A common European approach to the regulatory testing of nanomaterials

NANoREG

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Lung burden and particle detection and quantification in olfactory bulbs, blood – sub-acute exposure

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Lead beneficiary for this deliverable:

Bundesanstalt fuer Arbeitsschutz und Arbeitsmedizin (BAuA)

(German Federal Environmental Agency (UBA))

1 Description of task

Digestion and dilution of up to 1200 tissue samples in triplicate from lungs, olfactory bulbs, blood, lung-associated lymph nodes, spleen, liver, kidney, brain, heart, blood, feces for ICP-MS analysis starting with the high dose. The analyses will be targeted in a way that analyses will begin with the high dose in each organ to be studied. In case there are no granular biodurable particles (GBP) detectable, the lower dose will not be tested.

Preparation of ICP-MS equipment for analysis of up to 2600 organ tissue samples (installation of hydrofluoric acid kit). Running the analysis of organ samples. Maintenance of analytical equipment. The oral uptake will be quantified by feces, via feces analyses after 12 and 24 months (10 animals per dose and time, respectively).

Deliverable 4.3 is the first deliverable in this task and will be complemented by deliverables 4.4, 4.5 4.6. It describes the first results of a preceding 28-d study on lung burden. Such preceding studies are necessary for the dose-finding of the long-term study. Deliverable 4.4 will present further data from this study. Deliverables 4.5 and 4.6 will describe the respective results from the long-term study.

2 Description of work & main achievements

2.1 Summary

A 28-d inhalation study with cerium dioxide had been performed as preceding study to the planned long-term inhalation study (task 4.1). The main aim is the dose-finding for the long-term study and to assess the adequacy of the exposure atmosphere. This is achieved by the assessment of cerium dioxide deposited in the lung. i.e. the lung burden.

In the dose group 1 (low dose) a significant clearance effect was observed. Groups 2 (mid dose) and 3 (high dose), however, showed no pronounced decrease in the initial lung loading, which indicates lung overloading.

Based on the results of the study the exposure concentrations for the long-term inhalation study have been set on 0, 0.1, 0.3, 1 and 3 mg/kg.

2.2 Background of the task

A relevant and currently not clarified question is a putative carcinogenicity of nanomaterials. Due to reasons of feasibility, it will not be possible to test each single nanomaterial for this effect. Grouping approaches for safety testing can be chosen in case a common mode of action is known. A relevant group of nanomaterials are assumed to share a common mode of toxic action. These nanomaterials belong to a group of materials that can be described as poorly soluble, respirable granular biodurable particles without known significant specific toxicity (GBP). Prominent high production volume nanomaterials like carbon black or titanium dioxide belong to this group. Carbon black and nanosized titanium dioxide have been tested for chronic inhalation carcinogenicity in the rat, further respective data on other GBP nanomaterials are not available. Due to current knowledge, the induction of inflammation after inhalation and lung carcinogenicity appear to be the prominent health hazards for these materials. Up to now, there is no convincing evidence that further health hazards or oral/dermal exposure are relevant. There is a current scientific controversy, whether the lung tumours detected in the chronic rat inhalation studies induced by carbon black and nanosized titanium dioxide only appeared in artificially high exposure concentrations (i.e. so-called dust ‘overloading’ of the lungs) associated with inflammation. The planned study aims at verifying this hypothesis. The aim is to prove whether a dose-response curve with or without a threshold must be assumed for lung tumour induction. For this purpose, an inhalation carcinogenicity study with an extended protocol to enhance tumour detection sensitivity will be performed. Task 4.1 of the NANoREG project is a long-term inhalation study aiming to address this question. A 28-d inhalation study with cerium dioxide had been performed as

preceding study to the planned long-term inhalation study. The main aim is the dose-finding for the long-term study. Within this context, it is also necessary to assess the adequacy of the exposure atmosphere. This is achieved by the assessment of cerium dioxide deposited in the lung, i.e. the lung burden. The assessment of lung burden data over time also helps to assess whether particle clearance from lung is impaired. This is necessary information for the dose-finding in the long-term study which aims to study exposure concentrations below a relevant impairment of clearance. The lung burden results are described in the following paragraphs.

