A common European approach to the regulatory testing of nanomaterials

NANoREG

Grant Agreement Number 310584

Deliverable D 4.07

Histopathological evaluation (all organs)

Immunohistochemical detection of local and systemic genotoxicity

Due date of deliverable: *2016/08*

Actual submission date: *2016/08/09*

Author(s) and company:	BAuA, Tom Gebel
Work package/task:	WP4 / Task 4.1
Document status:	draft / final
Confidentiality:	confidential / restricted / public
Key words:	

DOCUMENT HISTORY

Version	*Date*	*Reason of change*
1	2017/02/21	Project Office harmonized lay-out
2		
3		
4		

This project has received funding from the European Union Seventh Framework Programme (FP7/2007-2013) under grant agreement no 310584

Lead beneficiary for this deliverable:

BUNDESANSTALT FUER ARBEITSSCHUTZ UND ARBEITSMEDIZIN; BAuA, 3

Owner(s) of this document	
Owner of the content	BAuA (consortium including BMUB, UBA, FhG ITEM), 3
Co-Owner 1	BASF, 13
Co-Owner 2	ENEA, 18
Co-Owner 3	
…..	

Table of Content

1 Description of task

This deliverable describes a part of results of a project that serves to experimentally study hypotheses on the assumed mode of action of selected nanomaterials. It aims at clarifying essential questions in the risk assessment of granular biopersistent particulate nanomaterials (GBP). As inhalation is considered to be the most relevant route of exposure a chronic study was performed using inhalation. An OECD TG 453 compliant study, conducted with OECD depository materials (NM-212 CeO_2 and NM-220 $BaSO_4$) under GLP, focused on investigating a putative inhalation carcinogenicity of GBP nanomaterials in low dose exposures. This study offers relevant information not only for occupational but also for environmental and consumer health. Moreover, systemic distribution and systemic toxicity will be studied as well.

2 Description of work & main achievements

2.1 Summary

A combined chronic/carcinogenicity whole body inhalation study was performed according to OECD TG 453 with several protocol extensions. Female rats (n=100/group) were exposed to cerium dioxide (NM-212, 0.1; 0.3; 1; 3 mg/m³) and barium sulfate (NM-220; 50 mg/m³) for 24 months. A control (n=100) was exposed to clean, filtered air in parallel. The aim is to investigate lung carcinogenicity and putative systemic effects of low-dose exposures to biopersistent nanoparticles. The histological examinations are ongoing, results are available from the 12-months interim section.

There was some evidence that an inflammation-mediated secondary local genotoxicity in the lung could not be excluded at the higher exposure concentrations. On the other hand, CeO2 inhalation exposure did not induce any significant effect on the analysed systemic genotoxicity endpoints, irrespective of dose and time. CeO2 exposure-related histopathological findings were exclusively observed in the respiratory tract but not systemically. In the nasal cavity, the incidence of age-related intra-epithelial eosinophilic globules was increased in the 3 mg/m³ high-dose CeO2 exposure group as compared to the control group and associated with minimal inflammatory cell infiltration. Adverse effects in the lung included dose-dependent alveolar/interstitial inflammatory cell infiltration, alveolar/interstitial granulomatous inflammation and interstitial fibrosis. Alveolar lipoproteinosis was observed in the 3 mg/m³ high-dose CeO2 exposure group only and cholesterol granulomas occurred in a single female each CeO2 the 1 and 3 mg/m³ CeO2 exposure groups. After 12 month of inhalation exposure neither neoplastic nor pre-neoplastic treatment-related findings were seen in the lungs of CeO2-exposed animals.

Although statistically not significant, some adverse effects such as alveolar/interstitial inflammatory cell infiltration, alveolar/interstitial granulomatous inflammation, and interstitial fibrosis have already been observed in the 0.1 mg/m³ low-dose CeO2 exposure group. Thus, a NOAEL (no observed adverse effect level) could not be established for the lung after 12 months of exposure to the present CeO2 nanoparticle concentrations.

2.2 Background of the task

see respective chapter in deliverable 4.2

2.3 Description of the work carried out

2.3.1 Study objectives

The objective of this combined chronic inhalation toxicity and carcinogenicity study is to determine the effects of two nanoparticles NM-212 CeO2 and NM-220 BaSO4 in female Wistar rats following prolonged and repeated whole-body inhalation exposure. The application of this guideline should generate data which identify the majority of chronic and carcinogenicity effects and determines concentration-response relationships. The design and conduct should allow determination the carcinogenic potential as well as general toxicity, including physiological, biochemical, and hematological effects and exposure-related morphological (pathology) effects after chronic exposure (12 month).

