SRI LANKA STANDARD 12005:2013
ISO 10808:2010

NANOTECHNOLOGIES - CHARACTERIZATION OF NANOPARTICLES IN INHALATION EXPOSURE CHAMBERS FOR INHALATION TOXICITY TESTING

SRI LANKA STANDARDS INSTITUTION

Sri Lanka Standard

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SLS 12005:2013
ISO 10808:2010

Gr. J

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This standard does not purport to include all the necessary provisions of a contract.

Sri Lanka Standard

NANOTECHNOLOGIES - CHARACTERIZATION OF NANOPARTICLES IN INHALATION EXPOSURE CHAMBERS FOR INHALATION TOXICITY TESTING

NATIONAL FOREWORD

This standard was approved by the National Mirror Committee on Nanotechnology and authorized for adoption and publication as a Sri Lanka Standard by the Council of the Sri Lanka Standards Institution on 2013.11.27.

This Sri Lanka Standard is identical with **ISO 10808:2010**, Nanotechnologies - Characterization of nanoparticles in inhalation exposure chambers for inhalation toxicity testing, published by the International Organization for Standardization (ISO).

TERMINOLOGY AND CONVENTIONS

The text of the International Standard has been accepted as suitable for publication, without any deviation as a Sri Lanka Standard. However, certain terminology and conventions are not identical with those used in Sri Lanka Standards. Attention is therefore drawn to the following:

a) Wherever the words “International Standard” appear referring to this standard they should be interpreted as “Sri Lanka Standard”.

b) The comma has been used throughout as a decimal marker. In Sri Lanka Standards, it is the current practice to use a full point on the baseline as the decimal marker.

Wherever page numbers are quoted, they are “ISO” page numbers.

CROSS REFERENCES

International Standard	Corresponding Sri Lanka Standard
ISO/TS 27687, Terminology and definitions for Nano-objects - Nanoparticle, nanofiber and nanoplate	**SLS 12000 - Part 1**, Terminology and definitions for nano-objects - nanoparticle, nanofiber and nanoplate

SLS 12005:2013

INTERNATIONAL STANDARD

ISO 10808

First edition
2010-12-15

Nanotechnologies — Characterization of nanoparticles in inhalation exposure chambers for inhalation toxicity testing

Nanotechnologies — Caractérisation des nanoparticules dans les chambres d'inhalation par exposition pour les essais de toxicité par inhalation

Reference number
ISO 10808:2010(E)

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Foreword

ISO (the International Organization for Standardization) is a worldwide federation of national standards bodies (ISO member bodies). The work of preparing International Standards is normally carried out through ISO technical committees. Each member body interested in a subject for which a technical committee has been established has the right to be represented on that committee. International organizations, governmental and non-governmental, in liaison with ISO, also take part in the work. ISO collaborates closely with the International Electrotechnical Commission (IEC) on all matters of electrotechnical standardization.

International Standards are drafted in accordance with the rules given in the ISO/IEC Directives, Part 2.

The main task of technical committees is to prepare International Standards. Draft International Standards adopted by the technical committees are circulated to the member bodies for voting. Publication as an International Standard requires approval by at least 75 % of the member bodies casting a vote.

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. ISO shall not be held responsible for identifying any or all such patent rights.

ISO 10808 was prepared by Technical Committee ISO/TC 229, *Nanotechnologies*.

Introduction

The number of nanotechnology-based consumer products containing silver, gold, carbon, zinc oxide, titanium dioxide and silica nanoparticles is growing very rapidly. The population at risk of exposure to nanoparticles continues to increase as the applications expand. In particular, workers in nanotechnology-based industries are at risk of being exposed to nanoparticles. If nanoparticles are liberated from products, the public could be exposed as well. Although toxicity screening using instillation of nanomaterials provides important information, it does not reflect the actual scenario of inhalation exposure and does not provide the data required for inhalation exposure risk assessment. In addition, while inhalation toxicology using rats is the norm at this time, it is desirable to replace this antiquated method with a human-relevant assay[10].

The inhalation toxicity of nanoparticles is of particular concern in ensuring the health of workers and consumers. In order to conduct inhalation toxicity studies of nano-sized particles, the monitoring of concentration, size and distribution of nano-sized particles in the inhalation chamber is necessary. The conventional methods of fine or coarse particle monitoring, such as weight-based mass dose monitoring, are considered insufficient for nanoparticles, since nano-specific parameters (particle surface area, particle number, etc.) might be critical determinants, and if so, should also be monitored.

This International Standard proposes a battery of inhalation toxicity testing chamber monitoring, including a differential mobility analyzing system (DMAS), for measuring particle number, size, distribution, surface area and estimated mass dose, as well as morphological examination using transmission electron microscopy (TEM) or scanning electron microscopy (SEM) equipped with an energy dispersive X-ray analyzer (TEM-EDXA) for chemical composition.

This International Standard also includes conventional mass dose monitoring and other physicochemical monitoring, for use when deemed a necessary parameter for toxicity determination. This method evaluates nano-sized particle surface area, mass dose, particle distribution, composition and dispersion to support effective analysis of inhalation toxicity testing results [13][17][18].

INTERNATIONAL STANDARD **ISO 10808:2010(E)**

Nanotechnologies — Characterization of nanoparticles in inhalation exposure chambers for inhalation toxicity testing

1 Scope

This International Standard specifies requirements for, and gives guidance on, the characterization of airborne nanoparticles in inhalation exposure chambers for the purpose of inhalation toxicity studies in terms of particle mass, size distribution, number concentration and composition.

2 Normative references

The following referenced documents are indispensable for the application of this document. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

ISO 10312, *Ambient air —Determination of asbestos fibres — Direct transfer transmission electron microscopy method*

ISO 15900, *Determination of particle size distribution — Differential electrical mobility analysis for aerosol particles*

ISO/TS 27687, *Nanotechnologies — Terminology and definitions for nano-objects — Nanoparticle, nanofibre and nanoplate*

OECD Test Guideline 403 (TG 403), *Acute Inhalation Toxicity*[1)]

OECD Test Guideline 412 (TG 412), *Subacute Inhalation Toxicity: 28-Day Study*[1)]

OECD Test Guideline 413 (TG 413), *Subchronic Inhalation Toxicity: 90-Day Study*[1)]

OECD Guidance Document 39 (GD 39), *Acute Inhalation Toxicity Testing*[1)]

3 Terms and definitions

For the purposes of this document, the terms and definitions given in ISO 15900 and ISO/TS 27687 and the following apply.