2.3 Description of the work carried out.

Analytical task and sample preparation

The analytical contract includes the determination of cerium in organic material (lung, lymph nodes (LALN)) after inhalation exposure (retention and clearance) in samples from the 28-day BASF CeO2-study as part of the UBA research project Z6-55410-31/8 (project code: 371261 206) and refers to studies for the GLP test "Assessment of chronic toxicity / carcinogenicity of selected nanomaterials" with the test number 05G12036 ITEM (subproject within the preliminary study, work package 1).

The quantitative determination of the cerium content in the samples is performed after appropriate sample preparation using the ICP-MS. For sample preparation, the steps freeze-drying, plasma ashing and microwave digestion and dilution of the respective digestion solution are required.

Freeze-drying

The (roughly chopped) lung material (for already prepared LALN no further preparation is needed) is freeze-dried in aluminium trays (flat) initially ≥ 6 h (0.37 mbar). The body weights before and after freeze-drying are logged.

Plasma ashing

The freeze-dried material is then subjected of a plasma ashing (cool plasma conditions, 400 W, about 1 mbar O2) overnight (≥ 12 h), homogenized and transferred to appropriate storage vessels. The ash weight is if fully implemented, usually by> 12 h less than 5% of the original wet weight and can be used as a parameter for assessing the ashing quality. The logging of ash weights is required.

Microwave digestion

The freeze-dried and plasma ashed samples (each time the total amount of present sample) are finally subjected to microwave digestion. To effect complete digestion, the use of sulfuric acid (96%, suprapur) is required. The following table shows the individual steps of the microwave program are summarized.

step	programme phase	power (W)	time (min)
1	pre-heating	250	5
2	rest	0	1
3	heat-up	500	5
4	rest	0	1
5	heat-up	400	8
6	rest	0	1
7	heat-up	400	8
8	ventilation/cooling	VENT	15

After cooling, the samples were quantitatively transferred into volumetric flasks 25 mL (KS), filled up to the mark with ultrapure water and then placed in 50 mL plastic containers until analysis stored cool.

To avoid analyte disappearances during the digestion of the material, the following system is used:

- cleaning digestion (before experiments start)
- air G0 exposure concentration: 0 mg/m^3 (control)
- CeO_2 G1 exposure concentration: 0.5 mg/m^3 (low dose)
- CeO_2 G2 exposure concentration: 5 mg/m^3 (mid dose)
- CeO_2 G3 exposure concentration: 25 mg/m^3 (high dose)

ICP-MS analysis:
Quantification is carried out with a quadrupole ICP-MS system, X-Series II (Thermo Fisher).

2.4 Results

In the following section, the results for the quantitative determination of cerium in organic material (lung) after inhalation exposure (retention and clearance) are summarized in samples from the 28-day BASF CeO_2 study.

All figures are averages and are based on each of 4 ICP-MS measurements of each of 5 independent samples (n = 5).

d: day of observation
FG: sample weight (fresh)
ASD: absolute standard deviation
RSD: relative standard deviation

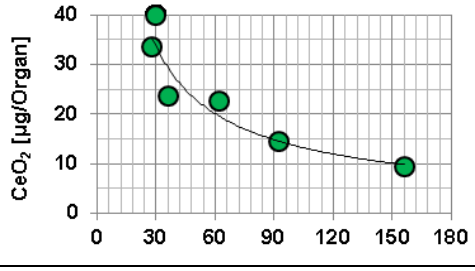
group zero: air control 0 mg/m^3
group one: low exposure group 0,5 mg/m^3
group two: mid exposure group 5 mg/m^3
group three: high exposure group 25 mg/m^3

group 0: 0 mg/m^3 (control)

group	animal	d	FG	CeO_2			
				mean	ASD	RSD	
			(g)	(µg/organ)		(%)	
0	6	28	0.92	< 0.03			For control animals that found in standard addition for all animals a (finite and reproducible) value of about 0.02 ng Ce / ml diluted sample. This concentration is therefore significantly below the defined in (5) limit of quantification of 0.1 ng Ce / ml, but well above the determined limit of detection (LOD) of approximately 0.01 ng Ce / ml.
0	12	30	1.06	< 0.03			
0	13	36	1.04	< 0.03			
0	24	62	0.97	< 0.03			
0	25	92	0.91	< 0.03			
0	26	156	0.95	< 0.03			