2.3.2 Study protocols

The conduct of inhalation exposures will be performed according to the following test guideline concerning repeated dose inhalation toxicity studies:

- Organization for Economic Cooperation and Development (OECD), OECD Guidelines for Testing of Chemicals, Section 4: Health Effects, No. 413 "Sub-Chronic Inhalation Toxicity: 90-day Study" adopted 07 September 2009.

In addition the study was carried out taking into account the following guidelines:

- Organization for Economic Cooperation and Development (OECD), OECD Guidelines for Testing of Chemicals, Section 4: Health Effects, Method 453 "Combined Chronic Toxicity/Carcinogenicity Study in Rodents" adopted 07 Sep 2009.
- Commission Regulation (EC) No 440/2008 of 30 May 2008 laying down test methods pursuant to Regulation (EC) No 1907/2006 of the European Parliament and of the Council on the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH), Part B.33.: Combined Chronic Toxicity/Carcinogenicity Test
- US Environmental Protection Agency (EPA), Health Effects Test Guidelines OPPTS870.4300, Combined Chronic Toxicity/Carcinogenicity, EPA 712-C-98-212, August 1998

In deviation to the guidelines, only females were exposed, because female rats are considered to be slightly more sensitive concerning carcinogenicity after inhalation exposure to dust aerosols (Nikula et al. 2000).

The chronic study was started with 100 rats per dose group. 50 animals per dose group were sacrificed after 24 months. The remaining animals were kept exposure-free till natural death or till month 30, i.e. December 2015. The intention for this extension was to enhance study sensitivity. It is known that a relevant portion of particle induced tumours become detectable first rather late in rats. The study sensitivity to detect lung tumours was further enhanced by an extended lung histopathology as 60 instead of 6 sliced will be studied per lung.

Satellite groups were sacrificed after 12 months (chronic group with 10 animals per dose for histopathology) and after 3 months, 12 months, and 24 months (for kinetic/organ burden evaluations).

Main exposure groups are used for histopathology examinations (carcinogenicity groups). Groups of 50 animals (of each exposure level) were sacrificed and examined after 24 months of exposure. Additional groups of 50 animals were kept without exposure up to 30 months and animals were sacrificed and examined after 30 months or if the only 25% or less animals were still alive. Animals of each group which died during the exposure or post-exposure period are examined as well.

2.4 Results

2.4.1 Local genotoxicity in the lung after 12 months

In this deliverable, the results of the 12-months immunohistochemical analyses on local lung genotoxicity of the chronic study obtained with cerium dioxide are described. After 12 months no increase in oxidative DNA damage could be detected for 8-OHdG (figure 1).

For the DNA double strand breakage marker γ-H2AX a significant difference could be detected beginning with 0.3 mg/m³ exposure (figure 2). Thus, an inflammation-mediated secondary local genotoxicity in the lung cannot be excluded at the selected exposure concentrations. It should be noted that γ-H2AX could also be induced through CeO_2-mediated induction of apoptosis or senescence.

