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Nanotechnologies — Lung burden mass measurement of nanomaterials for inhalation toxicity tests

Nanotechnologies — Mesure de la masse de la charge pulmonaire des nanomatériaux pour les études de toxicité par inhalation

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Foreword

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Introduction

Inhalation is a primary route of exposure to aerosolized nanomaterials and therefore appropriate inhalation toxicity tests are required to address risk assessment needs for these materials. For this reason, the Organisation for Economic Cooperation and Development (OECD) recently updated its inhalation toxicity test guidelines 412 (subacute) and 413 (subchronic) to make them applicable to nanomaterials.[1][2] These revised test guidelines require post-exposure lung burden measurements to be undertaken when a range-finding study or other relevant information suggests that inhaled test nanomaterials are poorly soluble with low dissolution rate and likely to be retained in the lung. The measurements of lung burden inform on pulmonary deposition and retention of nanomaterials in the lung. At least three lung burden measurements are needed to evaluate clearance kinetics.

This document gives information on how to derive clearance kinetic parameter values using lung burden measurement data. This document complements OECD TG 412[1] and OECD TG 413[2]. As References [1], [2] and [3] only provide limited information on methods for lung burden measurement for nanomaterials or the derivation of lung clearance kinetics, this document provides useful supporting information for conducting inhalation studies based on these test guidelines.

Nanotechnologies — Lung burden mass measurement of nanomaterials for inhalation toxicity tests

1 Scope

The document provides information on the measurement of nanomaterial mass in tissue after inhalation exposure, which can inform on lung clearance behaviour and translocation.

2 Normative references

The following documents are referred to in the text in such a way that some or all of their content constitutes requirements 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 80004 (all parts), *Nanotechnologies — Vocabulary*

3 Terms and definitions

For the purposes of this document, the terms and definitions given in the ISO 80004 series and the following apply.

ISO and IEC maintain terminology databases for use in standardization at the following addresses:

— ISO Online browsing platform: available at https://www.iso.org/obp

— IEC Electropedia: available at https://www.electropedia.org/

3.1
aerodynamic diameter
diameter of a spherical particle with a density of 1 000 kg/m^3 that has the same settling velocity as the particle under consideration

Note 1 to entry: Aerodynamic diameter is related to the inertial properties of *aerosol* (3.2) particles and is generally used to describe particles larger than approximately 100 nm.

[SOURCE: ISO/TR 27628:2007, 2.2[4]]

3.2
aerosol
metastable suspension of solid or liquid particles in a gas

[SOURCE: ISO/TR 27628:2007, 2.3[4]]

3.3
mass median aerodynamic diameter
MMAD
calculated *aerodynamic diameter* (3.1) which divides the particles of a measured *aerosol* (3.2) distribution in half based on the mass of the particles where fifty percent of the particles by mass will be larger than the median diameter and fifty per cent of the particles will be smaller than the median

[SOURCE: EPA IRIS Glossary[12]]

3.4
manufactured nanomaterial
nanomaterial (3.8) intentionally produced for commercial purposes to have specific properties or specific composition

[SOURCE: ISO/TS 80004-1:2015, 2.9[8], modified — "for commercial purposes" has been added to the definition.]

3.5
mixture
mixture composed of two or more substances in which they do not react

Note 1 to entry: A solution is a mixture as well.

[SOURCE: GHS, 2011[9]]

3.6
mobility
propensity for an *aerosol* (3.2) particle to move in response to an external influence, such as an electrostatic field, thermal field or by diffusion

[SOURCE: ISO/TR 27628:2007, 2.9[4], modified — the domain "<aerosols>" has been removed.]

3.7
nanofibre
nano-object with two similar external dimensions in the nanoscale and the third dimension significantly larger

Note 1 to entry: A nanofibre can be flexible or rigid.

Note 2 to entry: The two similar external dimensions are considered to differ in size by less than three times and the significantly larger external dimension is considered to differ from the other two by more than three times.

Note 3 to entry: The largest external dimension is not necessarily in the nanoscale.

[SOURCE: ISO/TS 80004-2:2015, 4.5[13], modified — Notes 1 and 2 to entry have been replaced.]

3.8
nanomaterial
material with any external dimension in the nanoscale or having internal structure or surface structure in the nanoscale

Note 1 to entry: This generic term is inclusive of *nano-object* and nanostructured material.

Note 2 to entry: See also engineered nanomaterial, manufactured nanomaterial and incidental nanomaterial.

[SOURCE: ISO/TS 80004-1:2015, 2.4[8]]

3.9
nanoparticle
nano-object with all external dimensions in the nanoscale where the lengths of the longest and the shortest axes of the nano-object do not differ significantly

Note 1 to entry: If the dimensions differ significantly (typically by more than 3 times), terms such as *nanofibre* (3.7) or *nanoplate* may be preferred to the term nanoparticle.

Note 2 to entry: Ultrafine particles may be nanoparticles.