1) Organization for Economic Cooperation and Development (OECD) publication.

3.1 Particle measuring systems

3.1.1
differential electrical mobility classifier
DEMC
differential electrical mobility spectrometer
DEMS
classifier that is able to select aerosol particle sizes from a distribution that enters it and pass only selected sizes to the exit

NOTE 1 A DEMC classifies aerosol particle sizes by balancing the electrical force on each particle in an electrical field with its aerodynamic drag force. Classified particles have different sizes due to their number of electrical charges and a narrow range of electrical mobility determined by the operating conditions and physical dimensions of the DEMC.

NOTE 2 Adapted from ISO 15900:2009, definition 2.7.

3.1.2
differential mobility analyzing system
DMAS
system used to measure the size distribution of submicrometre aerosol particles consisting of a DEMC, a particle charge conditioner, flow meters, a particle detector, interconnecting plumbing, a computer and suitable software

NOTE Adapted from ISO 15900:2009, definition 2.8.

3.1.3
condensation particle counter
CPC
instrument that detects particles and that can be used to calculate particle number concentration given the known flow rates into the detector

NOTE 1 The range of particles detected are usually smaller than several hundred nanometers and larger than a few nanometers. A CPC is one possible detector for use with a DEMC.

NOTE 2 In some cases, a condensation particle counter may be called a condensation nucleus counter (CNC).

NOTE 3 Adapted from ISO 15900:2009, definition 2.5.

3.2
inhalation exposure chamber
inhalation chamber
exposure chamber
system prepared to expose experimental animals to an inhaled test substance of predetermined duration and dose by either the nose-only or whole-body method

NOTE 1 The term “nose-only” is synonymous with “head-only” or “snout-only”.

NOTE 2 Adapted from OECD TG 403, 412, 413.

3.3
nanoparticle generation system
device used to make nanoparticle aerosol with controlled size distribution and concentration

3.4
breathing zone
location from which the experimental animal breathes

NOTE 1 For an unrestrained, non-caged animal, this will be the entire volume of the inhalation chamber. For a restrained or caged animal, this will be the range of motion for the animal's nose. For a masked animal, this will be the small volume in front of the nostrils.

NOTE 2 The term “breathing zone” is used to ensure test atmosphere samples are obtained from the same location as that in which the animal breathes. An undesirable sampling approach would be one where concentration measurements are obtained at the top of the inhalation chamber while the animal is exposed at the bottom.

3.5
geometric mean diameter
GMD
measure of central tendency of particle size distribution using the logarithm of particle diameters, computed for the DMAS by

$$\ln(\mathrm{GMD}) = \frac{\sum_{i=m}^{n} \Delta N_i \ln\left(d_i\right)}{N}$$

where

d_i is the midpoint diameter for the size channel, i;

N is the total concentration;

ΔN_i is the concentration within the size channel, i;

m is the first channel;

n is the last channel.

NOTE The GMD is normally computed from particle counts and when noted may be based on surface area or particle volume with appropriate weighting.

3.6
geometric standard deviation
GSD
measure of width or spread of particle sizes, computed for the DMAS by

$$\ln(\mathrm{GSD}) = \sqrt{\frac{\sum_{i=m}^{n} N_i \left[\ln d_i - \ln\left(\mathrm{GMD}\right)\right]^2}{N-1}}$$

3.7
count median diameter
CMD
diameter equal to GMD for particle counts assuming a logarithmic normal distribution

NOTE The general form of the relationship as described in ISO 9276-5 is

$$\mathrm{CMD} = x_{50,r} = x_{50,p}\mathrm{e}^{(r-p)s^2}$$

where

e is the base of natural logarithms, e = 2,718 28;

p is the dimensionality (type of quantity) of a distribution, where

p = 0 is the number,

p = 1 is the length,

p = 2 is the area, and

p = 3 is the volume or mass;

r is the dimensionality (type of quantity) of a distribution, where

$r = 0$ is the number,

$r = 1$ is the length,

$r = 2$ is the area, and

$r = 3$ is the volume or mass;

s is the standard deviation of the density distribution;

$x_{50,r}$ is the median particle size of a cumulative distribution of dimensionality, r.

4 Test substance monitoring method

4.1 Principle

4.1.1 Exposure

Precise characterization of the test substance exposure is essential for an inhalation toxicology study. The objective in nanoparticle inhalation toxicology is to establish a quantitative relationship between the observed toxicological outcome and the dose metrics used in terms of test substance physical and chemical properties.

4.1.2 Particle properties

The specific chemical and physical properties of the nanoparticle should be determined to the extent possible; however, because these may not be known *a priori*, as many parameters as possible should be determined. Nanoparticle composition, number and mass concentrations, median and mean size and size distribution, surface area, electrical charge, surface character, hygroscopicity and shape might be important parameters for dosimetry.

4.2 Preparation of system

4.2.1 During development of the nanoparticle generating system and prior to interfacing with the exposure chamber(s), measurements should be performed to verify aerosol particle composition and purity and to establish the stability. During exposure tests, analysis should be conducted continuously and/or intermittently depending on the method of analysis to determine the consistency of particle size distribution without disrupting the inhalation exposure.

NOTE A nanoparticle generating system for silver and other metals is described in ISO 10801[3].

4.2.2 Inhalation chambers and supporting equipment shall be prepared in accordance with OECD TG 403, OECD TG 412 and OECD TG 413.

4.2.3 Inhalation chambers and supporting equipment shall be prepared for nanoparticle exposure studies.

NOTE 1 Aerosolized nanoparticles can be deposited to walls by Brownian diffusion and particle size change due to aggregation/agglomeration. This deposition process depends on the particle size, electrostatic charge, particle number concentration and residence time. See standard texts on aerosol science, References [11], [19] and [20].