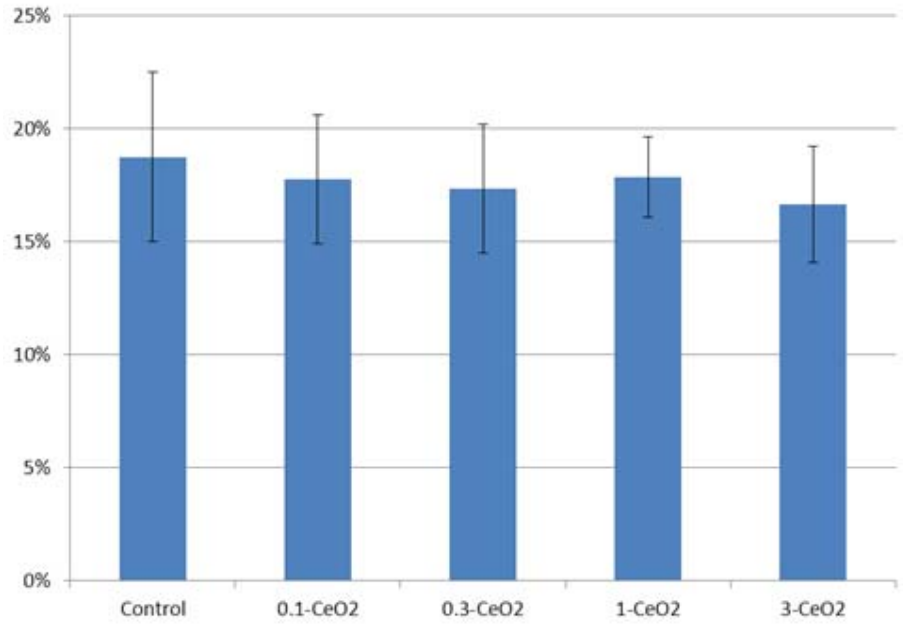

Figure 1

Proportion of 8-OHdG positive cells in lung tissue presented as means with standard deviation.

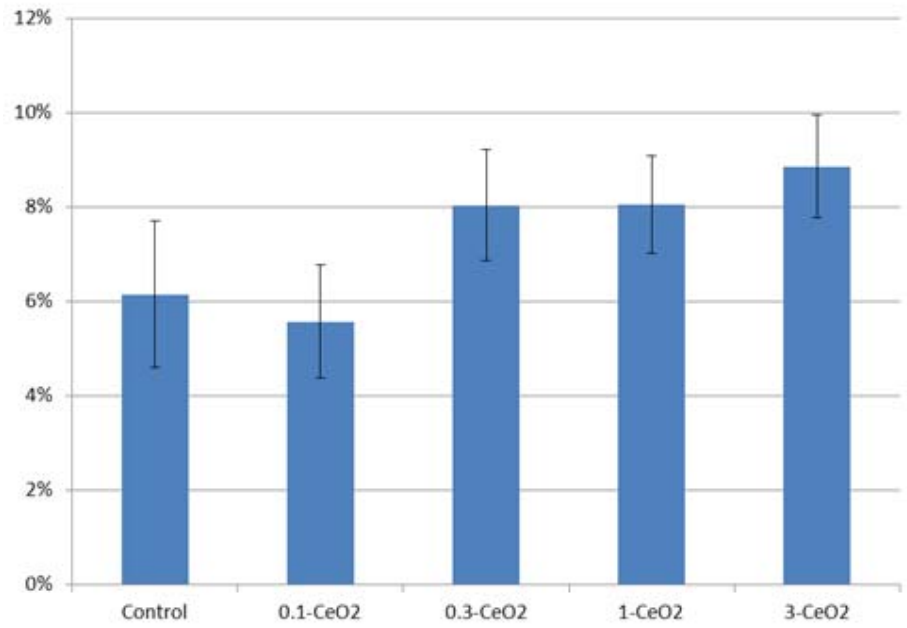

Figure 2

Proportion of γ-H2AX positive cells in lung tissue presented as means with standard deviation. *The effect was significant at 0.3, 1, and 3 mg/m³ (Dunnett's test, two-sided, p<0.01)*

2.4.2 Systemic genotoxicity after 3 and after 6 months

In this deliverable, the results of 3- and 6-months interim analyses on systemic genotoxicity of the chronic study are described (Cordelli et al. 2016).

Genotoxicity analyses on peripheral blood samples were carried out. Effects on DNA were analysed in leukocytes by the comet assay. Gene mutation and chromosome damage were measured in erythrocytes, by the flow cytometric pig-a (MutaFlow®, Litron) and micronucleus (MicroFlow®, Litron) tests, respectively. The analyses were carried out after 3 or 6 months of exposure at the concentration levels of 0.1, 0.3, 1 or 3 mg/m³ CeO_2 or 50 mg/m³ $BaSO_4$. An additional group of unexposed animals (clean air only) served as negative controls. Each experimental group was composed of 5 female rats.

CeO_2 inhalation exposure did not induce any significant effect on the analysed genotoxicity endpoints, irrespectively of dose and time (figures 3, 4, and 5). Similarly, $BaSO_4$ treatment did not significantly increase any of the genotoxicity parameters over the control values. No evidence of cytotoxicity was provided by a reduction of the percentage of reticulocytes over total red blood cells, as measured in the pig-a and micronucleus assays. The positive control N-ethyl-N-nitrosourea provided adequate results.