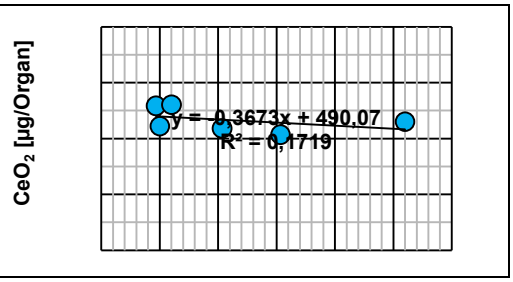
group 1: CeO_2 0.5 mg/m^3 (low dose)

1	36	28	0.93	33.7	0.7	2.0
1	42	30	1.03	39.9	0.8	2.1
1	43	36	0.97	23.7	0.5	2.2
1	54	62	1.00	22.7	0.5	2.2
1	55	92	0.96	14.5	0.3	2.3
1	56	156	1.13	9.5	0.2	2.5



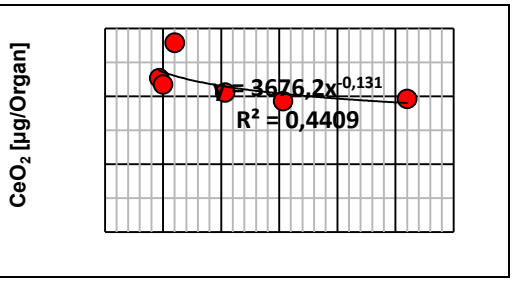
group 2: CeO_2 5 mg/m³ (mid dose)

2	66	28	1.20	517	3	0.6
2	72	30	0.97	444	2	0.5
2	73	36	1.12	521	4	0.8
2	84	62	1.07	436	4	0.9
2	85	92	0.99	414	3	0.7
2	86	156	1.12	460	1	0.2



group 3: CeO_2 25 mg/m³ (high dose)

3	96	28	1.16	2268	5	0.2
3	102	30	1.23	2174	6	0.3
3	103	36	1.31	2789	35	1.3
3	114	62	1.15	2056	3	0.1
3	115	92	1.13	1930	3	0.2
3	116	156	1.20	1961	9	0.5



2.5 Evaluation of the results

Unimpaired clearance in the rat lung is described by elimination half-lifes of 60-90 days. When determining the elimination half-lifes from the 5 and 25 mg/m³ exposure groups it became evident that 60-90 days are strongly exceeded which is already visually evident from the original results.

3 Deviations from the workplan

There were changes necessary due to changes in the coordination how the single partners adjusted and distributed their responsibilities within these tasks. This was discussed and coordinated after NANoREG started. The content of the deliverable had to focus on lung as organ. It was requested to change the scope of the deliverable into “Lung burden after sub-acute exposure”. The cerium content in other organs will be reported in deliverable 4.4 “Organ burden and particle detection pattern in other organs after sub-acute exposure”.

4 Conclusions

A 28-d inhalation study with cerium dioxide had been performed as preceding study to the planned long-term inhalation study. Exposure concentrations were 0, 0.5, 5, and 25 mg/m³. A strong impairment of clearance of cerium dioxide was found at 5 and 25 mg/m³. This indicates lung overload. The long-term study intends to investigate also exposure concentrations below lung overload to test whether tumour only become apparent above the overload threshold or also below

that threshold. Thus, the long term-study will be using the exposure concentrations of 0, 0.1, 0.3, 1 and 3 mg/kg.