NOTE 2 Charge neutralization might be required, depending on the purpose of the study.

If charge distribution is considered a characterization requirement, this shall be specified and measured in the study.

NOTE 3 To reduce deposition losses, conductive tubing of the minimum length practical to use with the tubing diameter is selected to interface with instrumentation.

4.2.4 An inhalation chamber or chambers and supporting equipment, such as sampling probes and manifolds, shall be characterized to ensure compliance with OECD TG 403, OECD TG 412 and OECD TG 413, for determining any sampling bias.

NOTE Sampling manifold consists of tubing, solenoid valves and/or other elements required for routing samples from each chamber to online monitoring equipment.

4.2.5 Measurement instruments used in inhalation testing should be calibrated and/or tested in accordance with ISO 15900.

The DMAS is usually calibrated at the factory and this should be documented in the report.

NOTE In addition, in the course of using the DMAS, it must be routinely calibrated as well.

4.3 Study

4.3.1 The study shall be conducted in accordance with OECD TG 403, OECD TG 412, OECD TG 413 and OECD GD 39.

4.3.2 During the exposure period the concentrations of the test substance should be held as constant as practicable and monitored continuously and/or intermittently depending on the method of analysis.

4.3.3 Breathing zone sampling shall be conducted to establish exposure.

4.3.4 The rate of air flow in the supply and chamber(s), should be monitored continuously in order to document compliance with OECD TG 403, OECD TG 412, OECD TG 413 and OECD GD 39.

Airflow meters should be employed to establish that the parameter is within limits.

4.3.5 Temperature and humidity inside the inhalation chamber and as close to the breathing zone as practical shall be monitored continuously.

Temperature and humidity sensors with transducers should be employed to establish that the parameter is within limits.

4.3.6 Exhaust air from the chambers containing nanoparticles shall be treated by appropriate filtration, and, if necessary or appropriate, chemical scrubbing, before being vented to the atmosphere.

5 Specific monitoring method

5.1 Requirements for number-based particle size distribution and mass concentration

Measurement of number-based particle size distribution and measurement of total particle mass concentration are two essential measurements in the characterization of nanoparticles in inhalation toxicity testing. Particle size distribution measurement is essential because the knowledge of particle size is crucial for the evaluation of the result of toxicity testing. Mass concentration, on the other hand, has been used as the dosimetric parameter in every inhalation toxicity test and is indispensable in nanoparticle toxicity testing. Therefore, these two measurements shall always be made in nanoparticle inhalation toxicity testing and carried out using appropriate methods.

5.2 Measurement of number-based particle size distribution

5.2.1 The method used shall be able to monitor particle size distribution in a continuous manner during particle exposures with time resolution appropriate to checking the stability of particle size distribution and concentration.

5.2.2 The measurable range of particle sizes and concentrations in the animal's breathing zone shall cover those of the nanoparticle aerosols exposed to the test system during the toxicity test.

5.2.3 Particle size and concentration measurements in the animal's breathing zone should be accurate for nanoparticle toxicity testing, and can be validated by means such as calibration against appropriate reference standards (see ISO/IEC 17025).

5.2.4 The resolution of particle sizing shall be accurate and the range of particle sizes measured shall be sufficiently broad to permit conversion from number-based distribution to surface area-based or volume-based distribution.

NOTE For particle size distribution, measurement with DMAS is the only currently available method that meets all the above requirements in the size range below 100 nm (see ISO 15900).

Particles larger than 100 nm may be measured by other instruments using optical or electrical properties, time of flight, or other aerodynamic properties[8].

5.3 Mass concentration measurement

The method selected shall be accurate and sensitive, and defined by the limit of quantification, for nanoparticle aerosols exposed to test subject during the toxicity test.

NOTE 1 Beta attenuation monitor (BAM), tapered element oscillating microbalance (TEOM), piezoelectric microbalance, filter gravimetric, and other methods based on chemical analysis of particles collected on filter media may meet the requirements for nanoparticle mass concentration measurement[4].

NOTE 2 Mass concentration can be derived from number-based size distribution measurement data by making an assumption regarding particle density, particularly for spherical particles which may match bulk material density[14]. However, significant errors in calculated mass concentration may result if particle density is inaccurate or unknown.

Derived mass concentration from number-based size distribution data should be accepted only when no other accepted methods meet the measuring requirements.

5.4 Inhalation exposure chamber

5.4.1 Air flow shall be 10 h^{-1} to 15 h^{-1} air changes for whole body chamber. For nose-only exposure chambers, the air flow shall be at least twice the respiratory minute volume of animals exposed (e.g. at least 0,5 l/min per exposure port for rats).

The chamber should be prepared such that distribution of nanoparticles inside it is uniform.

5.4.2 Temperature and humidity shall remain within study established limits.

NOTE OECD TG 413 prescribes that the temperature at which the test is performed be maintained at 22 °C (±3°).

Ideally, the relative humidity should be maintained between 30 % and 70 %, but in certain instances (e.g. tests of aerosols) this may not be practicable.

5.4.3 Pressure inside the chamber shall remain slightly negative ($\leqslant$ 5 mm water) to prevent leakage outside the testing boundaries.

For nose-only exposure, pressure should be slightly positive to ensure animals will be properly exposed. Due to potential leakage from this positive pressure, nose-only experiments should be conducted inside the boundaries of an adequately designed fume hood (see OECD GD 39).

5.4.4 Supply air shall ensure an adequate oxygen content of at least 19 % as well as uniform conditions throughout the exposure chamber.

6 Assessment of results

6.1 The following nanoparticle data shall be obtained to assist with interpretation of the study results:

a) nanoparticle number-size (in nanometres) distribution, geometric mean diameter (GMD), and geometric standard deviation (GSD) in each exposure chamber using DMAS, TEM, SEM, etc.;

b) particle morphology using TEM or SEM with an adaptation of ISO 10312;

c) number concentration (in particles per cubic centimetre) in each exposure chamber using DMAS, etc;

d) mass concentration of nanoparticles administered into each exposure chamber as measured by membrane filters (micrograms per cubic metre), or other methods such as estimation by DMAS;

e) chemical composition of nanoparticles.

