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Nanotechnologies — Compilation and description of sample preparation and dosing methods for engineered and manufactured nanomaterials

Nanotechnologies — Compilation et description de la préparation des échantillons et des méthodes de dosage pour les nanomatériaux d'ingénierie et manufacturés

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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.

The procedures used to develop this document and those intended for its further maintenance are described in the ISO/IEC Directives, Part 1. In particular the different approval criteria needed for the different types of ISO documents should be noted. This document was drafted in accordance with the editorial rules of the ISO/IEC Directives, Part 2 (see www.iso.org/directives).

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. Details of any patent rights identified during the development of the document will be in the Introduction and/or on the ISO list of patent declarations received (see www.iso.org/patents).

Any trade name used in this document is information given for the convenience of users and does not constitute an endorsement.

For an explanation on the meaning of ISO specific terms and expressions related to conformity assessment, as well as information about ISO's adherence to the World Trade Organization (WTO) principles in the Technical Barriers to Trade (TBT) see the following URL: www.iso.org/iso/foreword.html.

The committee responsible for this document is ISO/TC 229, *Nanotechnologies.*

Introduction

This document provides guidance regarding the preparation of nanomaterials for toxicological, including eco-toxicological, testing. The goal of this document is to assist health and environmental scientists and scientists and experts from other disciplines to understand, plan, choose and address issues relevant to nanomaterials before and during conducting toxicological tests. These issues include the effects of the properties of the material on preparation methods and of the media into which the samples of nanomaterials will be added. Failure to consider these effects might lead to erroneous conclusions regarding the relationship between the nature of the nanomaterial and observed toxicological responses. In particular, the composition and other characteristics of test media can affect the dose to which an organism that is the subject of a test will be exposed. Information on preparation of the test material is necessary prior to any biological or ecological evaluation. Information such as this is consistent with other ISO documents. For example, ISO 10993-18[1] specifically addresses the evaluation of the chemical characterization of materials used in medical devices, ISO 14971[2] specifies that a toxicological risk analysis should take into account the chemical nature of the materials, ISO/TR 13014[3] addresses issues pertaining to the materials themselves and ISO/TS 19337[55] points out the need to clarify whether observed toxic effects come from tested nano-objects themselves or from other uncontrolled sources. Some examples are provided of methods that establish test conditions that are relatable to environmentally relevant conditions.

This document uses a number of technical terms which have been defined earlier in other documents. Some of these terms have been defined in multiple documents, in different areas of science and technology, providing potentially or seemingly conflicting definitions. This document does not provide new, authoritative definitions for the terms used herein. Instead, this clause provides short descriptions for the terms used. Where possible, reference is made to existing documents.

Nanotechnologies — Compilation and description of sample preparation and dosing methods for engineered and manufactured nanomaterials

1 Scope

This document provides guidance regarding the preparation of nanomaterials for eco- and bio-toxicological testing. It provides guidance regarding factors pertaining to sample preparation and dose determination that might be useful in toxicological, including ecotoxicological, testing of engineered and manufactured nanoscale materials.

The descriptions of sample preparation method factors for both *in vitro* and *in vivo* toxicological testing of engineered and manufactured nanoscale materials include considerations about physico-chemical properties, media, methods for transformation and accumulation studies, health effects and dosimetry. The document is not intended to be a literature review nor a thorough assessment of the quality of the methods or data generated. The document is intended to complement other international efforts.

The focus of this document is on factors that might lead to results that are not relevant to safety evaluations. When featured, referenced methods are considered for their general interest and potential applicability. It is likely that most of the described methods are not generally applicable to all nanomaterials but they do demonstrate important factors and limitations that are common for a variety of nanomaterials.

2 Normative references

There are no normative references in this document.

3 Terms and definitions

For the purposes of this document, the following terms and definitions apply.

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

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

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

3.1
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 particle definition applies to nano-objects.

