Short-Term Inhalation Study in Rats for Testing of Nanomaterials

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Version
1.0

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1. STUDY DESIGN

Route of administration: Inhalation

Exposure mixture: Dust aerosol

Type of exposure: Head-nose exposure

No. of test concentrations: 3 and concurrent air control

Frequency of exposure: On 5 consecutive days

Duration of one exposure: 6 hours

Number of exposures: 5

Recovery period 3 weeks

The parameter to be examined and the exact time points of the examinations are presented in Figure 1. The details of the examined parameters are described in the following sections.

Study day ⟶

1	2	3	4	5	6	7	8	9 - 27	28
x	x	x	x	x	R	R	R	R	R
				E			L		E+L

x: head/nose exposure
R: recovery period
E: lung burden, gross necropsy, organ weights, preservation of organs and tissues for histopathology
L: broncho alveolar lavage (BAL)

2. ANIMALS

Animal species:	Rat
Gender:	Male
Age at	About
supply:	7 weeks
start of preflow:	8 weeks
start of exposure:	9 weeks
Test groups:	Lavage: 5 animals per concentration and time point
	Pathology and histopathology: 3 animals per concentration and time point
	Lung burden: 3 animals per concentration and time point

3. GENERATION OF THE TEST ATMOSPHERES

3.1. Generating the test atmosphere by brush dust generator

Dust aerosols were produced at target concentrations by dry dispersion of powder pellets with a brush dust generator (developed by the Technical University of Karlsruhe in cooperation with BASF, Germany). Each concentration was generated with compressed air in a mixing stage, mixed with conditioned dilution air and passed via a cyclone (to separate particles > 3 µm) into a head-nose inhalation system (Fig. 2). To reduce electrostatic charging, brushes made of stainless steel were used. The generator itself and all conducting tubes were grounded.

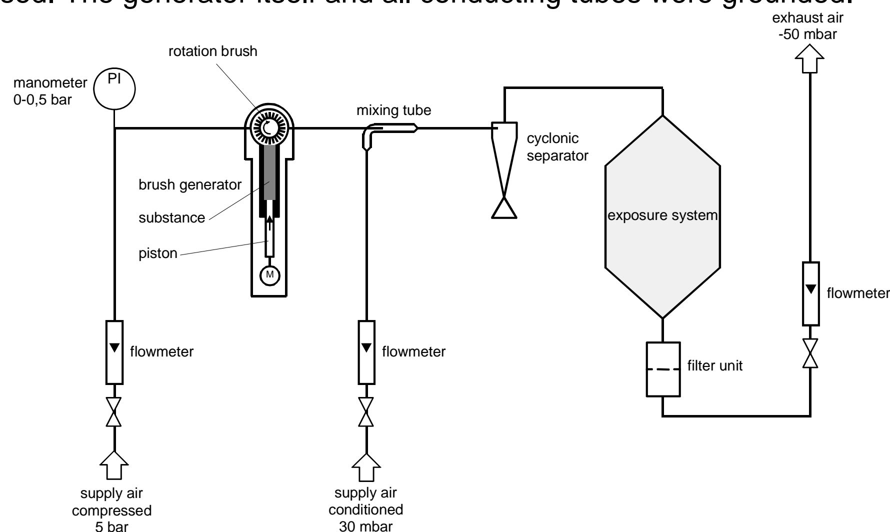

Figure 2: Flow diagram of dust generation and exposure system using brush dust generator

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3.2. Generating the test atmosphere by atomizing suspension

For each concentration the test substance suspension was supplied to a two-component atomizer at a constant rate by means of a metering pump. The aerosol was generated with compressed air in a mixing stage, mixed with conditioned dilution air and passed via the cyclonic separator into the inhalation system (Figure 3).

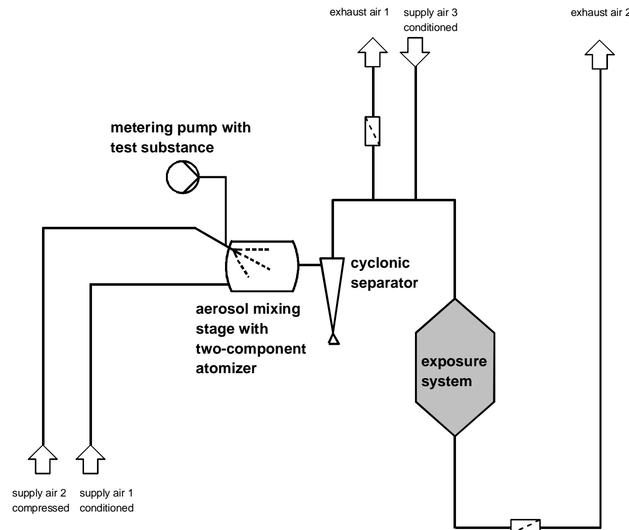

Figure 3: Flow diagram of dust generation and exposure system using two-component atomizer.

4. CHARACTERIZATION OF THE TEST ATMOSPHERES

4.1. Determination of atmospheric concentration of the test item

Method:	Gravimetrical measurement
Sampling site:	In adjacent to the animals snouts
Sampling velocity:	1.25 m/s, at 3 L/min
Sampling probe:	7 mm
Sampling device:	Gas sampling station with glass fibre filter (d = 4.7 cm)
Sampling volume:	Depending on atmospheric concentration. The total sample amount on the filter should be between 1 and 5 mg.

4.2. Determination of the mass median aerodynamic diameter

Device:	Eight-stage Marple Personal Cascade Impactor (Sierra-Andersen)
Method:	gravimetrical measurement with appropriate balance
Sampling site:	In adjacent to the animals snouts
Sampling velocity:	1.25 m/s, at 3 L/min
Sampling probe:	7 mm
The effective aerodynamic cutoff diameters (EACD):	21, 15, 10, 6.5, 3.5, 1, 0.7, or 0.4 µm and the backup filter
Sampling volume:	Depending on atmospheric concentration. The total sample amount on collectors should be between 0.8 and 1.2 mg.

4.3. Determination of particle size distribution

In addition to the cascade impactor measurement, the atmospheres are further characterized by scanning mobility particle sizer (SMPS) and an optical particle counter.

5. EXAMINATIONS

In-life:	Clinical observation (3 times/day during exposure, once daily during the non-exposure days) body weight (weekly)
Sacrificing:	Exsanguination under Narcoren® anaesthesia and opening of the abdominal vessels
BAL:	2 flushes with 6 mL (0.9 % NaCl) each, combined BAL fluid will be examined
BALF parameters	Recovered BALF volume
	Total cell count Cell differential analysis of cytospin preparations
	Protein Lactate dehydrogenase Alkaline phosphatase N-acetyl-ß-D-glucosaminidase γ-Glutamyltransferase
	MCP-1 IL-8 M-CSF Osteopontin Clusterin
Parameters in lavaged lung tissue:	IL-1 α TNF-α
Hematology	Leukocytes Troponin Erythrocytes Hemoglobin Hematocrit Mean corpuscular volume (MCV) Mean corpuscular hemoglobin (MCH) Mean corpuscular hemoglobin concentration (MCHC) Platelets Differential blood cell count
Akute Phase Proteins	Haptoglobin CRP
Pathology and	Gross necropsy
	Organ weight (lung, mediastinal lymph nodes, liver, kidney, spleen, thymus and brain)
Histopathology:	Nasal cavity (Level 1- IV) Larynx (Level I-III) Trachea with bifurcation, longitudinal section Lungs (five lobes) Mediastinal lymph node