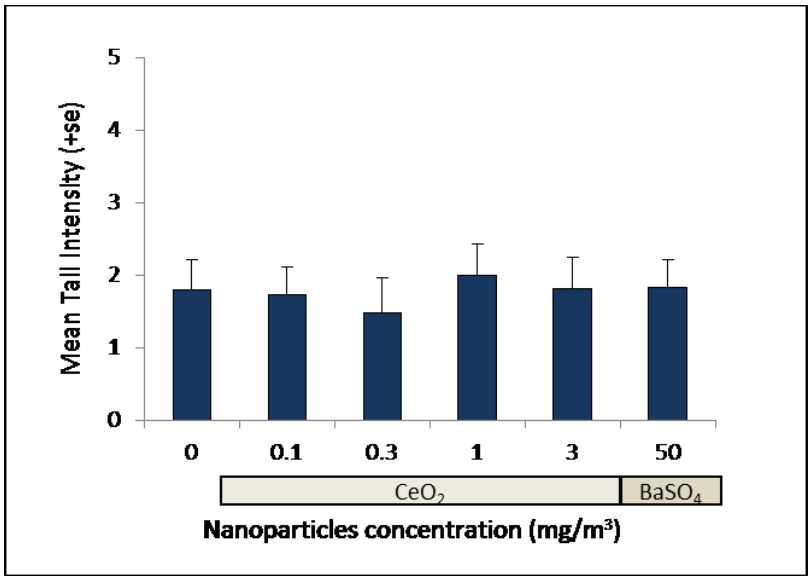

Figure 3 Summary of comet assay results.

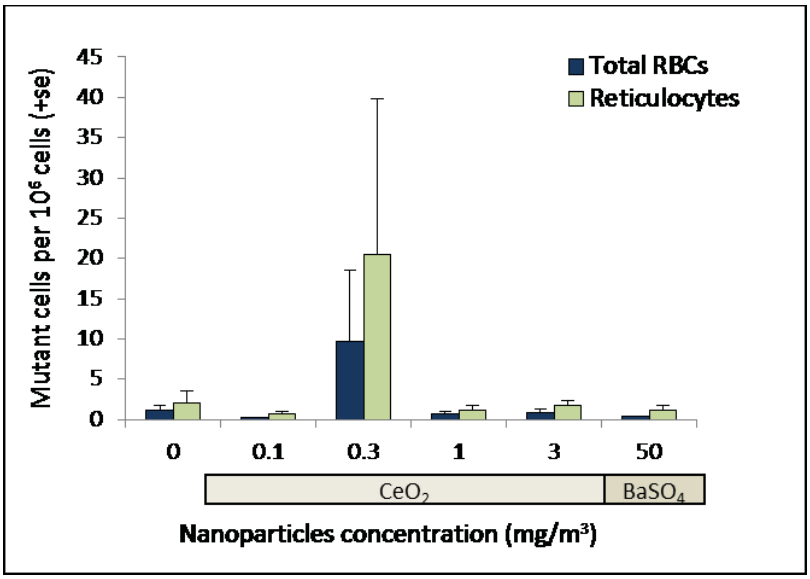

Figure 4 Summary of pig-a assay results. No statistically significant increase of the mutant frequency was detected either on total red blood cells (RBCs) or on reticulocytes. The higher mean values at 0.3 mg/m³ are due to a single outlier (45 and 98 mutants in 10^6 RBCs and reticulocytes respectively) that could reflect the clonality of a spontaneous mutation.

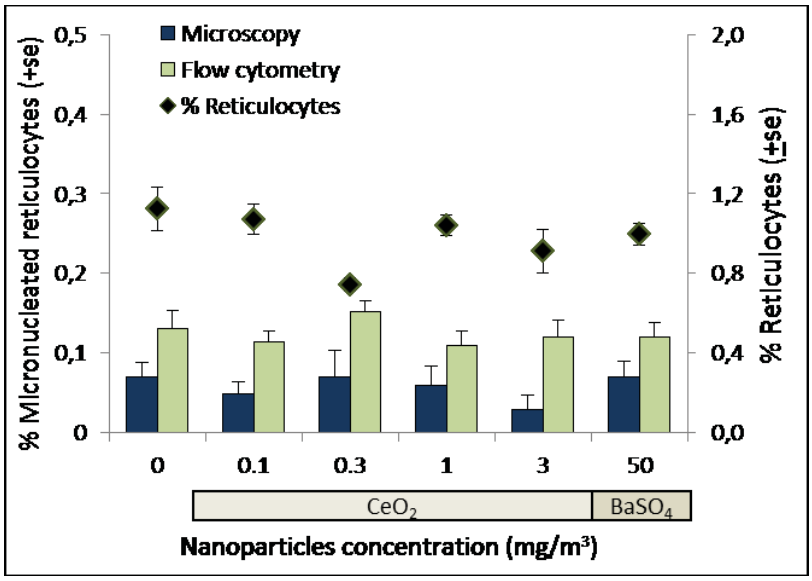

Figure 5

Summary of micronucleus assay results. Micronuclei were scored by two independent methods: microscopically or by flow cytometry. No evidence of cytotoxicity was provided by a reduction of the percentage of reticulocytes over total red blood cells.