[SOURCE: ISO/TS 80004-2:2015, 4.4[13]]

3.10
nanotube
hollow *nanofibre* (3.7)

[SOURCE: ISO/TS 80004-2:2015 4.8[13]]

3.11
single-walled carbon nanotube
SWCNT
SWCNT single-walled carbon nanotube consisting of a single cylindrical graphene layer

Note 1 to entry: The structure can be visualized as a graphene sheet rolled into a cylindrical honeycomb structure.

[SOURCE: ISO/TS 80004-1:2015, 4.4[8]]

3.12
multi-wall carbon nanotube
MWCNT
MWCNT multi-walled carbon nanotube composed of nested, concentric or near-concentric graphene sheets with interlayer distances similar to those of graphite

Note 1 to entry: The structure is normally considered to be many *single-walled carbon nanotubes* (3.11) nesting each other, and would be cylindrical for small diameters but tends to have a polygonal cross-section as the diameter increases.

[SOURCE: ISO/TS 80004-1:2015, 4.6[8]]

3.13
particle
minute piece of matter with defined physical boundaries

Note 1 to entry: A physical boundary can also be described as an interface.

Note 2 to entry: A particle can move as a unit.

Note 3 to entry: This general definition applies to particle *nano-objects*.

[SOURCE: ISO/TS 26824:2013, 1.4[5]]

3.14
poorly soluble particle
inhaled test particles that are likely to be retained in the lung

[SOURCE: OECD TG 412, paragraph 2 [1]]

3.15
primary particle
original source particle of *agglomerates* or *aggregates* or mixtures of the two

Note 1 to entry: Constituent particles of agglomerates or aggregates at a certain actual state may be primary particles, but often the constituents are aggregates.

Note 2 to entry: Agglomerates and aggregates are also termed secondary particles.

[SOURCE: ISO 26824:2013, 1.4[5]]

3.16
secondary particle
particle formed through chemical reactions in the gas phase (gas to particle conversion)

[SOURCE: ISO/TR 27628:2007, 2.17[4]]

4 Abbreviated terms

AAS	Atomic absorption spectroscopy
AgNP	Silver nanoparticles
AuNP	Gold nanoparticles
BALF	Bronchoalveolar lavage fluid
CoO	Cobalt oxide
CuO	Copper oxide
DEMC	Differential electrical mobility classifier
DEMS	Differential electrical mobility spectrometer
ECA	Elemental carbon analysis
GD	Guidance document
GHS	Globally harmonized system
HPLC	High performance liquid chromatography
ICP-MS	Inductively coupled plasma mass spectrometry
LALN	Lung-associated lymph node
MMAD	Mass median aerodynamic diameter
NDIR	Non-dispersive infrared
OECD	Organisation for Economic Cooperation and Development
PEO	Post-exposure observation
sp-ICP-MS	Single particle ICP-MS
TG	Test guideline
TiO_2	Titanium dioxide
UV-Vis	Ultraviolet-visible spectrophotometer
WPMN	Working Party on Manufactured Nanomaterials
ZnO	Zinc oxide

5 Use of lung burden measurements for risk assessment of nanomaterials

The concept of lung overload hypothesis was first proposed in Reference [15]. The determination of lung burden of inhaled nanomaterials is therefore of great relevance to assess a possible lung overload.[16] [17] Morrow[15] has also proposed that a continuously increasing prolongation of particle lung clearance occurs when the retained lung burden exceeds a certain threshold. Decreased clearance capacity of alveolar macrophages will lead to inflammatory reactions and to an increase in the translocation of the inhaled particles to interstitium and lung-associated lymph nodes[18].

Lung burden data can be used for the risk assessment of poorly soluble with low dissolution rate particles (e.g. as obtained from tests according to References [1] and [2]). When pulmonary effects are

driving the human health risk assessment, risk assessors need to evaluate whether the occurrence of the pulmonary effects is better characterized by administered exposure concentration or by retained dose in the lungs. The human equivalent dose and lifetime human exposure may be calculated for risk estimation. Applications of such principles are available in literature, e.g. References [19] and [20]. Another value of lung burden data is the possibility of reading across hazard data from studies using the same material with different primary particle sizes.[21] The same external concentrations can result in differences in retained dose. Conversely, different external concentrations can result in the same retained dose for different particle sizes[22].