IMPORTANT — Estimation of particle size from DMAS measurements can result in significant error for non-spherical particles. Application of DMAS for non-spherical particles is not recommended.

Mass concentration estimation by DMAS based on particle size can produce error for non-spherical particles.

6.2 The following data should be obtained to interpret study results:

a) surface area[15][16] in accordance with ISO 10312 or ISO/TR 13014;

b) volume of nanoparticle in accordance with ISO 10312;

c) shape and dispersion by TEM and SEM image in accordance with ISO/TR 13014 and ISO 9276-6.

d) surface chemistry by energy dispersive X-ray analyzer in accordance with ISO/TR 13014; ISO 10312 or electron spectroscopy for chemical analysis in accordance with ISO/TR 18394;

e) measurement of net electrical charge on the particles or documentation that an aerosol neutralizer was employed and its location in the system.

7 Test report

7.1 The test report shall be in accordance with the test procedures used.

7.2 The test report shall include the following:

a) complete identification of the nanomaterial(s) as outlined in Clause 6 for each exposure chamber;

b) test substance (manufacturer's code, catalogue or formulation number, batch number or date of manufacture, trade-name, etc.) and, in the case of resuspension of particles, processing and operating parameters;

c) all equipment and instrumentation used (manufacturer's model or catalogue number, serial number or date of manufacture, brand-name, etc.);

d) air flow rates through the inhalation chamber;

e) temperature and humidity of air in the inhalation chamber;

f) all supporting information developed during preparation of tests (sample line losses, etc.).

7.3 The test report should also include:

a) surface chemistry in accordance with ISO 10312, or other methods, and

b) either net charge or documentation of use of charge neutralization.

Annex A
(informative)

