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Nanotechnologies — Characterization of carbon nanotube and carbon nanofibre aerosols to be used in inhalation toxicity tests

Nanotechnologies — Caractérisation des aérosols de nanotubes de carbone et de nanofibres de carbone à utiliser dans les tests de toxicité par inhalation

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Foreword

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

Any feedback or questions on this document should be directed to the user’s national standards body. A complete listing of these bodies can be found at www.iso.org/members.html.

Introduction

Inhalation is the primary route of exposure to aerosolised carbon nanotubes (CNTs) and carbon nanofibres (CNFs). Exposure to CNTs or CNFs can occur in consumer settings as well as in occupational settings. Occupational exposure to CNTs or CNFs can occur at all phases of the manufacturing, handling, and formulation of the material into final products[1,2]. Consumers are potentially exposed to CNTs or CNFs released as products of degradation, weathering, or mechanical processes (e.g. grinding or polishing) from consumer products that contain CNT or CNF embedded into a matrix[3,4].

Similar to other nanomaterials, the physicochemical properties of CNTs or CNFs are greatly diverse in terms of diameter, length, shape, arrangement of carbon atoms, surface chemistry, defects, and impurities. Their different physicochemical characteristics are responsible for different functional properties such as mechanical, electrical, optical, and thermal properties. Many previous inhalation toxicity studies of CNT and CNF aerosols reported various hazards from acute inflammation to carcinogenicity and the toxicological responses to CNT and CNF aerosols vary depending on their physicochemical characteristics[5].

Among the various physicochemical characteristics, morphological factors such as length and rigidity have been suggested as key parameters related to the toxicity of CNT and CNF aerosols[6,7]. CNT and CNF aerosols can consist of individual primary fibres in the nanoscale[8] and aggregated or agglomerated structures, including those with diameters larger than 100 nm[9]. Among various types of CNT and CNF, the asbestos-like pathogenicity has been observed only in long (>5 µm) and rigid fibres, but not in short or tangled CNT[6]. Thus, a better understanding of the characteristics of generated CNT or CNF aerosols in relation to toxicity end points is key for risk assessment and safer-by-design approaches.

The framework for material characterization for inhalation studies consists of (1) characterization of as-produced (pristine) or supplied material, (2) characterization of administered material, (3) characterization of material following administration, and (4) human exposure characterization[10]. This document focuses on the first two characterization needs, which include physicochemical properties (e.g. size, size distribution, aggregation/agglomeration, and shape) and measurement of concentration (e.g. mass, number, surface area, and volume). These parameters can be measured by direct (online) or indirect (off-line) methods and each technique needs specific sampling procedures. However, the limited technologies in the generation and characterization of nanofibres make it difficult to perform inhalation toxicity studies, although the inhalation exposure to CNT and CNF is highly likely in the workplace[9,11], and research facilities[8], where they are in use. In this regard, this document provides the current status of CNT and CNF aerosol characterization used in the inhalation toxicity tests as well as the physicochemical properties of CNTs and CNFs and their relationship with toxicity end points.

This document complements the work of other international organizations including the Organization for Economic Co-operation and Development (OECD) which has published guidelines and guidance on the performance of inhalation toxicity studies[12,13]. ISO 10808 describes the characterization of nanoparticles in inhalation exposure chambers for inhalation toxicity testing. This document is different from ISO 10808 and focuses on different types of nanomaterials (nanotubes and nanofibres opposed to nanoparticles) because many characterization methods and important physicochemical parameters related to the toxicity of CNT and CNF are different from those of nanoparticles. Recommendations and guidelines to assist investigators in making appropriate choices for the characterization of CNT and CNF aerosols to be studied are presented in this document.

Nanotechnologies — Characterization of carbon nanotube and carbon nanofibre aerosols to be used in inhalation toxicity tests

1 Scope

This document reviews characterization of CNT and CNF aerosols for inhalation exposure studies. The document also provides useful information on appropriate characterization of CNT and CNF, which is required to evaluate and understand the inhalation toxicity of CNT and CNF aerosols. This document neither provides guidance on aerosol characterization for other carbon nanomaterials, nor provides guidance for characterization of carbon nanotube and nanofibre aerosols in the workplace or ambient air.