6 Inhalation exposure and tissue sampling to determine lung burden

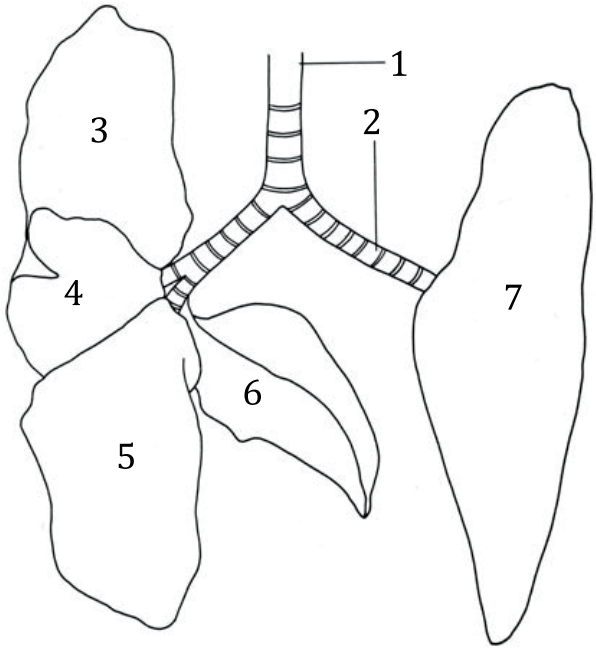
6.1 Inhalation exposure

For inhalation exposure of nanomaterials, nanomaterials are frequently generated in situ or powdered forms of nanomaterials are dispersed and generated and delivered into the inhalation chamber. The generation of nanomaterials aerosol for inhalation toxicity testing is described in ISO/TR 19601[23] and ISO 10801[24] and monitoring of such aerosols in the inhalation chamber was described in ISO 10808[11]. These bibliographical references also provide methods of aerosol concentration monitoring and physicochemical characterization as well as OECD test guidelines.

6.2 Lung burden evaluation in single or multiple lobes

For inhalation toxicity testing of nanomaterials, please refer to References [1] and [2]. Depending on the type of nanomaterial, the study director can use data from a range-finding study to determine the appropriate post-exposure duration as well as the optimal number and timing of sampling intervals for a repeated exposure inhalation study. Although the TGs require using one lung (right lung) for lung burden measurement and the other lung (left lung) for histopathological evaluation, recent studies with AgNPs and AuNPs demonstrated that nanoparticles deposit in the rat lung lobes evenly, thus, any lobe can be used for lung burden measurement.[25][26] As shown in Figure 1, the right lung lobe consisted of four lobes. Soluble nanoparticles with high dissolution rate such as silver nanoparticles[25][27] as well as poorly soluble with low dissolution rate particles such as gold nanoparticles[26] were evenly deposited in rat lungs after subacute (5 d) inhalation exposure. Using any lobe for lung burden measurements opens the opportunity to use the remaining lobes for other measurements, such as histopathological tissue preparation and bronchoalveolar lavage fluid (BALF) assay, in the same rat. Such an approach can maximize the number of endpoints measured and has the potential to reduce the number of animals used in testing. Although fibrous or plate forms of nanomaterials such as carbon nanotubes and graphenes were not tested and proven for even deposition throughout the lung lobes, some lung burden and thereafter lung clearance kinetic study has been conducted for carbon nanotubes.

A recent study given in Reference [28]. on the lung deposition and retention of multi-walled carbon nanotubes (MWCNTs) (mass median aerodynamic diameter (MMAD) is 1,015 µm) after 28 d of inhalation and for 28 d post-exposure showed that the lung clearance kinetics of MWCNTs can be effectively evaluated using one lobe from the right lung.[28] The BAL fluid was collected from the right lung after occluding the post-caval lobe and left lung. The left lung was then used to evaluate the histopathology and the post-caval lobe to evaluate the lung burden.[26] In another recent study, quantitative analyses of lung burdens on various shapes of carbon nanomaterials including printex-90 carbon black (50 mg/m^3), nanomaterial NM-401 (0,5 mg/m^3 and 1,5 mg/m^3), NM-403 (1,5 mg/m^3), and MWCNT-7 (1,5 mg/m^3) nanotubes were conducted after 28-d inhalation exposure. Their MMAD was 940 nm for printex-90 carbon black, 790 nm for NM-401, 1 940 nm for NM-403, and 1 780 nm for MWCNT. The middle right lobe was separated and used for lung burden analysis successfully[29].



Key

1 trachea
2 left bronchus
3 superior lobe
4 middle lobe
5 inferior lobe
6 post-caval lobe
7 left lung

Figure 1 — Rodent trachea and lungs[3]

6.3 Post-exposure observation points

Although References [1] and [2] prescribe only one mandatory sampling point [post-exposure observation (PEO)-1)], it is recommended to conduct two additional sampling points (PEO-2 and PEO-3) right after the termination of exposure (PEO-1, post-exposure observation) to conduct toxicokinetic or particokinetics studies. The concept of "particokinetics" is introduced to address the dynamic biological behaviour of ENMs at the molecular level (including gravitational sedimentation, dispersion, aggregation, and interaction with biomolecules in suspending media), cellular level (including cellular uptake, transport, biotransformation and elimination), and whole-organism level (including absorption, distribution, metabolism and excretion in vivo).[30][31][32][33][34] In addition, lung burden measurement at exposure d-1 (E-1, 6-h exposure) can provide information about the solubility of test nanomaterials and the retention trend after the designed exposure period, because lung retention time and biopersistence increases as particles are insoluble.

Additional satellite groups can be added to the main study to evaluate recovery, persistence, delayed occurrence of toxicity, or lung burden for a post-treatment period of an appropriate length. Designs of main studies with satellite groups are shown in Annexes A and B. The study director should modify the design of a study based on the physicochemical characteristics and kinetics of a test chemical to achieve the most robust data.

All satellite groups are exposed concurrently with the experimental animals in the main study and at the same concentration levels and there should be concurrent air or vehicle controls as needed. The scheduling and design of satellite groups depend on whether the test chemical is a solid aerosol and is likely to result in lung retention following Annexes A and B. If the test chemical is likely to result in lung