Example of nanoparticle characterization for inhalation toxicity testing

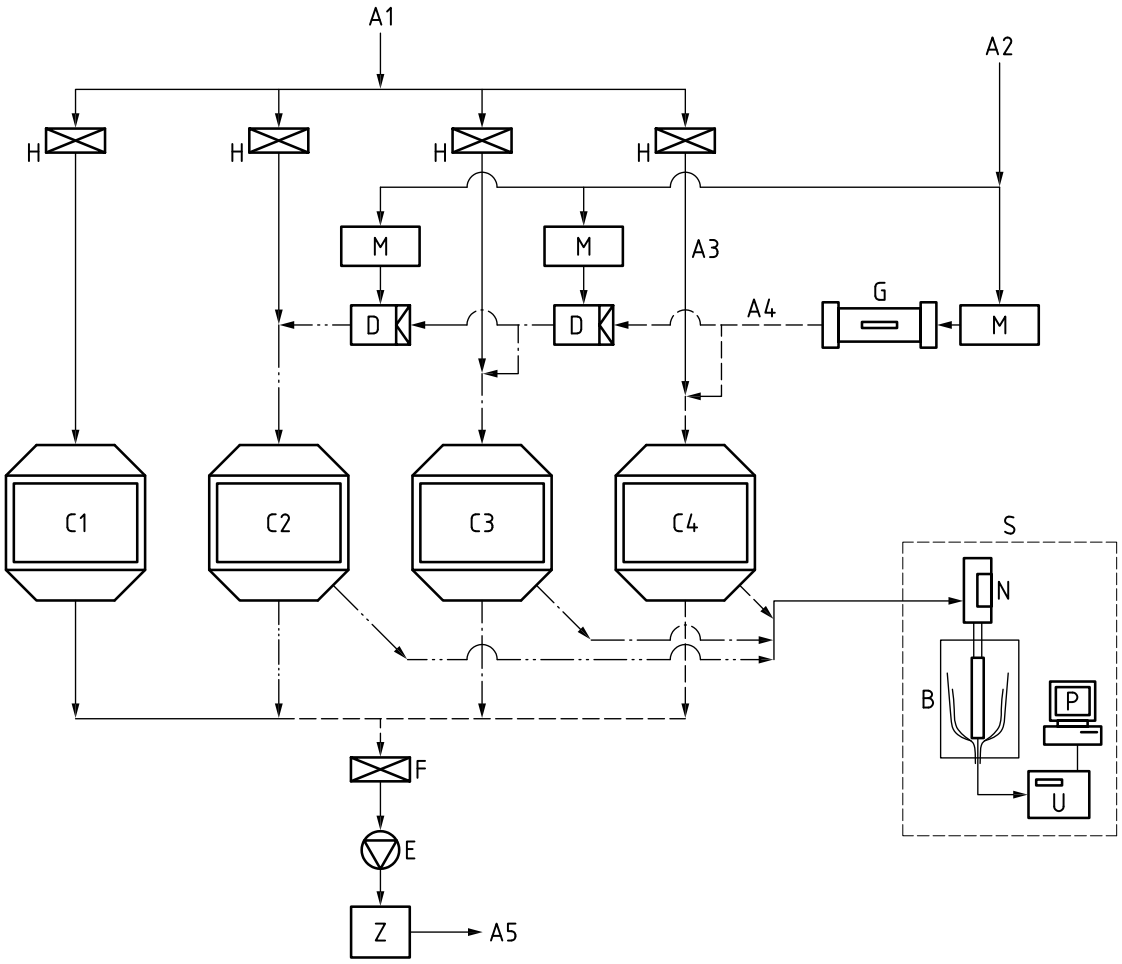
A.1 System set-up

A.1.1 General

Figure A.1 shows a system for distributing three different nanoparticle concentrations for investigation of the toxic effects of inhaling silver nanoparticles, generated from 99,99 % pure bulk silver wire. A Venturi-type dilutor and integral particle diverting probe was used, consisting of two concentric cylinders where the space between the tubes provides a passage for diluting the gas[9]. As such, the gas passes through the narrow gap formed by the inner tube and inner surface of the diverting tip, and the high velocity gas flow creates a pressure drop around the diverting hole positioned at the centre of the diverting probe tip. This pressure drop draws source aerosol through the diverting hole, allowing the aerosol to mix with clean, filtered air inside the diverting probe. Since the suction (negative) pressure increases as the dilution flow increases, the flow of diverting gas through the hole also increases. The system in this example produced different concentrations of nanoparticles (high, medium and low) in three separate chambers. Dilutor 1 produced a medium particle concentration by using mass flow controller 2 (MFC) to control mixing of sheath air with the aerosol diverted from the high particle concentration chamber. In the same way, Dilutor 2 produced a low particle concentration by mixing sheath air controlled by MFC 3 with the aerosol diverted from the medium particle concentration chamber. The nanoparticle generator operated at 30 l/min and the output mixed with 200 l/min supply air through the high-concentration chamber[12][13]. If three generators were to be used, one for each exposure chamber, the diluters would not be necessary. Nanoparticle monitoring was performed using two sample probes in each chamber. Sample probes operate using the same principle as diverting probes in the dilutors. Sampling from each chamber was controlled by solenoid valve manifold upstream of the DMAS.

A.1.2 Inhalation chamber concentration monitoring

The distribution of nanoparticles with respect to size was measured directly for each individual chamber that contained a different nanoparticle concentration. Measurements were conducted using a DMAS consisting of a Polonium 210 charge neutralizer, a differential mobility analyzer (DMA) and a condensation particle counter (CPC). Nanoparticles from 1,98 to 64,9 nm were measured using sheath air at 15 l/min and poly-dispersed aerosol air at 1,5 l/min, these values being the operational conditions for DMA and CPC[13]. The filters on which the nanoparticles were sampled were coated with carbon, mounted on an electron microscope grid (200 mesh), and visualized under a transmission electron microscope (TEM). Hundreds of randomly selected particles were measured at 100 000 × magnification and analyzed using an energy dispersive X-ray analyzer (EDXA) at an accelerating voltage of 75 kV[13].



Key

Component and process description

B differential electrical mobility classifier (DEMC)
C1 inhalation chamber, control
C2 inhalation chamber, low concentration
C3 inhalation chamber, medium concentration
C4 inhalation chamber, high concentration
D dilutor
E exhaust fan
F dust filter
G nanoparticle generator
H HEPA filter
M mass flow controller (MFC)
N neutralizer (^{210}Po)
P personal computer
S differential mobility analyzing system (DMAS)
U ultrafine condensation particle counter (UCPC)
Z chemical scrubber

Airflows

A1 fresh, pre-filtered air
A2 dried filtered air
A3 HEPA filter air 200 l/min
A4 nanoparticles in air at 30 l/min
A5 clean exhaust air

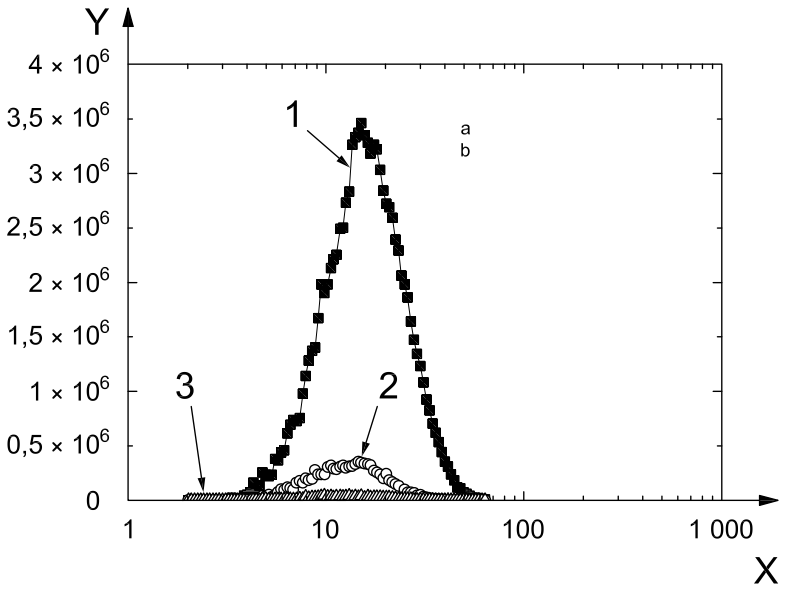
Nanoparticle concentrations

– – – – → high
— · — · → medium
——·· → low

Figure A.1 — System for distributing and monitoring discrete nanoparticle concentrations for inhalation toxicity study

A.2 Example results

Figures A.2. and A.3 illustrate the size distributions of the silver nanoparticles measured in the three positive exposure chambers. Nanoparticle generator conditions were 85 V applied voltage (1 130 °C) and carrier air flow rate of 30 l/min. In the high-concentration chamber, the geometric mean diameter (GMD), geometric standard deviation (GSD) and total number concentration of silver nanoparticles were 15,38 nm, 1,58, and $1{,}63 \times 10^6$ particles/cm^3, respectively, in the medium-concentration chamber, they were 12,60 nm, 1,53, and $1{,}60 \times 10^5$ particles/cm^3, respectively, and in the low-concentration chamber, they were 12,61 nm, 1,52, and $1{,}66 \times 10^4$ particles/cm^3, respectively.



Key

X mobility diameter, Dp (nm)

Y dN/dlog(Dp), particles/cm^3

1 high concentration group

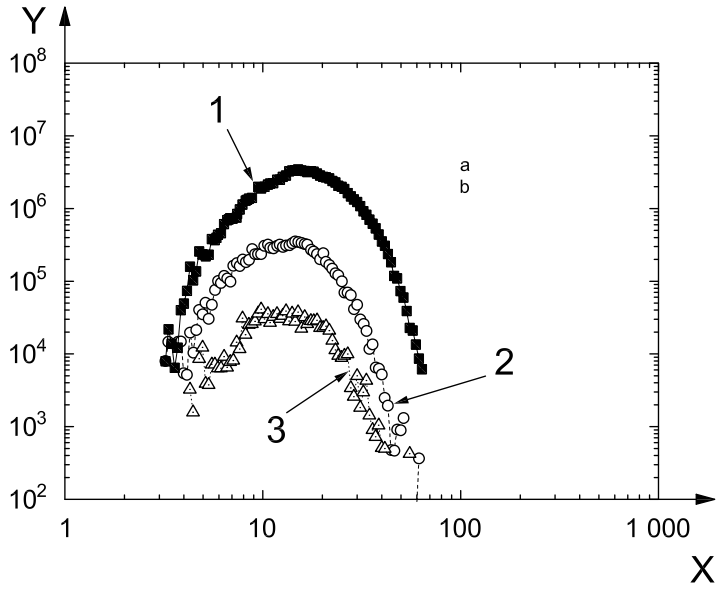
2 medium concentration group

3 low concentration group

a First diluter flow rate: 5,75 l/min.

b Second diluter flow rate: 7,50 l/min.