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 ISO 80004 (all parts), 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
carbon nanotube
nanotube composed of carbon

Note 1 to entry: Carbon nanotubes usually consist of curved graphene layers, including single-wall carbon nanotubes and multiwall carbon nanotubes.

[SOURCE: ISO/TS 80004-3:2020, 3.3.3]

3.2
multiwall carbon nanotube
MWCNT
multi-walled *carbon nanotube* (3.1) 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-wall carbon nanotubes 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 80004-3:2020, 3.3.6]

3.3
single-wall carbon nanotube
SWCNT
carbon nanotube (3.1) 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 80004-3:2020, 3.3.4]

3.4
carbon nanofibre
CNF
nanofibre (3.5) composed of carbon

[SOURCE: ISO/TS 80004-3:2020, 3.3.1]

3.5
nanofibre
nano-object (3.28) with two similar external dimensions in the nanoscale and the third dimension significantly larger

Note 1 to entry: A *nanofibre* (3.5) 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]

3.6
aerosol
metastable suspension of solid or liquid particles in a gas

[SOURCE: ISO TR 27628:2007, 2.3]

3.7
inhalation chamber system
system prepared to expose experimental animals to an inhaled test substance of predetermined duration and dose by either nose-only or whole-body method

Note 1 to entry: This system consists of chamber, head-only and nose-only.

Note 2 to entry: The term “nose-only” includes head-only, nose-only, or snout-only.

Note 3 to entry: [SOURCE: OECD TG 403[18], 412[12], 413[13]]

3.8
nanoparticle generation system
device to make nanoparticle aerosol with controlled size distribution and concentration

[SOURCE: ISO 10808:2010, 3.3]

3.9
aspect ratio
ratio of length to width of a particle

[SOURCE: ISO 10312:2019, 3.8]

3.10
rigidity
inability to be to bent or forced out of shape or ability of a material to resist deformation

Note 1 to entry: This term applies to CNT or CNF.

Note 2 to entry: Asbestos fibres and MWNT-7 are examples of rigid structures.

3.11
aggregate
particle comprising strongly bonded or fused particles where the resulting external surface area is significantly smaller than the sum of calculated surface areas of the individual components

Note 1 to entry: The forces holding an aggregate together are strong forces, for example, covalent bonds, or those resulting from sintering or complex physical entanglement, or otherwise combined former primary particles.

Note 2 to entry: Aggregates are also termed secondary particles and the original source particles are termed primary particles.

[SOURCE: ISO 26824:2013, 1.3]

3.12
agglomerate
collection of weakly or medium strongly bound particles where the resulting external surface area is similar to the sum of the surface areas of the individual components

Note 1 to entry: The forces holding an agglomerate together are weak forces, for example van der Waals forces or simple physical entanglement.

Note 2 to entry: Agglomerates are also termed secondary particles and the original source particles are termed primary particles.

[SOURCE: ISO/TS 80004-2:2015, 3.4]

3.13
biodurability
ability of a material to resist *dissolution* (3.14) and mechanical disintegration from chemical and physical clearance mechanisms

[SOURCE: ISO/TR 19057:2017, 3.3]

3.14
dissolution
process of obtaining a solution containing the analyte of interest

Note 1 to entry: Dissolution is the act of dissolving and the resulting species may be molecular or ionic.

[SOURCE: ISO/TR 19057:2017, 3.6]

3.15
aerodynamic diameter
diameter of a sphere of 1 g cm^{-3} density with the same terminal settling velocity in calm air as the particle, under the prevailing conditions of temperature, pressure and relative humidity

Note 1 to entry: The particle aerodynamic diameter depends on the size, density and shape of the particle.

Note 2 to entry: Aerodynamic diameter is related to the inertial properties of aerosol particles.

