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Nanotechnologies — Generation of metal nanoparticles for inhalation toxicity testing using the evaporation/condensation method

Nanotechnologies — Génération de nanoparticules de métal pour essais de toxicité par inhalation en utilisant la méthode de condensation/évaporation

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Foreword

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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 10801 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 manufactured nanoparticles. If nanoparticles are liberated from products, the public could be exposed as well.

There is currently limited, but growing, knowledge about the toxicity of nano-sized particles. The processes of nanoparticle production include gas-phase, vapour-phase, colloidal and attrition processes. Potential paths of exposure include inhalation, dermal and ingestion. Inhalation may arise from direct leakage from gas-phase and vapour-phase processes, airborne contamination of the workplace from deposition or product recovery and handling of product, or post-recovery processing and packing[7]. Exposure to manufactured nano-sized particles might occur during production, use and disposal in the ambient air or workplace and is of concern for public and occupational health.

There are currently neither generally accepted methods of inhalation toxicology testing for nano-sized particles nor specific nanoparticle generation methods for such testing. The ability to disperse respirable nano-sized particles from powders has been an obstacle to evaluating the effects of inhalation of nano-sized particles on the respiratory system. Although it is possible to disperse nanoparticles in air from powders, the size of the particles so generated may be larger than desired due to aggregation and agglomeration. In order to gain vital information for evaluating the health effects of nanoparticles by inhalation, nano-sized particles need to be generated and transported to a test environment containing experimental animals for testing short- or long-term inhalation toxicity. The nanoparticle generation method based on evaporation of metal (silver in this example) and subsequent condensation is capable of providing a consistent particle size distribution and stable number concentrations, suitable for short- or long-term inhalation toxicity study.

This International Standard provides a method for stable silver nanoparticle generation with particle sizes up to 100 nm. A detailed method is described in Annex A. The generation method provided here has sufficient stability for continuous inhalation toxicity testing up to 90 days. The generated nanoparticles can be used in various experimental systems, including high-throughput human cell-based labs-on-a-chip, a variety of additional *in-vitro* methods [8][9][10][11], as well as the animal experiments that may still be performed at this time, which include, but are not limited to, whole-body, head-only and nose-only. The method is not limited to the silver nanoparticles used in this example and may be used to generate other metallic nanoparticles with a similar melting temperature and evaporation rate, such as gold. However, this method is not applicable to the generation of nanoparticles of all metals.

Nanotechnologies — Generation of metal nanoparticles for inhalation toxicity testing using the evaporation/condensation method

1 Scope

This International Standard gives requirements and recommendations for generating metal nanoparticles as aerosols suitable for inhalation toxicity testing by the evaporation/condensation method. Its application is limited to metals such as gold and silver which have been proven to generate nanoparticles suitable for inhalation toxicity testing using the technique it specifies (see Annex A).

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

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

ISO/IEC 17025, *General requirements for the competence of testing and calibration laboratories*

OECD Test Guideline (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)]

3 Terms and definitions

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

3.1
differential mobility analysing 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.

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

3.2
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.

3.3
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 This definition is different from the one given in ISO 15900.

3.4
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, OECD TG 412, OECD TG 413.

3.5
evaporation/condensation nanoparticle generator system
device used to make a nanoparticle aerosol by the evaporation/condensation method, which can be connected to an inhalation chamber or other toxicity testing device

3.6
geometric mean diameter
GMD
measure of the central tendency of particle size distribution using the logarithm of particle diameters, computed for the DMAS by

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

where

d_i is the midpoint diameter for size channel i;

N is the total concentration;

ΔN_i is the concentration within 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.

3.7
geometric standard deviation
GSD
measure of width or spread of particle sizes, computed for the DMAS by

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

3.8
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 Principle

4.1 Generation

The test airborne nanoparticles are generated by heating solid silver to evaporate silver from the solid silver precursor. The entrained silver vapour is then cooled to nucleate and the vapour condensed to form a silver nanoparticle aerosol. One experimental method that describes the generation of silver nanoparticles with the evaporation/condensation method is described in Annex A.

4.2 Preparation of system

4.2.1 Prior to interfacing the nanoparticle generating system with the exposure chamber or chambers, nanoparticle size analysis should be performed to establish the number concentrations and size distribution of nanoparticles and to determine the stability of the generated aerosol. For this process, parameters selected to generate the silver nanoparticle aerosol include flow rate, evaporation temperature, quench-zone length and temperature gradients, among others. During exposure tests, analysis should be conducted continuously and/or intermittently, depending on the method of analysis, so as to determine the consistency of particle size distribution without disrupting the inhalation exposure.

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, viz. Reference [12].

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 minimum length and diameter consistent with instrument tube diameters is selected to interface with instrumentation and thereby avoid expansions and restrictions.

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 or US EPA Guidelines[31], for determining any sampling bias.

NOTE The sampling manifold consisting of conductive tubing, solenoid valves and/or other elements required for routing samples from each inhalation chamber to on-line monitoring equipment may increase particle losses and alter downstream particle size distributions if losses are dependent upon particle size.

4.2.5 Measurement instruments used in inhalation testing shall be calibrated and/or tested in accordance with ISO/IEC 17025.

The differential mobility analysing system (DMAS) is usually calibrated at the factory and this should be documented in the report.

5 Requirements

5.1 Capacity and control

Output, reliability and control of the generator shall be adequate for the planned study, as follows:

a) metal evaporation rate (μg/h);

b) air flow rate (m^3/h);

c) continuous operation of generator at target evaporation and air flow rates for study duration to be considered.

