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)

ISO copyright office
Case postale 56 • CH-1211 Geneva 20
Tel. + 41 22 749 01 11
Fax + 41 22 749 09 47
E-mail copyright@iso.org
Web www.iso.org

Published in Switzerland

Contents

Page

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].

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

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

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

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

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.