Figure A.2 — Particle size distribution in high, medium, and low particle concentration chambers, log-linear scale[13]



Key

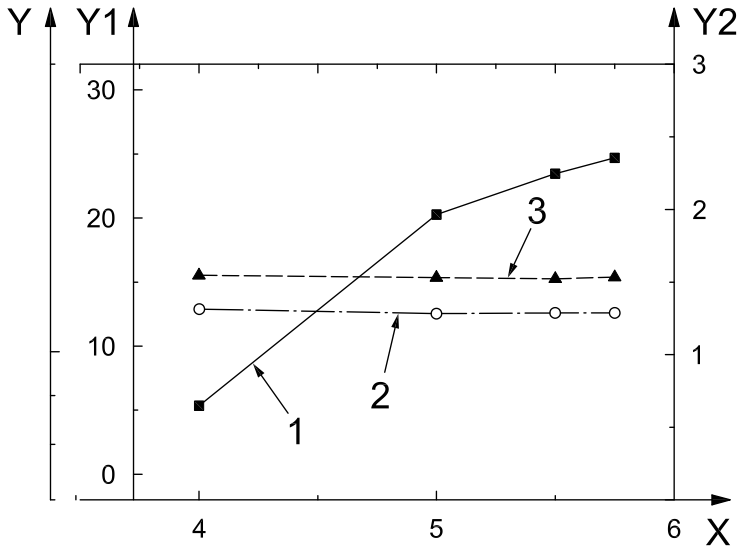
X mobility diameter, Dp (nm)
Y dN/dlog(Dp), particles/cm^3
1 high concentration group
2 medium concentration group
3 low concentration group

a First diluter flow rate: 5,75 l/min.

b Second diluter flow rate: 7,50 l/min.

Figure A.3 — Particle size distribution in high, medium and low particle concentration chambers, log-log scale[13]

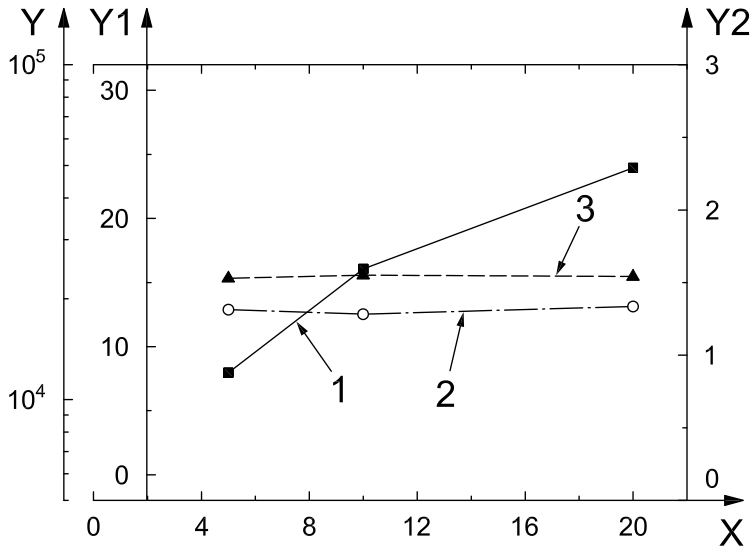
Figures A.4 and A.5 show only a slight variation in particle GMD and GSD for each sampler flow rate.



Key

X first sampler flow rate (l/min)
Y total number concentration (particles/cm^3)
Y1 geometric mean diameter (nm)
Y2 geometric standard deviation
1 total number concentration
2 geometric mean diameter
3 geometric standard deviation

Figure A.4 — Medium concentration chamber distribution in total number concentration (GMD and GSD) when varying sampler flow rate[13]

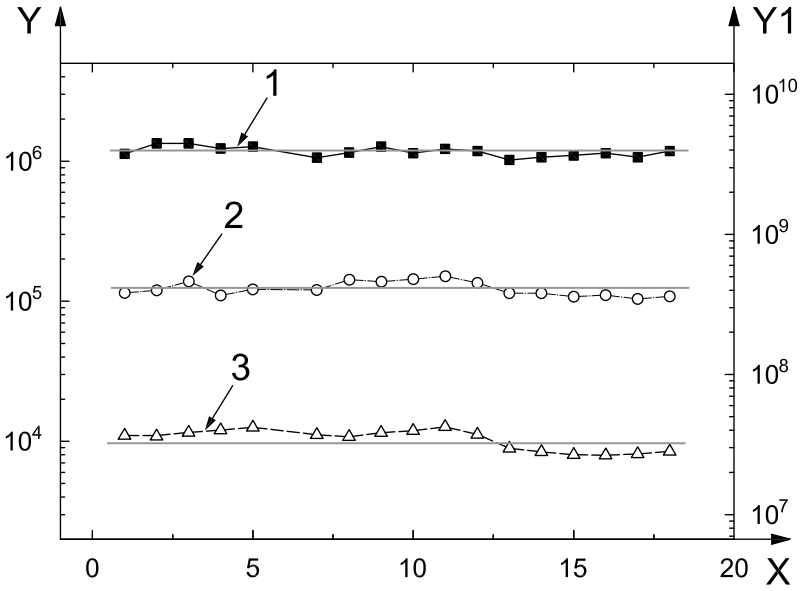


Key

X second sampler flow rate (l/min)
Y total number concentration (particles/cm^3)
Y1 geometric mean diameter (nm)
Y2 geometric standard deviation
1 total number concentration
2 geometric mean diameter
3 geometric standard deviation