5.2 Nanoparticle properties

5.2.1 The geometric mean diameter (GMD) of nanoparticles shall be less than 100 nm. This is accomplished primarily by controlling the metal evaporation and condensation rates and the residence time in each of the reactor zones. If, despite all reasonable effort, this requirement is unable to be met, expert judgement will need to be provided.

5.2.2 The geometric standard deviation (GSD) shall be less than 2 (as proposed in OECD TG 403, OECD TG 412 and OECD TG 413).

5.2.3 Test article purity, including particle purity and particle surface purity, shall be established to meet the objective of the study. Preferably prior to the start of the study, there should be a characterization of the test article that includes its purity and, if technically feasible, the name and quantities of unknown contaminants and impurities (OECD GD 39).

NOTE Determination of the chemical purity may require characterization of the surface chemistry of the generated particles in addition to bulk chemical purity.

5.3 Exposure chamber atmosphere

5.3.1 Air delivered to test animals shall be breathable, with an adequate oxygen content of at least 19 % (OECD TG 403, OECD TG 412 and OECD TG 413; US EPA Guidelines[31]).

This may be accomplished by supplying appropriate dilution air to the generator.

5.3.2 Care shall be taken that contaminants are not generated by evaporation of volatile compounds in binders, lubricants, finishes and sealants used in the aerosol generator. This can be accomplished by selection of appropriate materials and adequate bake-out of the system.

5.3.3 The temperature of the air delivered to the test inhalation chamber shall be within the limits for inhalation studies (OECD TG 403, OECD TG 412 and OECD TG 413; US EPA Guidelines[31]).

5.3.4 Supply air to both the generator and inhalation chambers shall be free of oil, volatile compounds and other contaminants, and shall be HEPA-filtered to remove aerosols, including nanoparticles, dust and microorganisms.

5.4 System operational safety

5.4.1 All local safety requirements shall be respected.

5.4.2 Contact with hot surfaces and electrical conductors associated with the electrical heater or other components shall be prevented.

5.4.3 Gas discharged to the atmosphere from the system shall be HEPA-filtered.

5.4.4 There shall be no measurable leaks to the atmosphere from the aerosol generator.

5.4.5 Exposure chambers should be maintained at negative pressure ($\leqslant$ 5 mm water) with respect to ambient conditions in order to avoid worker exposure in case of leakage. This pressure differential should be monitored on a continuous basis and arranged to be kept within alarm limits. An alternative approach is to maintain the apparatus at positive pressure with respect to ambient conditions to ensure that aerosols or airborne contaminants cannot enter the exposure chamber. The apparatus at positive pressure should be enclosed within ventilated secondary containment in order to minimize worker exposure.

For nose-only exposure, pressure should be slightly positive so as to ensure that 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 (OECD GD 39).

NOTE Frequent leak checks, e.g. by the soap bubble method, or the installation of permanent leak detectors may be necessary when there is a risk of nanomaterial leakage. In nose-only exposure systems, the test atmosphere could leak around the animal where it meets the exposure apparatus. Leaks can be prevented by using a restraint system that seals the tube, although heat and moisture buildup in the tube is a concern[29].

6 Characterization of generator performance

6.1 Requirements for particle size distribution and mass concentration

Measurement of particle size distribution and total particle mass concentration are essential for the characterization of nanoparticles for inhalation toxicity testing. In the case of particle size distribution, this is because the knowledge of particle size influences dose and dose distribution while mass concentration is the dosimetric parameter used routinely in inhalation toxicity testing. In evaluating the nanoparticle aerosol generator used for inhalation toxicity testing, these particle size distribution and total particle mass-concentration measurements shall always be made.

6.2 Particle size distribution measurement

The method shall include near-continuous monitoring based upon the scan speed of the classification and detection instruments, with a time resolution appropriate for verifying the stability of the nanoparticle generator in terms of particle size distribution and concentration. The measurement method used shall be comprehensive for nanoparticle aerosols produced by the generator. The accuracy of particle size and concentration measurements shall be sufficient for nanoparticle toxicity testing, and may be validated by methods such as calibration against appropriate reference standards. The particle diameter range of particle sizing shall be sufficiently broad so that all relevant data are recorded to reduce errors in conversion from number-weighted distribution to surface-area-weighted or volume-weighted distribution.

NOTE For number-based particle size distribution, measurement using DMAS is the only currently available method that meets all the above requirements in the size range below 100 nm.

6.2.1 Sampling with DMAS

Nanoparticles should be measured following manufacturer's recommendations and in accordance with ISO 15900.

6.2.2 Sampling for microscopy

The filters on which the particles are sampled shall be coated with carbon (to reduce charging during analysis), mounted on an electron microscope grid (200 mesh), and observed under a transmission electron microscope (TEM). Diameters of randomly selected particles should be measured at 100 000× magnification, and analysed using an energy-dispersive x-ray analyser (EDXA) at an appropriate accelerating voltage for the target. ISO 10312[3] can be adapted for the sampling and analysis to determine nanoparticle morphology. Additional details on sampling may be obtained from other sources [6][13][15][17][18][27].

6.3 Mass concentration measured by filter sampling

Gravimetric filter analysis is the method used for measuring total nanoparticle concentrations, in which the test atmosphere is sampled from the animal's breathing zone. The mass concentration is calculated by dividing the mass of the nanoparticles collected on the filter by the volume of air passed through the filter.

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

Obtaining adequate mass loading to generate data above lower detection limits should be considered [5].