TECHNICAL REPORT

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Nanotechnologies — Guidance on physico-chemical characterization of engineered nanoscale materials for toxicologic assessment

Nanotechnologies — Directives relatives à la caractérisation physico-chimique des matériaux machinés à l'échelle nanométrique pour l'évaluation toxicologique

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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

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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.

In exceptional circumstances, when a technical committee has collected data of a different kind from that which is normally published as an International Standard ("state of the art", for example), it may decide by a simple majority vote of its participating members to publish a Technical Report. A Technical Report is entirely informative in nature and does not have to be reviewed until the data it provides are considered to be no longer valid or useful.

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/TR 13014 was prepared by Technical Committee ISO/TC 229, *Nanotechnologies*.

Introduction

The last few years have seen a large increase in the use of nanomaterials in consumer and other products, and this increase has been accompanied by growing concern about the possible health and environmental impacts of exposure to nanomaterials, in particular to nano-objects, and their agglomerates, and aggregates (NOAA). While a large number of toxicological studies on materials in NOAA form have been reported, many have failed to provide detailed physico-chemical characterization of what has been tested, to evaluate the results obtained and to compare test results. Given the diversity of NOAAs that can be produced with seemingly similar composition, detailed physico-chemical characterization is critical for the precise identification of test materials and to support the development of understanding the toxicological impact of nanomaterials.

This Technical Report provides guidance for the physico-chemical characterization of manufactured nano-objects (those nano-objects that are intentionally produced for commercial purposes) prior to toxicological assessment, including both human and ecological-based assessments. The purpose of this Technical Report is to assist health scientists and experts from other disciplines to understand, plan, identify and address relevant physico-chemical characterization of such materials before conducting toxicological tests on them. Such activity should be seen as a prerequisite to any biological evaluation and is consistent with other ISO documents. For example, ISO 10993-18[1] specifically addresses the chemical characterization of materials used in medical devices, and ISO 14971[2] points out that a toxicological risk analysis takes into account the chemical nature of the materials.

Characterization is expected to provide valuable information about the influence of physico-chemical properties on the responses observed in toxicological testing. This Technical Report provides the following information which will be of value in the physico-chemical characterization of manufactured nano-objects submitted for toxicological assessment:

— how physico-chemical characterization fits within the framework of toxicological testing of NOAAs;

— physico-chemical characteristics deemed critical for assessment before toxicological testing; and

— what should be measured to assess the physico-chemical characteristics.

Nanotechnologies — Guidance on physico-chemical characterization of engineered nanoscale materials for toxicologic assessment

1 Scope

This Technical Report provides guidance for the physico-chemical characterization of manufactured nano-objects and their aggregates and agglomerates (NOAA) greater than 100 nm presented for toxicological testing in order to aid in assessing and interpreting the toxicological impact of manufactured nano-objects and to allow the material under test to be differentiated from seemingly similar materials. For each of the selected properties, a description, clarification, relevance, measurand and example measurement methods are provided.

This Technical Report will be of value to parties (e.g. toxicologists, ecotoxicologists, regulators, health and safety professionals) interested in assessing and interpreting the potential toxicological effect of manufactured NOAAs.

2 Terms and definitions

For the purposes of this document, the terms and definitions given in ISO/TS 27687, ISO/TS 80004-1, ISO/TS 80004-3, ISO/IEC Guide 99 and the following apply.

2.1
aggregate
particle comprising strongly bonded or fused particles where the resulting external surface area may be significantly smaller than the sum of calculated surface areas of the individual components

NOTE 1 The forces holding an aggregate together are strong forces, for example covalent bonds, or those resulting from sintering or complex physical entanglement.

NOTE 2 Aggregates are also termed “secondary particles” and the original source particles are termed “primary particles”.

[ISO/TS 27687:2008, definition 3.3]

2.2
agglomerate
collection of weakly bound particles or aggregates or mixtures of the two where the resulting external surface area is similar to the sum of the surface areas of the individual components

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

NOTE 2 Agglomerates are also termed “secondary particles” and the original source particles are termed “primary particles”.

[ISO/TS 27687:2008, definition 3.2]

2.3
carbon nanotube
CNT
nanotube composed of carbon

NOTE Carbon nanotubes usually consist of curved graphene layers, including single-wall carbon nanotubes and multiwall carbon nanotubes.