2.4.3 Histopathology

In this deliverable, the results of the 12-months interim histopathological analyses of the chronic study obtained with cerium dioxide are described.

Exposure-related microscopic changes were observed in the nasal cavity, larynx, lung, tracheobronchial and mediastinal lymph nodes.

Nasal cavity

The presence of (multi)focal intracytoplasmic eosinophilic globules within the olfactory epithelium was increased in incidence and grade in the 3 mg/m³ CeO_2 exposure group (9/10; 5/10 very slight, 3/10 slight, 1/10 moderate) as compared to the clean air control (6/10; 5/10 very slight, 1/10 slight). A similar trend was observed for eosinophilic globules in the respiratory epithelium. The incidence in the clean air group 40 was 5/10 (all very slight) whereas 9/10 (7/10 very slight, 2/10 slight) females in the high dose group were affected (table 1). Although the difference between the control and CeO_2 high-dose test group was statistically not significant, the increase in incidence and severity of this change in both types of epithelium is considered to be exposure-related. The same is true for (multi)focal very slight subepithelial (mixed) inflammatory cell infiltration which occurred in 3/10 and 7/10 females in the low and high dose groups, respectively.

Table 1 Summary of histological changes in the nasal cavity and in the larynx

Nasal cavity 10 animals per group (♀)	Clean	CeO_2
	Air	3 mg/m^3
Eosinophilic globules, Olfactory epithelium	0.7	1.4
Eosinophilic globules, Respiratory epithelium	0.5	1.1
Infiltration. Inflammatory Cell, Subepithelial	0.3	0.7
Accumulation. Particle-Laden Macrophages, NALT	0	1
Intraepithelial. Intracytoplasmic Particles	0	1
Larynx 10 animals per group (♀)		
Accumulation, Particle-Laden Macrophages, Subepithelial	0	0.5

Grading: 1, minimal; 2, slight; 3, moderate; 4, severe; 5, very severe

Further exposure-related findings such as (multi)focal very slight accumulation of particle-laden macrophages within the NALT (nasal mucosa-associated lymphoid tissue) and multifocal very slight amounts of intraepithelial (intracytoplasmic) particles were diagnosed in all (10/10) animals of the high dose group. Occasional particles were seen not only in the respiratory and olfactory epithelium, but also in epithelial cells of the submucosal glands (Bowman's glands). Incidental findings in the nasal cavity which were considered to be unrelated to particle exposure included dilatation of submucosal glands, mucous cell hyperplasia, subepithelial mononuclear cell infiltration and subepithelial mineralization and were seen in up to 3/10 animals in both test groups.

Larynx

In 4/10 animals of the 3 mg/m³ CeO_2 high exposure test group, (multi)focal subepithelial accumulation of particle-laden macrophages (3/10 very slight, 1/10 slight) was observed as exposure-related finding (table 1). Spontaneous findings included very slight to slight subepithelial mononuclear cell infiltration (4/10 each, low and high dose) as well as very slight to slight dilatation of submucosal glands in 2/10 females of the high dose test group.

Lung

CeO_2 exposure-related pulmonary findings included accumulation of particle-laden macrophages and giant cells, cell-free intra-alveolar agglomerations of CeO_2 particles and bronchiolo-alveolar hyperplasia of the bronchiolar type (alveolar bronchiolization) (tables 2 and 3). (Multi)focal alveolar/interstitial accumulation of particle-laden macrophages was observed dose-dependently in 10/10 females each of the 0.1 mg/m³ (8/10 very slight, 2/10 slight), 0.3 mg/m³ (8/10 very slight, 2/10 slight), 1 mg/m³ (1/10 very slight, 9/10 slight) and 3 mg/m³ (7/10 slight, 3/10 moderate) CeO_2 exposure test groups. Deposits of particle-laden macrophages were present not only in alveoli but also in interstitial (intraseptal, peribronchiolar and perivascular) compartments.