[SOURCE: ISO/TS 80004-2:2015, 3.1]

3.2
structure
arrangement defined by four different aspects (crystallinity, crystal structure, molecular structure and microstructure)

3.2.1
crystallinity
presence or absence of crystalline structure in the arrangement of the atoms of which a material consists

3.2.2
crystal structure
lattice structure in which atoms of an individual crystal are arranged, using lattice parameters and lattice type, such as face-centred cubic, hexagonal close-packed, body-centred, cubic, etc.

3.2.3
molecular structure
arrangement of atoms of an individual molecule

3.2.4
microstructure
arrangement of individual crystals or amorphous phases in a polycrystalline or multiphase material

3.3
measurand
quantity intended to be measured or a quantity that is being determined by measurement

Note 1 to entry: 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. The measurement, including the measuring system and the conditions under which the measurement is carried out, might change the phenomenon, body, or substance so that the quantity being measured may differ from the measurand as defined.

[SOURCE: ISO/IEC Guide 99, 2007, 2.3 — modified]

3.4
nanomaterial
NM
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, incidental nanomaterial.

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

3.5
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]

3.6
nanoparticle
NP
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 three times), terms, such as *nanofibre* or *nanoplate*, may be preferred to the term nanoparticle.

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

3.7
nanoscale
length range approximately from 1 nm to 100 nm

Note 1 to entry: Properties that are not extrapolations from a larger size are predominantly exhibited in this length range.

[SOURCE: ISO/TS 80004-2:2015, 2.1]

4 Abbreviated terms

BET	Brunauer–Emmett–Teller isotherm
CNT	carbon nanotube
DLS	dynamic light scattering
ICP-MS	inductively coupled plasma mass spectrometry
NOAA	nano-objects, and their aggregates and agglomerates greater than 100 nm
NOM	natural organic material
TEM	transmission electron microscopy

5 Background

5.1 Discussion of the importance of sample preparation and dosing

Nanomaterials are diverse, being based on endless combinations of composition, particle size and distribution, surface chemistry and many other key properties. With this diversity, nanomaterials cannot be treated as a single class of substances. Just as in other areas of toxicology, the assessment of biological effects should consider how samples and doses are prepared and dosimetry is assured so that the observed effects are meaningful and test results can be used in a realistic way such as in safety assessments.

Screening tests are used for rapid evaluations and are typically conducted using cell culture or other *in vitro* techniques due to fast response time, cost, infrastructure and time constraints, factors that limit most whole animal studies. The purpose of a screening test is to provide an indicator of potential adverse outcomes and effects on human health or the environment. Although there are many definitions available for the term screening test, for the purposes of this document, a screening test can be generally defined as a relatively simple, inexpensive test that can be administered easily and provides rapid results. The screening tests should reflect the compromise between simplicity, rapidity and low-cost while still providing results that have meaning to safety-relevant situations, the way that samples are prepared and the doses administered will ideally be relatable to realistic situations. Therefore, the points considered in this document also apply to screening tests and should also be taken into account in tiered testing to ensure consistent conditions with each step of the tier.

5.2 Discussion of specific issues when testing the toxicology of manufactured nanomaterials

5.2.1 Physical properties

5.2.1.1 General

The subject that is sometimes referred to as “nanotoxicology” considers the same issues as the broader subject of toxicology with additional scrutiny paid to elements based on a very small size. These include, but are not limited to, physical properties that are described in detail below in 5.2.1.2 to 5.2.1.5.

5.2.1.2 Size

Smaller particles can have the ability to reach target sites that larger forms cannot. In addition, smaller particles present larger surface areas possibly increasing their chemical reactivity. An example might be inhaled particles (<100 nm) that can reach the alveolar region of the lungs.

5.2.1.3 Size distribution

Most nanoparticles are not of a single size, therefore there is a distribution of sizes. For some materials, there might be nano-sized fractions and aggregated larger particles present of the same composition. The toxicological contributions of each should be distinguished where possible.

5.2.1.4 Dissolution

Nanomaterials have a greater surface to mass ratio so sparingly soluble nanomaterials more readily dissolve in solutions than the same material in bulk form. Examples include amorphous silicon dioxide, zinc oxide and silver. When a material has any appreciable solubility, the relative contributions of toxicological effects due to particulates versus dissolved species should be considered. In some cases where dissolution is complete, observed effects might be due to the ionized/dissolved fraction rather than the nanoparticles, even though the initial test substance was a nanoparticle.