[ISO/TS 80004-3:2010, definition 4.3]

2.4
colloid
heterogeneous substance consisting of a liquid (dispersion medium) in which nanoscale (1 nm to 100 nm) particles are uniformly retained in suspension by their electrical charge, and which exhibits Brownian movements and is subject to cataphoresis

NOTE 1 Colloidal means having the properties of a colloid.

NOTE 2 Adapted from ISO 1942-2.

2.5
composition
property of the nanomaterial given by the identity and content of each specific component

NOTE Adapted from ISO 6141.

2.6
crystallinity
presence of three-dimensional order at the level of molecular dimensions

[ISO 472]

2.7
combined standard measurement uncertainty
combined standard uncertainty (deprecated)
standard measurement uncertainty that is obtained using the individual standard measurement uncertainties associated with the input quantities in a measurement model

NOTE In case of correlations of input quantities in a measurement model, covariances must also be taken into account when calculating the combined standard measurement uncertainty; see also ISO/IEC Guide 98-3:2008, 2.3.4.

[ISO/IEC Guide 99:2007, definition 2.31]

2.8
dispensability
level of dispersion when it has become constant under the defined conditions

NOTE 1 Dispersion is defined as a suspension of discrete particles.

NOTE 2 Adapted from ISO 8780-1 and ISO 1213-1.

2.9
expanded measurement uncertainty
expanded uncertainty (deprecated)
product of a combined standard measurement uncertainty and a factor larger than the number one

NOTE 1 The factor depends upon the type of probability distribution of the output quantity in a measurement model and on the selected coverage probability.

NOTE 2 The term “factor” in this definition refers to a coverage factor. A coverage factor is a number by which a standard measurement uncertainty of a measurement result is multiplied to obtain an expanded measurement uncertainty.

NOTE 3 Adapted from ISO/IEC Guide 99.

2.10
fullerene
molecule composed solely of an even number of carbon atoms, which form a closed cage-like fused-ring polycyclic system with 12 five-membered rings and the rest six-membered rings

NOTE 1 Adapted from the definition in the IUPAC Compendium of Chemical Terminology.

NOTE 2 A well-known example is C60, which has a spherical shape with an external dimension of about 1 nm.

[ISO/TS 80004-3, definition 3.1]

2.11
measurement model
mathematical relation among all quantities known to be involved in a measurement

NOTE 1 A general form of a measurement model is the equation $h(Y, X_1, \ldots, X_n) = 0$, where Y, the output quantity in the measurement model, is the measurand, the quantity value of which is to be inferred from information about input quantities in the measurement model $X_1, \ldots, X_n$.

NOTE 2 Adapted from ISO/IEC Guide 99.

2.12
metrological traceability
property of a measurement result whereby the result can be related to a reference through a documented unbroken chain of calibrations, each contributing to the measurement uncertainty

NOTE 1 For this definition, a 'reference' can be a definition of a measurement unit through its practical realization, or a measurement procedure including the measurement unit for a non-ordinal quantity, or a measurement standard.

NOTE 2 Metrological traceability requires an established calibration hierarchy.

NOTE 3 Adapted from ISO/IEC Guide 99.

2.13
measurand
quantity intended to be measured

NOTE 1 The specification of a measurand requires knowledge of the kind of quantity, description of the state of the phenomenon, body, or substance carrying the quantity, including any relevant component, and the chemical entities involved.

NOTE 2 In the second edition of the VIM and in IEC 60050-300:2001, the measurand is defined as the 'quantity subject to measurement'.

NOTE 3 The measurement, including the measuring system and the conditions under which the measurement is carried out, might change the phenomenon, body, or substance such that the quantity being measured may differ from the measurand as defined. In this case, adequate correction is necessary.

NOTE 4 In chemistry, "analyte", or the name of a material or compound, is a term sometimes used for measurand. This usage is erroneous because these terms do not refer to quantities.

NOTE 5 For further information, see Reference [8].

NOTE 6 Adapted from ISO/IEC Guide 99.

2.14
nanofibre
nano-object with two similar external dimensions in the nanoscale and the third dimension significantly larger

NOTE 1 A nanofibre can be flexible or rigid.

NOTE 2 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 The largest external dimension is not necessarily in the nanoscale.