Table 2 Summary of histological changes in the lung

	Clean	CeO_2	CeO_2	CeO_2	CeO_2
Lung	Air	0.1 mg/m^3	0.3 mg/m^3	1 mg/m^3	3 mg/m^3
No. of animals (♀)	10	10	10	10	10
Accumulation, Particle-Laden Macrophages, Alveolar/Interst., grade 1–3	0	10*	10*	10*	10*
Accumulation, Particle-Laden Macrophages, BALT, grade 1–4	0	10*	10*	10*	10*
Giant Cells, Syncytial, BALT, present, no grade	0	0	3	9*	10*
Hyperplasia, Bronchiolo-Alveolar; Bronchiolar type, grade 1–2	0	1	2	10*	10*
Infiltration, Inflammatory Cell, Alveolar/Interstitial, grade 1–2	1	4	10*	10*	10*
Inflammation, Granulomatous, Alveolar/Interstitial, grade 1–2	0	1	3	10*	10*
Fibrosis, Interstitial, grade 1	0	3	4	10*	10*
Lipoproteinosis, Alveolar, grade 1–4	0	0	0	0	4
Granuloma, Cholesterol, grade 1–2	0	0	0	1	1

**p<0.001, Chi-Quadrat/Fisher-Test, 2-sided. Grading: 1, minimal; 2, slight;L 3, moderate; 4, severe; 5, very severe*

In addition, agglomerates of CeO_2 particles were lying freely within alveoli at very slight to slight degrees in 3/10 animals of test group 0.1 mg/m³ and in 10/10 females each of the higher exposure test groups. (Multi)focal aggregates of particle-laden macrophages were also observed dose-dependently within the bronchus-associated lymphoid tissue (BALT) at incidences of 10/10 each in test groups 0.1 mg/m³ (all very slight), 0.3 mg/m³ (8/10 very slight, 2/10 slight), 1 mg/m³ (8/10 slight, 2/10 moderate) and 3 mg/m³ (1/10 slight, 7/10 moderate, 2/10 severe). Syncytial giant cells - mainly particle-laden - were present in the BALT of 3/10, 9/10 and 10/10 females of test groups 0.3, 1, and 3 mg/m³, respectively. The amount of the intracellular particle-load in both single-nucleated macrophages and multinucleated giant cells corresponded well to the used CeO_2 exposure dose. (Multi)focal bronchiolo-alveolar hyperplasia of the bronchiolar type was observed in a single animal of test group 0.1 mg/m³ (very slight) and in 2/10 (all very slight), 10/10 (all very slight) and 10/10 (9/10 very slight, 1/10 slight) females of test groups 0.3, 1, and 3 mg/m³, respectively.

Table 3 Summary of histological changes in the lung, average grading

Lung / 10 animals per group (♀)	Clean Air	CeO_2 0.1 mg/m³	CeO_2 0.3 mg/m³	CeO_2 1 mg/m³	CeO_2 3 mg/m³	
Accumulation, Particle-Laden Macrophages, Alveolar/Interstitial	0	1.2	1.2	1.7	2.9	non-adverse lesions
Accumulation, Particle-Laden Macrophages, BALT	0	1	1.2	2.2	3	
Giant Cells, Syncytial, BALT	0	0	0.3	0.9	1	
Hyperplasia, Bronchiolo-Alveolar; Bronchiolar type	0	0.1	0.2	1	1.1	
Infiltration, Inflammatory Cell, Alveolar/Interstitial	0.1	0.4	1.1	1.3	1.6	adverse lesions
Inflammation, Granulomatous, Alveolar/Interstitial	0	0.1	0.3	1.3	1.6	
Fibrosis, Interstitial	0	0.3	0.4	1	1	
Lipoproteinosis, Alveolar	0	0	0	0	0.8	
Granuloma, Cholesterol	0	0	0	0.1	0.2	

