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Nanotechnologies — Compilation and description of toxicological screening methods for manufactured nanomaterials

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National foreword

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TECHNICAL REPORT

ISO/TR 16197

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2014-05-15

Nanotechnologies — Compilation and description of toxicological screening methods for manufactured nanomaterials

Nanotechnologies — Compilation et description des méthodes de criblage toxicologiques pour les nanomatériaux 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).

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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 WTO principles in the Technical Barriers to Trade (TBT) see the following URL: Foreword - Supplementary information

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

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Introduction

This Technical Report provides a compilation of methods intended to aid the process of toxicological screening of engineered and manufactured nanomaterials prior to full-scale toxicological testing, analysis, and risk assessment. Toxicological screening methods focus on providing information and tools that can be used in decision-making processes. For instance, this Technical Report provides information on methods that can be used to screen nanomaterials in order to determine whether to continue development of a nanomaterial itself and/or a product containing a nanomaterial; determine whether to take on the cost of performing the remaining tiers within a complete tiered-testing strategy; or determine whether appropriate controls are in place to continue nanomaterial research in the laboratory.

This Technical Report is not intended to supplant or compete with any existing regulatory requirements regarding nanomaterial testing, use, and disposal, nor does it provide a list of validated tests for this purpose.

The information provided is consistent with other International Standards. For example, its sister document 'Compilation and Description of Sample Preparation and Dosing Methods for Manufactured NMs' is developed in concert and discusses methods used to prepare samples in various relevant media for toxicological studies. ISO 10993-18[1] specifically addresses the evaluation of the chemical characterization of materials used in medical devices, ISO 14971[2] points out that a toxicological risk analysis should take into account the chemical nature of the materials, and ISO/TR 13014[3] addresses issues pertaining to the materials themselves. ISO/TR 13121[4] describes a process for identifying, evaluating, and communicating the potential risks of manufactured nanomaterials and provides guidance on tiered nanomaterial toxicity testing.

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Nanotechnologies — Compilation and description of toxicological screening methods for manufactured nanomaterials

1 Scope

This Technical Report provides a compilation and description of *in vitro* and *in vivo* methods that can be useful for the toxicological, including ecotoxicological screening of engineered and manufactured nanomaterials. Toxicological screening tests included in this Technical Report can be used for such purposes as early decision-making in research and product development, rapid feedback on potential toxicological/safety concerns, or for the preliminary assessment of manufactured nanomaterials. This Technical Report is divided between screening assays related to humans and screening assays related to the environment. A screening test is a relatively simple, inexpensive test that can be administered easily and provides an indication of potential adverse outcomes and effects on human health or the environment.

The Technical Report is intended to complement other international efforts that address nanomaterial toxicology by focusing on screening methods suited for preliminary assessment and is not intended to duplicate similar efforts in other international organizations such as the Organization for Economic Cooperation and Development (OECD). If screening provides an early indication of hazard, the guidance will refer to other organizations' approaches for full-scale toxicological assessment or further tiered studies.

2 Normative references

The following documents, in whole or in part, are normatively referenced in this document and are indispensable for its application. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

ISO/TS 27687:2008, *Nanotechnologies — Terminology and definitions for nano-objects — Nanoparticle, nanofibre and nanoplate*

ISO/TS 80004-1, *Nanotechnologies — Vocabulary — Part 1: Core terms*

3 Terms and definitions

For the purposes of this document, the terms and definitions given in ISO/TS 27687:2008 and ISO/TS 80004-1 and the following apply.

3.1
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 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 27867:2008, 3.2]

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3.2
aggregate
particle comprising strongly bonded or fused particles where the resulting external surface area can be 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 bods, or those resulting from sintering or complex physical entanglement.

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

[SOURCE: ISO/TS 27867:2008, 3.3]

3.3
manufactured nanomaterial
nanomaterial intentionally produced for commercial purposes to have specific properties or specific composition

[SOURCE: ISO/TS 80004-1, 2.9]

3.4
nanofibre
nano-object with two 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 27687:2008, 4.3]

3.5
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, 2.4]

3.6
nano-object
material with one, two, or three external dimensions in the nanoscale

Note 1 to entry: Generic term for all discrete nanoscale objects.

[SOURCE: ISO/TS 27687:2008, 2.2]

3.7
nanoparticle
nano-object with all three external dimensions in the nanoscale

Note 1 to entry: If the lengths of the longest to the shortest axes of the nano-object differ significantly (typically by more than three times), the terms nanorod or nanoplate are intended to be used instead of the term nanoparticle.

[SOURCE: ISO/TS 27687:2008, 4.1]

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3.8
nanoscale
size range from approximately 1 nm to 100 nm

Note 1 to entry: 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 to entry: 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.

[SOURCE: ISO/TS 27687:2008, 2.1]

3.9
nanotube
hollow nanofibre

[SOURCE: ISO/TS 27687:2008, 4.4]

3.10
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 27687:2008, 3.1]

4 Symbols (and abbreviated terms)

ASTM American Society for Testing and Materials

ESR Electron Spin Resonance

IT Intracheal

ITS Integrated Testing Strategies (or in some cases, Intelligent Testing Strategies)

LTE Lymphoid Tissue Equivalent Module

NMs nanomaterials

OECD Organization for Economic Cooperation and Development

PBS phosphate buffer saline

PTE peripheral tissue equivalent

ROS reactive oxygen species

STS standard toxicity studies

5 Background

5.1 Role and relevance of toxicological screening for the safety evaluation of manufactured NMs

It is a challenge to keep up with the number of emerging NMs with novel properties and to toxicologically assay new materials prior to human exposure, including occupational and environmental exposure.