[ISO/TS 27687:2008, definition 4.3]

2.15
nanomanufacturing
intentional synthesis, generation or control of nanomaterials, or fabrication steps in the nanoscale, for commercial purposes

[ISO/TS 80004-1:2010, definition 2.11]

2.16
nanomaterial
material with any external dimension in the nanoscale or having internal structure or surface structure in the nanoscale

NOTE 1 This generic term is inclusive of nano-object and nanostructured material.

NOTE 2 Adapted from ISO/TS 80004-1.

2.17
nano-object
material with one, two or three external dimensions in the nanoscale

NOTE Generic term for all discrete nanoscale objects.

[ISO/TS 80004-1:2010, definition 2.5]

2.18
nanoparticle
nano-object with all three external dimensions at the nanoscale

NOTE If the lengths of the longest to the shortest axes of the nano-object differ significantly (typically by more than three times), the terms “nanofibre” or “nanoplate” are intended to be used instead of the term “nanoparticle”.

[ISO/TS 27687:2008, definition 4.1]

2.19
nanoplate
nano-object with one external dimension in the nanoscale and the two other external dimensions significantly larger

NOTE 1 The smallest external dimension is the thickness of the nanoplate.

NOTE 2 The two significantly larger dimensions are considered to differ from the nanoscale dimension by more than three times.

NOTE 3 The larger external dimensions are not necessarily in the nanoscale.

[ISO/TS 80004-3:2010, definition 2.4]

2.20
nanoscale
size range from approximately 1 nm to 100 nm

NOTE 1 Properties that are not extrapolations from a larger size will typically, but not exclusively, be exhibited in this size range. For such properties the size limits are considered approximate.

NOTE 2 The lower limit in this definition (approximately 1 nm) is introduced to avoid single and small groups of atoms from being designated as nano-objects or elements of nanostructures, which might be implied by the absence of a lower limit.

[ISO/TS 80004-1:2010, definition 2.1]

2.21
nanostructured material
material having internal nanostructure or surface nanostructure

NOTE This definition does not exclude the possibility for a nano-object to have internal structure or surface structure. If external dimension(s) are in the nanoscale, the term “nano-object” is recommended.

[ISO/TS 80004-1:2010, definition 2.7]

2.22
nanotechnology
application of scientific knowledge to manipulate and control matter in the nanoscale to make use of size- and structure-dependent properties and phenomena distinct from those associated with individual atoms or molecules or with bulk materials

NOTE Manipulate and control include material synthesis.

[ISO/TS 80004-1:2010, definition 2.3]

2.23
nanotube
hollow nanofibre

[ISO/TS 27687:2008, definition 4.4]

2.24
particle size
size of a sphere having the same physical properties in the method of analysis as the particle being described

NOTE 1 See also equivalent particle diameter.

NOTE 2 There is no single definition of particle size. Different methods of analysis are based on the measurement of different physical properties. The physical property to which the equivalent diameter refers is indicated using a suitable subscript or reference to the documentary measurement standard according to which the particle size was measured. In ISO 9276 the symbol x is used to denote the particle size or the diameter of a sphere. However, it is recognized there that the symbol d is also widely used to designate these values. Therefore the symbol x may be replaced by d where it appears.

[ISO 21501-1:2009, definition 2.3]

2.25
particle size distribution
cumulative distribution of particle concentration as a function of particle size

[ISO 14644-6:2007, definition 2.107]

2.26
shape
particle shape
external geometric form of a particle

NOTE Adapted from ISO 3252.

2.27
solubility
maximum mass of a nanomaterial that is soluble in a given volume of a particular solvent under specified conditions

NOTE 1 Solubility is expressed in grams per litre of solvent.

NOTE 2 Adapted from ISO 7579.

2.28
surface area
area of external surface plus the internal surface of its accessible macro- and mesopore

NOTE Includes mass-specific surface area or volume-specific surface area.

2.29
surface charge
electrical charge on a surface

2.30
surface chemistry
chemical nature of a surface

2.31
validation
verification, where the specified requirements are adequate for an intended use

NOTE Adapted from ISO/IEC Guide 99.

2.32
verification
provision of objective evidence that a given item fulfils specified requirements

NOTE 1 When applicable, measurement uncertainty should be taken into consideration.

NOTE 2 The item might be, e.g. a process, measurement procedure, material, compound, or measuring system.

NOTE 3 Adapted from ISO/IEC Guide 99.