Figure A.5 — Low concentration chamber distribution in total number concentration (GMD and GSD) when varying sampler flow rate[13]

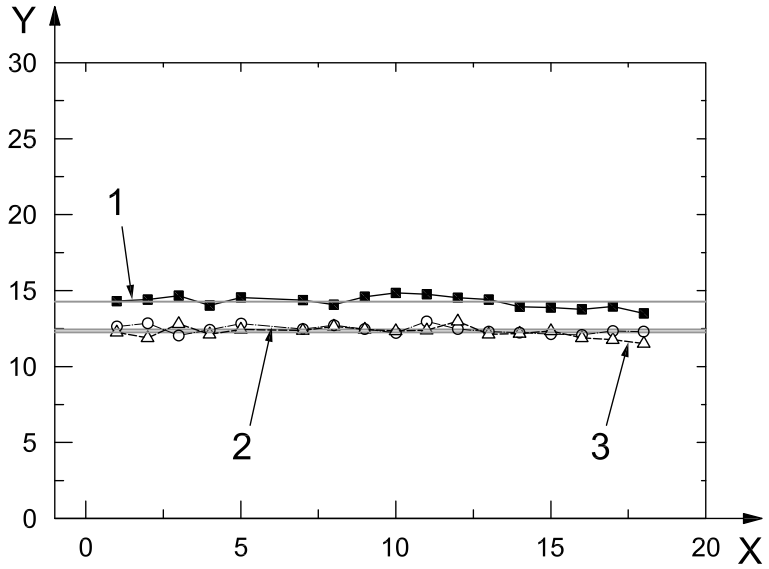
Size distribution of the silver particles was monitored over 18 h to measure variability. Figures A.6, A.7 and A.8 illustrate the variation in total-number concentration (GMD and GSD) with respect to time in the individual exposure chambers. See also Figure A.9 for the EDX profile and A.10 for cumulative size distribution.



Key

X time (hours)
Y total number concentration (particles/cm^3)
Y1 total particle generation (particles/second)
1 high concentration chamber: 8,2 %
2 medium concentration chamber: 12,1 %
3 low concentration chamber: 16,2 %

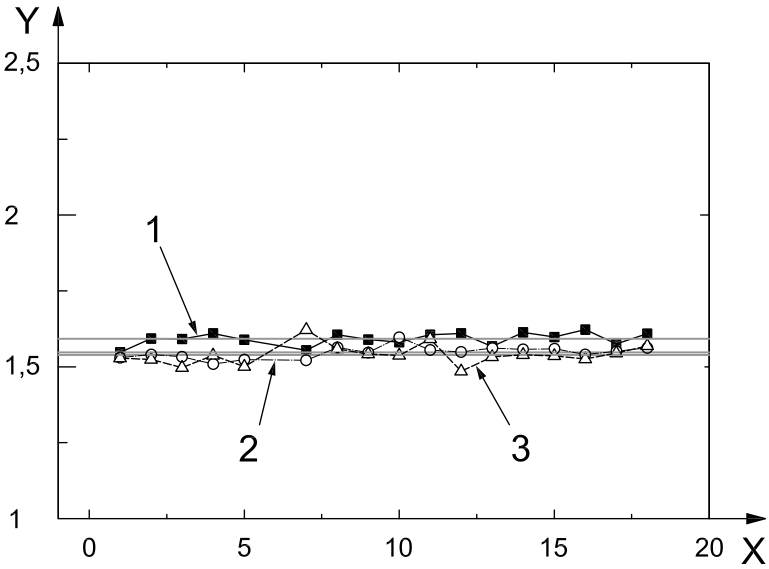
Figure A.6 — Variation in total number concentration/particle generation (GMD and GSD) of silver nanoparticles over time[13]



Key

X time (hours)
Y geometric mean diameter (nm)
1 high concentration chamber: 2,6 %
2 medium concentration chamber: 2,2 %
3 low concentration chamber: 3,0 %

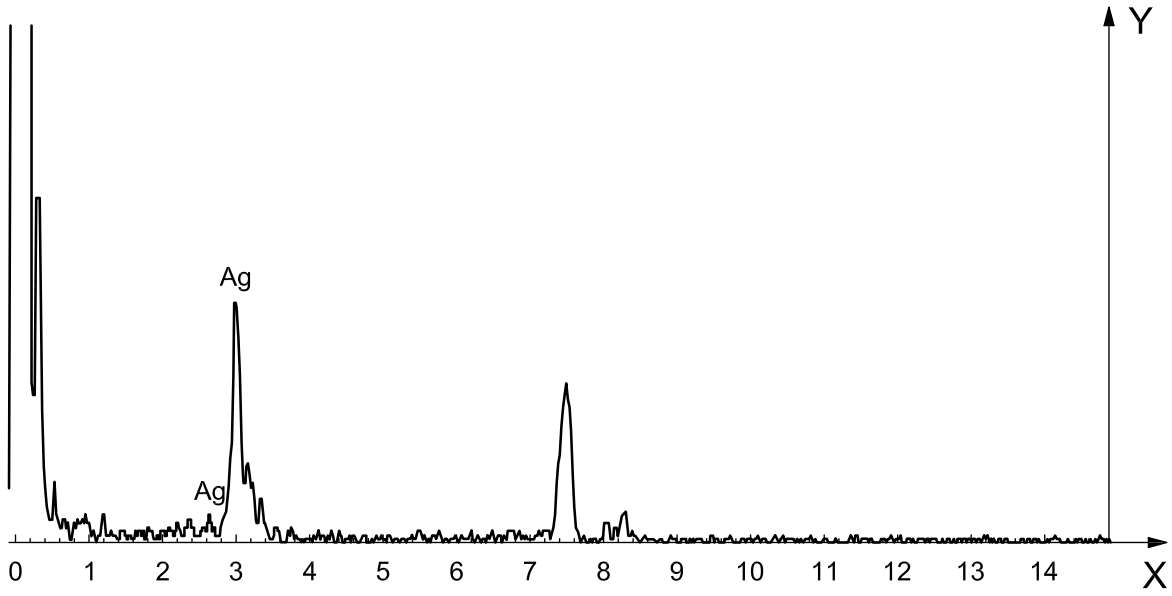
Figure A.7 — Variation in geometric mean diameter (GMD and GSD) of silver nanoparticles over time[13]