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Because virtually every element on the periodic table could be explored in nanotechnology, the potential diversity of NMs makes it impractical to utilize our current testing paradigms to evaluate every new nanomaterial. High-throughput toxicological screening is essential to keeping pace with the rate of NMs currently emerging on the market. Such screening is typically conducted using cell culture or other *in vitro* techniques due to cost, infrastructure, and time constraints; such considerations prevent most whole animal studies. In addition, there is a worldwide effort to diminish the use of *in vivo* animal studies as presented in the 3R (replacement, reduction, refinement) principle.[5]

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 Technical Report, a screening test can be generally defined as a relatively simple, inexpensive test that can be administered easily and provides rapid results.

A screening test might include the following:

— uses no (or a very limited number of) sentient animals;

— produces a quantifiable end point or a yes/no screen that is well-accepted and reliable;

— has demonstrated repeatability in multiple laboratories;

— is reproducible with appropriate positive and negative controls.

Screening tests often provide specific mechanistic data that should be used in the context of either an adverse outcome pathway framework or a chemical signature analysis. This Technical Report deals solely with screening tests that have been used for the purposes of assessing nanomaterial toxicity; therefore, results from this type of screening test could be used to determine whether or not to pursue the continued development of a particular nanomaterial product. For example, NMs predicted to be particularly hazardous can be removed from development.

When used in a tiered testing strategy, high throughput screening methods have the potential to eliminate further *in vivo* testing or to identify hazardous materials for targeted *in vitro* or *in vivo* investigation, thus streamlining the hazard identification process. The fact that screening assays can be conducted in a high-throughput fashion has especially important human health implications considering the complexity and the large number of NMs already on the market and currently in development.

However, there are also limitations of screening assays relative to more detailed, confirmatory assays; therefore, screening assays should be designed to be incorporated into an integrated testing strategy. Limitations of screening assays include

— screening assays often lack validated human predictability,

— extrapolation of dose-response relationships from screening assays to human exposure is complex, and

— prediction of human chronic exposure hazard from an acute exposure screen is difficult.

While screening tests are not meant to be used as stand-alone methods, they can eliminate further studies if the results suggest a NM is particularly toxic or non-toxic.

This means that screening assays in some instances will undoubtedly overestimate or underestimate human hazards.

5.2 Toxicological screening as part of tiered approaches to toxicological assessment of manufactured NMs

A tiered testing approach is based on a stepwise assessment, with each step of the assessment providing data/information that might be required for the subsequent step or will be of use for the whole testing approach.

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Often screening tests are included in a tiered testing strategy, and they are generally carried out in one of the earliest tiers. This allows an efficient use of resources at several levels, e.g. both for identifying further testing needs and for decisions on product development in view of an early hazard profile.

Toxicological screening is part of most early tiers in weight-of-evidence-based, intelligent testing strategies (ITS).[6] ITS take into account the data available for the nanomaterial of interest and provide a rational testing strategy to understand the hazard properties of that nanomaterial without resorting to undue animal testing.

In addition to *in vitro* and *in vivo* methods, *in silico* methods could form part of ITS. In principle, the methods of Quantitative Structure–Activity Relationship (QSAR) analysis can be applied to nanoparticles, provided that suitable descriptors can be found to associate the structural and physicochemical characteristics of nanoparticles with their biological activity.[7][8] These could result in models that make qualitative (e.g. the potential for oxidative stress) or quantitative predictions (e.g. cytotoxic potency), depending on the data and modelling approach. To date, however, only a few studies have been published, probably due to a lack of suitable data sets.[9]-[17] The challenges and recent successes of developing QSARs for nanoparticles are further outlined elsewhere.[18][19]

5.3 Discussion of relevant dosing for toxicological screening

Humans and the environment are exposed to NMs via a limited number of routes, e.g. inhalation, ingestion, dermal contact, or, for the environment, water, air, and soil. The exposure concentrations for some of these exposure scenarios can be determined, e.g. airborne NMs in the workplace or particles per gram of oil-in-water emulsions applied to the skin. Currently, the exposure concentrations of engineered NMs for the environment are 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. As with all studies, 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 overly interpreted. Whenever possible, investigators should use dose levels that approximate the estimated dose to which the species of concern might be exposed; thus, *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 dose levels that are consistent with concentrations applied to the skin. Other dose levels could be used to demonstrate dose-related responses. For potential hazard identification, the dose-response is generally used. The lowest dose of such study might be in the range of expected human exposure. These additional dose levels are frequently exaggerated levels of real exposures, but they serve to stress the system investigated and to identify potential hazards.

There are many factors that need to be considered in sample preparation to ensure that the targeted doses are delivered. Methods should consider if dispersing agents are needed so that particles do not form agglomerates in media unless the agglomeration is intended to be considered in evaluations. If dispersing agents are used they need to be evaluated to ensure that their contribution to observed effects is understood. For example, if a nanoparticle is being evaluated for toxic effects the dispersing agent should not also contribute to the toxic effects. High energy methods such as ultrasonication are used to disperse particles. Such dispersion should be considered if the resulting particles are representative of particles in the environment.

As is noted at several locations in this Technical Report, sufficient physico-chemical information should be taken to allow investigators to consider the several dose-metrics (number, mass, and surface area) found in the literature. In the case of environmental studies, need should also be taken to differentiate among environmental concentration, exposure, and dose. Particular attention should be paid to situations where dispersion stability is shorter than the test duration and then consider alternative dosing stratagems.

By their nature, screening tests are intended for hazard identification and for range-finding when preparing for the more involved, exacting test protocols. Readers should be reminded that the dose levels (and environmental concentrations and exposure levels) used for risk assessment will likely differ from those appropriate to hazard identification.

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