Grading: 1, minimal; 2, slight; 3, moderate; 4, severe; 5, very severe

Besides these reactive/adaptive (= non-adverse) pulmonary findings, several adverse changes were also diagnosed (tables 2 and 3). These included alveolar/interstitial (mixed) inflammatory cell infiltration, alveolar/interstitial granulomatous inflammation, interstitial fibrosis, alveolar lipoproteinosis and cholesterol granuloma(s). Except alveolar lipoproteinosis and cholesterol granuloma(s), all changes were seen at dose-dependent incidences and severity grades in all CeO_2 exposure test groups.
(Multi)focal alveolar/interstitial (mixed) inflammatory cell infiltration occurred in a single control animal (very slight) as a spontaneous finding, in 4/10 females of test group 0.1 mg/m³ (all very slight) and in 10/10 animals each of exposure test groups 0.3 mg/m³ (9/10 very slight, 1/10 slight), 1 mg/m³ (7/10 very slight, 3/10 slight) and 3 mg/m³ (4/10 very slight, 6/10 slight). In test groups with exposures higher than 0.1 mg/m³, the difference to the control was statistically significant.
Probably as correlate to the macroscopic finding 'focus in the lung', multifocal alveolar/interstitial granulomatous inflammation was observed in 1/10 females of test group 0.1 mg/m³ (very slight), in 3/10 females of test group 0.3 mg/m³ (all very slight) and at significantly increased incidences in 10/10 animals each of test groups 1 mg/m³ (7/10 very slight, 3/10 slight) and 3 mg/m³ (4/10 very slight, 6/10 slight). The term 'granulomatous inflammation' was used only, if (mixed) inflammatory cell infiltration, syncytial giant cells and interstitial fibrosis were present in conjunction to form a granuloma-like focal lesion.
(Multi)focal very slight interstitial (mainly intraseptal) fibrosis was diagnosed with increasing exposure in 3/10, 4/10, 10/10 and 10/10 females, respectively. For test groups 1 mg/m³ and 3 mg/m³, the difference to the control group was statistically significant.
Multifocal alveolar lipoproteinosis was seen exclusively in 4/10 animals of the 3 mg/m³ CeO_2 test group (2/10 very slight, 1/10 slight, 1/10 severe). The intra-alveolar lipoproteinaceous material was mainly granular, eosinophilic and mixed with particle agglomerations reflecting

basically an origin from degenerating particle-laden macrophages. A similar pathogenesis can be assumed for development of focal cholesterol granuloma(s) occurring in a single female each of test groups 1 mg/m³ (very slight) and 3 mg/m³ (slight).

Incidental pulmonary findings occurring in single animals of different exposure groups as well as in the control group consisted of focal very slight osseous metaplasia, focal very slight neuroendocrine cell hyperplasia and focal very slight hair granuloma. In addition, 4/10 control animals revealed focal very slight alveolar macrophage aggregation. All these findings were considered to be unrelated to particle exposure.
After 12 month of inhalation exposure neither neoplastic nor pre-neoplastic findings were seen in the lungs of CeO_2 exposed animals.

Tracheobronchial and Mediastinal lymph nodes

As correlate to the macroscopic findings 'enlargement' and 'discoloration', the lymph nodes at both sites showed a dose-dependent (multi)focal very slight to severe accumulation of particle-laden macrophages (table 4). Regarding the tracheobronchial lymph node, the incidences were 8/8 (all very slight) in test group 0.1 mg/m³, 9/9 (1/9 very slight, 7/9 slight, 1/9 moderate) in test group 0.3 mg/m³ and 10/10 each in test group 1 mg/m³ (2/10 slight, 8/10 moderate) and 3 mg/m³ (5/10 moderate, 5/10 severe). In addition, particle-laden syncytial (multinucleated) giant cells were present in the tracheobronchial lymph node of 1/8, 6/9, 10/10 and 10/10 females with increasing dose, respectively.
The incidences of (multi)focal accumulation of particle-laden macrophages in the mediastinal lymph nodes were 6/10 (all very slight) in test group 0.1 mg/m³, 10/10 (all very slight) in test group 0.3 mg/m³, 9/9 (3/9 slight, 6/9 moderate) in test group 1 mg/m³ and 10/10 (5/10 moderate, 5/10 severe) in test group 3 mg/m³, while syncytial giant cells were only observed in 9/9 and 10/10 females of test groups 1 and 3 mg/m³, respectively.