Key

X time (hours)
Y geometric standard deviation
1 high concentration chamber
2 medium concentration chamber
3 low concentration chamber

Figure A.8 — Variation in geometric standard deviation for total number concentration/particle generation (GMD and GSD) of silver nanoparticles over time[13]



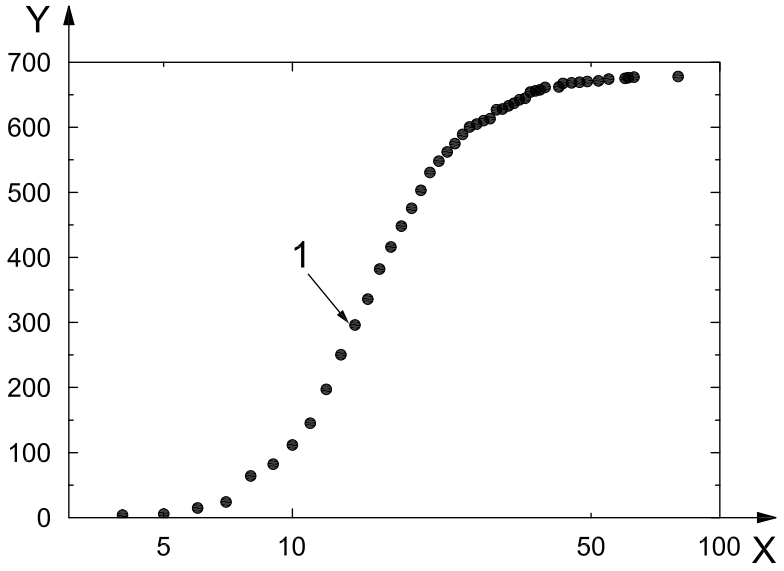
Key

X keV

Y spectrum 1

Substrate material supporting nanoparticles also appears in this profile. Non-agglomerated silver nanoparticles are seen in TEM observation.

Figure A.9 — EDX profile of distribution of nanoparticles demonstrating their pure silver composition[13]



Key

X diameter (nm)

Y cumulative requency

1 CMD

Count mean diameter (CMD) and GSD were 18 nm and 2,1 respectively. Arrow indicates CMD.

Figure A.10 — Cumulative size distribution of silver nanoparticles showing variation in median diameter[13]

Actual exposure conditions for the animal experiment — including the geometric mean diameter, total number concentration, total surface area concentration, and total volume and mass concentration for the high, medium, and low-dose chambers — is shown in Table A.1. DMAS software was used to calculate surface area, volume, and mass based on particle diameter, assuming spherical particles and using the bulk density of silver for mass concentration. The approximate surface-area concentration was obtained from the particle-number size distribution as silver nanoparticles were non-aggregate and nearly spherical.

Table A.1 — Distribution of silver nanoparticles (mean ± SE) during 28 days of inhalation toxicity testing[13]

Group	Sample Site	Diameter GMD nm	Number particles/cm^3	Surface nm^2/cm^3	Volume nm^3/cm^3	Mass µg/m^3
Control	—	0	0	0	0	0
Low	Up[a]	11,80 ± 0,17	$2,21 \times 10^4 \pm 1,12 \times 10^4$	$1,75 \times 10^7 \pm 1,03 \times 10^7$	$6,27 \times 10^7 \pm 3,95 \times 10^7$	0,65 ± 0,41
	Down[b]	12,13 ± 0,47	$1,03 \times 10^4 \pm 3,60 \times 10^2$	$6,83 \times 10^6 \pm 2,72 \times 10^5$	$2,18 \times 10^7 \pm 9,85 \times 10^5$	0,22 ± 0,01
	Total	11,93 ± 0,22	$1,73 \times 10^4 \pm 6,64 \times 10^3$	$1,32 \times 10^7 \pm 2,34 \times 10^7$	$4,60 \times 10^7 \pm 2,34 \times 10^7$	0,48 ± 0,25
Middle	Up	12,57 ± 0,23	$1,31 \times 10^5 \pm 1,33 \times 10^5$	$1,04 \times 10^8 \pm 1,65 \times 10^7$	$3,72 \times 10^8 \pm 7,58 \times 10^7$	3,89 ± 0,79
	Down	12,15 ± 0,13	$1,21 \times 10^5 \pm 7,23 \times 10^3$	$8,60 \times 10^7 \pm 8,16 \times 10^6$	$2,74 \times 10^8 \pm 2,71 \times 10^7$	2,88 ± 0,28
	Total	12,40 ± 0,15	$1,27 \times 10^5 \pm 8,45 \times 10^3$	$9,68 \times 10^7 \pm 1,04 \times 10^7$	$3,33 \times 10^8 \pm 4,66 \times 10^7$	3,48 ± 0,49
High	Up	14,74 ± 0,14	$1,35 \times 10^6 \pm 2,58 \times 10^4$	$1,43 \times 10^9 \pm 3,86 \times 10^7$	$5,92 \times 10^9 \pm 1,93 \times 10^8$	62,12 ± 2,02
	Down	14,82 ± 0,18	$1,28 \times 10^6 \pm 2,24 \times 10^4$	$1,38 \times 10^9 \pm 4,25 \times 10^7$	$5,73 \times 10^9 \pm 2,21 \times 10^8$	60,00 ± 2,31
	Total	14,77 ± 0,11	$1,32 \times 10^6 \pm 1,79 \times 10^4$	$1,41 \times 10^9 \pm 2,87 \times 10^7$	$5,84 \times 10^9 \pm 1,45 \times 10^8$	61,24 ± 1,52

[a] Animal breathing zone.

[b] Under breathing zone of caged animals.

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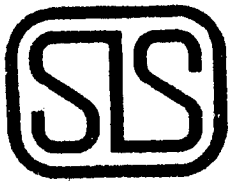

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