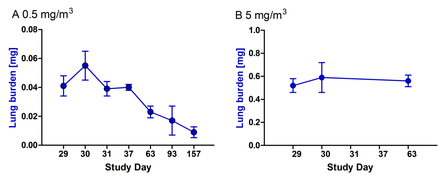
5-day

0.5 mg/m³ CeO_2 NM-212	SD 5	SD 26
Lung burden [mg]	0.011 ± 0.001	0.006 ± 0.001

5 mg/m³ CeO_2 NM 212	SD 5	SD 26
Lung burden [mg]	0.1 ±0.009	0.088 ±0.009

25 mg/m³ CeO_2 NM-212	SD 5	SD 26
Lung burden [mg]	0.53 ± 0.12	0.4 ± 0.07

28-day



28-day study Course of lung particle content (A-C) and lymph nodes content (C, green)

Retention half-life time
of 0.5 mg/m³: $t_{1/2}$= 68 days
of 25 mg/m³: $t_{1/2}$= 245 days

90-day

CeO_2 NM-212	0.1 mg/m³	3 mg/m³
	SD 94	SD 94
Lung burden [mg]	**0.012** ± 0.0028	**1.39** ± 0.16

CeO_2 NM-212	3 mg/m³
Lymph node burden [µg]	SD 94
Tracheobronchial	**11.93** ± 14.21
Mediastinal	**13.78** ± 15.85
Total	**25.71**

Summary and Conclusions

- Inhalation of aerosols containing 5 and 25 mg/m³ (but not 0.5 mg/m³) CeO_2 (NM-212) was associated with high CeO_2 retention in the lungs, translocation of the particles to the regional lymph nodes and a retarded lung clearance already after 5-days of exposure.
- These exposure conditions caused inflammation in the lung and findings in the draining lymphoid tissue.
- Dose-response curves using PMN counts in BALF as response and surface area of retained particles appear to be an appropriate dose metrics. Among 5-day, 28-day and 90-day studies shorter exposure periods demonstrated a steeper effect.
- The short-term inhalation test (STIS, 5 days of exposure) demonstrated an earlier and greater neutrophil (PMN) influx in the lung compared to the 28-day study. STIS is thus the inhalation test with the highest sensitivity.
- In histopathology, 5-day exposure revealed comparable pulmonary effects with less severity than 28-day exposure. Granulomatous inflammations in the lungs were, however, only observed by histopathology 4 weeks post-exposure in the 28-day study.