Table 4
Summary of histological changes in the lung-associated lymph nodes, average grading

Lung-associated-lymph nodes 10 animals per group (♀)	Clean	CeO_2	CeO_2	CeO_2	CeO_2
	Air	0.1 mg/m³	0.3 mg/m³	1 mg/m³	3 mg/m³
Accumulation, Particle-Laden Macrophages	0	0.8	1.8	2.8	3.5
Giant Cells, Syncytial	0	0.1	0.7	1	1

Grading: 1, minimal; 2, slight; 3, moderate; 4, severe; 5, very severe

Other organs

Several sporadic neoplastic and non-neoplastic findings were observed in the other organs examined histopathologically. These occurred either incidentally or were similar in distribution pattern and severity in control rats compared to the CeO_2 high-dose test group. Sporadic findings in the other CeO_2 exposure groups were recorded only as correlates of macroscopic findings. All of the observed findings were considered to be without any relation to CeO_2-exposure.
A total number of 6 neoplasms were observed: an adenoma of the pars distalis in the pituitary gland of single females each of group 0, 0.1 and 0.3 mg/m³, a sebaceous adenoma and a

lipoma of the skin/subcutaneous tissue in single animals of test group 0.3 mg/m³, and an endometrial stromal polyp of the uterus in a female control animal.
Findings such as epithelial degeneration (incidence up to 6/10 rats per test group) and interstitial inflammation (incidence up to 7/10 rats per test group) of the Harderian glands are most likely considered to be related to the blood sampling procedure. Further common spontaneous findings included (multi)focal very slight intratubular mineralization of the kidneys (incidence up to 8/10 rats per test group), (multi)focal very slight mononuclear cell infiltration of the liver (incidence up to 7/10 rats per test group), chondromucinous degeneration of sternebral cartilage (incidence up to 7/10 rats per test group), epithelial hyperplasia (incl. hyperplasia of the type 'epithelial tubules and cords') at incidences of up to 8/10 rats per test group in the thymus and acinar cell hypertrophy of the salivary glands (incidence up to 4/10 rats per test group). Estrous cycle-depending luminal dilatation of the uterus, C-cell hyperplasia of the thyroids, and parasites (nematodes) in the rectum, colon and/or cecum were observed in up to 3/10 animals per test group. In addition, various other incidental findings occurred in single or in up to 2/10 rats per test group.

2.5 Evaluation and conclusions

There was some evidence that an inflammation-mediated secondary local genotoxicity in the lung could not be excluded at the higher exposure concentrations. On the other hand, CeO_2 inhalation exposure did not induce any significant effect on the analysed systemic genotoxicity endpoints, irrespective of dose and time. CeO_2 exposure-related histopathological findings were exclusively observed in the respiratory tract and included reactive/adaptive changes such as accumulation of particle-laden macrophages in the nasal cavity, larynx, lung, tracheobronchial and mediastinal lymph nodes. In the nasal cavity, the incidence of age-related intra-epithelial eosinophilic globules was increased in the 3 mg/m³ high-dose CeO_2 exposure group as compared to the control group and associated with minimal inflammatory cell infiltration. Non-adverse findings consisted of accumulation of particle-laden macrophages in the alveolar/interstitial areas and in the BALT as well as particle-laden syncytial giant cells in the BALT. In addition, bronchiolo-alveolar hyperplasia of the bronchiolar type graded no more than "very slight" (grade 1) or "slight" (grade 2) was considered as a non-adverse finding. Adverse effects in the lung included dose-dependent alveolar/interstitial inflammatory cell infiltration, alveolar/interstitial granulomatous inflammation and interstitial fibrosis. Alveolar lipoproteinosis was observed in the 3 mg/m³ high-dose CeO_2 exposure group only and cholesterol granulomas occurred in a single female each CeO_2 the 1 and 3 mg/m³ CeO_2 exposure groups. After 12 month of inhalation exposure neither neoplastic nor pre-neoplastic treatment-related findings were seen in the lungs of CeO_2 -exposed animals.
Although statistically not significant, some adverse effects such as alveolar/interstitial inflammatory cell infiltration, alveolar/interstitial granulomatous inflammation, and interstitial fibrosis have already been observed in the 0.1 mg/m³ low-dose CeO_2 exposure group. Thus, a NOAEL (no observed adverse effect level) could not be established for the lung after 12 months of exposure to the present CeO_2 nanoparticle concentrations.

2.6 Data management

Excel files for the ISA-TAB-Nano templates have been prepared, the available results from the histological and genotoxicity analyses were entered and uploaded to CIRCABC on July 21 2016 to be included into the database.

3 Deviations from the work plan

No major or relevant deviations from the work plan were necessary. Thus, there is nothing to report.

4 References / Selected sources of information (optional)

Cordelli E, Keller J, Eleuteri P, Villani P, Ma-Hock L, Schulz M, Landsiedel R, Pacchierotti F. No genotoxicity in rat blood cells upon 3- or 6-month inhalation exposure to CeO2 or BaSO4 nanomaterials. Mutagenesis. 2016 Feb 9 [Epub ahead of print].