[SOURCE: ISO 4225:2020, 3.1.5.13]

3.16
differential mobility analysing system
DMAS
system to measure the size distribution of submicrometer aerosol particles consisting of a *DEMC* (3.19), a particle charge conditioner, flow meters, a particle detector, interconnecting plumbing, a computer, and suitable software

[SOURCE: ISO 15900: 2020, 3.12]

3.17
geometric mean diameter
GMD
measure of the central tendency of particle size distribution using the logarithm of particle diameters

Note 1 to entry: The GMD is normally computed from particle counts and when noted may be based on surface area or particle volume with appropriate weighting, as:

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

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

[SOURCE: ISO 10808:2010, 3.5]

3.18
geometric standard deviation
GSD
measure of the width or spread of particle sizes, computed for the *DMAS* (3.16) by

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

[SOURCE: ISO 10808:2010, 3.6]

3.19
differential electrical mobility classifier
DEMC
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 to entry: A DEMC is sometimes called a Differential Electrical Mobility Spectrometer (DEMS). A DEMC classified 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.

[SOURCE: ISO 10801: 2010, 3.2]

3.20
count median diameter
CMD
diameter equal to *GMD* (3.17) for particle counts assuming a logarithmic normal distribution

Note 1 to entry: The general form of the relationship as described in ISO 9276-5:2005 is

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

where

e	is the base of natural logarithms, e = 2,718 28;
p	is the dimensionality (type of quantity) of a distribution
	$p = 0$ 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.

[SOURCE: ISO 10808:2010, 3.7]

3.21
mass median aerodynamic diameter
MMAD
calculated aerodynamic diameter which divides the particles of an aerosol in half based on mass of the particles

Note 1 to entry: Fifty percent of the particles by mass will be larger than the median diameter and 50 per cent of the particles will be smaller than the median.

[SOURCE: EPA IRIS Glossary; ISO 15779:2011, 3.30]

3.22
mobility diameter
diameter of a spherical particle that has the same mobility as the particle under consideration

Note 1 to entry: Mobility diameter is generally used to describe particles smaller than approximately 500 nm, and is independent of the density of the particle

[SOURCE: ISO/TR 27628:2007, 2.10]

3.23
particle density
ratio obtained by dividing the mass of a sample of aggregate particles by the volume, including both permeable and impermeable pores within the particles (but not including the voids between the particles)

Note 1 to entry: It is expressed as mass per unit volume, i.e. kilograms per cubic meter (kg/m^3)

[SOURCE: ISO 20290-1, 3.2]

3.24
specific surface area
surface area per unit mass of a particle or material

[SOURCE: ISO/TR 27628:2007, 2.19]

3.25
respirable fraction
mass fraction of inhaled particles which penetrate to the unciliated airways

[SOURCE: ISO 7708:1995, 2.11]

3.26
inhalable fraction
fraction of total airborne particles of given particle size inhaled through the nose and mouth

Note 1 to entry: Adapted from ISO 7708:1995, 2.3.

Note 2 to entry: The fractions specified in 3.3 to 3.8, as defined at specific particle size (characterized by thermodynamic and aerodynamic diameters), are independent of the basis of measurement, e.g. mass, area or particle count.

Note 3 to entry: A significant portion of the inhaled particles may be exhaled, but since these are smaller particles their effect on the mass deposited may be minimal.

[SOURCE: ISO 13138:2012, 3.3]

3.27
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]

3.28
nano-object
discrete piece of material with one, two or three external dimensions in the nanoscale

Note 1 to entry: The second and third external dimensions are orthogonal to the first dimension and to each other.

[SOURCE: ISO/TS 80004-2:2015, 2.2]

3.29
nanoparticle
nano-object (3.28) 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* (3.5) or *nanoplate* (3.30) may be preferred to the term nanoparticle.

Note 2 to entry: Ultrafine particles may be nanoparticles.

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

3.30
nanoplate
nano-object (3.28) with one external dimension in the nanoscale and the two other external dimensions significantly larger

Note 1 to entry: The larger external dimensions are not necessarily in the nanoscale.

[SOURCE: ISO/TS 80004-2:2015, 4.6]

3.31
nanotube
hollow *nanofibre* (3.5)

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

3.32
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 26824:2013, 1.1]

3.33
primary particle
original source *particle* (3.32) of *agglomerates* (3.12) or *aggregates* (3.11) 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]

3.34
hazard
source with a potential to cause injury and ill health

Note 1 to entry: Hazards can include sources with the potential to cause harm or hazardous situations, or circumstances with the potential for exposure leading to injury and ill health.

[SOURCE: ISO 45001:2018, 3.19]