5.2.1.5 Transformation

Because nanomaterials are not a single class of materials there are an enormous number of uses for them. Some of these uses might involve intentional and unintentional transformations. An example of an intentional transformation is the use of aluminium nanomaterials in energetic applications where at least some of the aluminium is converted to alumina[4]. An example of an unintentional transformation is the release of CNTs from matrices into environmental settings in which they would be subject to photochemical processes, oxidation, biotransformation, etc. CNTs might be subject to combustion processes if they are part of matrices that are burned. With each transformation it is important to consider if changes have occurred that require recharacterization of the test nanomaterial.

5.2.2 Sample preparation

Toxicological studies are generally performed to evaluate the potential hazards of materials. For sample preparation, care should be taken to ensure that the material is prepared in a way that is appropriate for the toxicological evaluation that will be applied. The OECD “Guidance on Sample Preparation and Dosimetry for the Safety Testing of Manufactured Nanomaterials”[5] provides information regarding the critical aspects that should be considered for preparation of nanomaterial samples for testing and the use of relevant dose and dose metrics. It is important to prepare and administer nanomaterials in a way that is representative of a potential exposure, i.e. which can be linked to an exposure scenario. As with assays in general, potential interferences should be considered[6][7][8][9].

5.2.3 Administration of doses

After sample preparation it is important to quantify and characterize the administered dose and the received dose.

5.2.4 Discussion of relationship between this document and ISO/TR 13014

ISO/TR 13014 provides essential guidance to all researchers who attempt to assess relationships between nanomaterial exposures and linked biological responses. However, as of the time of writing, the practices described in ISO/TR 13014 are not widely practiced. It is not possible to find many references in literature where materials are characterized as described in ISO/TR 13014. ISO/TR 13014 was used as a benchmark in considering whether the studies cited in the references contained in this document should be considered.

5.3 Discussion of relevant dosing for toxicological screening

Humans and the environment may be exposed to nanomaterials via a number of routes, e.g. inhalation, ingestion, dermal for humans, or environment via water. The exposure concentrations for some of these scenarios can be determined, e.g. airborne nanomaterials in the workplace or particles per mass unit of oil-in-water emulsions applied to the skin. Currently, the exposure concentrations of engineered and manufactured nanomaterials for the environment is unknown. While certainty of these concentrations is required for quantitative risk assessment, it is not required for the hazard characterization typically associated with screening level assays.

However, care should be employed by the investigator conducting screening assays and by the assessors evaluating the data so that the relationship between effect and dose is not over-interpreted. Whenever possible, investigators should use dose levels that approximate the estimated concentrations to realistic exposure dose; therefore, *in vitro* studies of cell cultures from the respiratory tract should use concentrations that relate to lung burden observed following inhalation, or *in vitro* studies of keratinocytes should use concentration levels that are consistent with dose applied to the skin. Other dose levels might and should be used to demonstrate dose-related responses. These additional dose levels are frequently exaggerated levels of real exposures, but they serve to stress the system investigated. The use of single dose level studies is discouraged.

5.4 Discussion of the relationship between this document and ISO/TR 16197

This document was developed in concert with a sister document ISO/TR 16197. ISO/TR 16197 discusses methods used to prepare samples in various relevant media for toxicological studies, and also discusses issues of relevant dose metrics for toxicological testing considering the various routes of administration. ISO/TR 16197 complements this document by moving from sample preparation and dosimetry into a more detailed discussion of the various methods used to perform toxicological screening. When using ISO/TR 16197, it is important to consider this document since difficulties associated with sample preparation and dosimetry are often the bottleneck in performing a good toxicological assessment of nanomaterials.

5.5 Review of other relevant international activities and documents

Readers/users of this document should also take note of an OECD document, “Guidance on Sample Preparation and Dosimetry for the Safety Testing of Manufactured Nanomaterials”[5]. This document provides a perspective based on a detailed review of the literature at the time of the document’s publication including potential issues to be addressed.