3 Symbols and abbreviated terms

ADME absorption, distribution, metabolism, and excretion

AFM atomic force microscopy

BIPM Bureau International des Poids et Mesures

CNT carbon nanotube

EHS environment, health and safety

GMP Good Manufacturing Practices

GUM Guide to the Expression of Uncertainty in Measurement

OECD Organization for Economic Cooperation and Development

NOAA nano-objects, and their aggregates and agglomerates greater than 100 nm

SEM scanning electron microscopy

SPM scanning probe microscopy

TEM transmission electron microscopy

UV ultraviolet

4 Importance of physico-chemical properties to toxicological assessment

4.1 The purpose of toxicological experimentation

When the introduction of new materials into commerce is preceded by risk assessment, depending on the nature of the material(s) under consideration, it will require toxicology and ecotoxicology data acquired for the purpose of assessing the potential effects on humans and the environment.

The purpose of toxicological experimentation is to assess the potential effects to humans and the environment resulting from exposure to a chemical substance, including nano-objects, and their aggregates and agglomerates. The toxicological risk of a substance is its capacity to cause harm to a living organism and is generally resulting from the hazardous properties of the substance combined with the exposure to it. Properly designed experimental studies in toxicology are helpful in reducing the uncertainty associated with the test result. The intention for all toxicological experiments is to obtain reliable information that includes data related to:

— dose-response;

— any differences in responses associated with distinct inherent properties of the substance;

— any differences in responses associated with different exposure routes;

— the types and severity of adverse effects;

— the mode and mechanism of action (including upstream biochemistry);

— any period(s) of time when the organism is particularly sensitive to exposure (e.g. foetal development);

— carcinogenicity, mutagenicity and teratogenicity;

— time course of response; and

— use of control samples.

4.2 General methods of toxicological testing and risk assessment

4.2.1 General

Scientists have developed and adopted procedures for assessing possible risk(s) of harmful effects of materials, and conversely the degree of safety, by conducting a toxicological risk assessment. Scientists in government, industry, and academia can make these assessments for human health as well as for the environment. As described in the National Research Council [United States] publication (1983), *Risk Assessment in the Federal Government: Managing the Process*, the risk assessment process comprises four steps. These are: (1) hazard identification; (2) dose-response / concentration-effect assessment; (3) exposure assessment; and (4) risk characterization[13]. Toxicological testing provides fundamental data for hazard identification, dose-response assessment, and exposure assessment. Risk assessment data are used to derive other information such as occupational, general public, or consumer exposure limits, recommendations for personal protective equipment, and hazard communication documents.

4.2.2 Hazard identification

Hazard identification is the first step in the risk assessment process and is the process of determining whether a chemical substance can cause toxic effect(s). The types of scientific information that are often used in this step include: *in vivo* studies, *in vitro* studies, epidemiologic data, and human clinical data. Well-conducted experimental studies assume the use of the scientific method, for example, the design of experiments that test the toxicity of a material must be such that experiments are repeatable and reproducible. To assist in attaining this goal, the use of standardized toxicological testing protocols is recommended.

Recently, in line with current ethical and scientific thinking, there is a global trend toward replacing, where possible, traditional *in vivo* studies (involving laboratory animals) with improved *in vitro* (exposing the tested material to simple organisms such as bacteria, tissue cultures, or live tissue slices) and *in silico* (computer simulation) methods. Such studies limit the use of animals, and mechanistic information (e.g. biochemical chain of events) can be obtained. An example of an *in vitro* study is the examination of the mechanism by which chemical substances bind to cell membrane receptors (e.g. in a lock and key manner) and how that event activates second messengers to interact with cellular components. Furthermore, *in vitro* study results could also be relevant to *in vivo* study design.

A material's inherent ability to cause an effect (desirable and undesirable) is related to the chemical and physical properties of the material in question, including its impurities. Obtaining physico-chemical information for NOAAs is a fundamental component of a well-executed toxicological experiment using the scientific method. With accurate physico-chemical information, scientists are able to clearly characterize and describe the NOAAs being tested so that they can identify the same material and test it in the same manner to obtain reproducible toxicological results.

4.2.3 Dose-response assessment

Dose-response assessment is the second step in the risk assessment process and examines the relationship between the magnitude of exposure and the response of the test system (such as an adverse effect). This step of the process characterizes the relationship between the dose of material administered or received and the incidence of an adverse health effect in exposed populations (environments). The assessment considers the