SRI LANKA STANDARD 12008:2013
ISO/TR 13014:2012

NANOMATERIALS - PREPARATION OF MATERIAL SAFETY DATA SHEET (MSDS)

SRI LANKA STANDARDS INSTITUTION

Sri Lanka Standard

NANOMATERIALS - PREPARATION OF MATERIAL SAFETY DATA SHEET (MSDS)

SLS 12008:2013
ISO/TR 13014:2012

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SRI LANKA STANDARDS INSTITUTION
17, Victoria Place
Elvitigala Mawatha
Colombo - 08
Sri Lanka.

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This standard does not purport to include all the necessary provisions of a contract.

Sri Lanka Standard
NANOMATERIALS - PREPARATION OF MATERIAL SAFETY DATA SHEET (MSDS)

NATIONAL FOREWORD

This standard was approved by the National Mirror Committee on Nanotechnology authorized for adoption and publication as a Sri Lanka Standard by the Council of the Sri Lanka Standards Institution on 2013.11.27.

This Sri Lanka Standard is identical with **ISO/TR 13014:2012**, Nanomaterials - Preparation of material safety data sheet (MSDS), published by the International Organization for Standardization (ISO).

TERMINOLOGY AND CONVENTIONS

The text of the International Standard has been accepted as suitable for publication, without any deviation as a Sri Lanka Standard. However, certain terminology and conventions are not identical with those used in Sri Lanka Standards. Attention is therefore drawn to the following:

a) Wherever the words “International Standard” appear referring to this standard they should be interpreted as “Sri Lanka Standard”.

b) The comma has been used throughout as a decimal marker. In Sri Lanka Standards, it is the current practice to use a full point on the baseline as the decimal marker.

Wherever page numbers are quoted, they are “ISO” page numbers.

SLS 12008:2013

TECHNICAL REPORT

ISO/TR 13014

First edition
2012-05-15

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

Reference number
ISO/TR 13014:2012(E)

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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

Published in Switzerland

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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.

TECHNICAL REPORT ISO/TR 13014:2012
TECHNICAL CORRIGENDUM 1

Published 2012-07-15

INTERNATIONAL ORGANIZATION FOR STANDARDIZATION • МЕЖДУНАРОДНАЯ ОРГАНИЗАЦИЯ ПО СТАНДАРТИЗАЦИИ • ORGANISATION INTERNATIONALE DE NORMALISATION

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

TECHNICAL CORRIGENDUM 1

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

RECTIFICATIF TECHNIQUE 1

Technical Corrigendum 1 to ISO/TR 13014:2012 was prepared by Technical Committee ISO/TC 229, *Nanotechnologies*.

Page 2, 2.8

Replace the term "dispensability" with "dispersibility".

Page 29, Table B.1

In the last row, in the "measurement methods" column, replace the word "dispensability" with "dispersibility".

ICS 07.030 **Ref. No. ISO/TR 13014:2012/Cor.1:2012(E)**

Published in Switzerland

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

strength, age, sex, sensitivity or susceptibility, size, uniqueness, diversity and other modifying factors related to the exposed populations (environments), as well as the amount, duration, frequency, and route of exposure. For environmental assessments, fate and behaviour is taken into account. Human health toxicological testing for dose-response assessment includes the decision to test a material for acute, chronic or subchronic effects. Short-term, acute responses are tested in general by higher doses and short durations (e.g. hours or days) of exposure. Some adverse effects are thought to be related to the length of exposure. For example, some chronic diseases are generally thought to be caused by repeated exposures over extended periods of time. A common example is cancer. Long term, chronic responses are tested with lower doses and long periods of exposures, such as a 2-year bioassay with mice. Subchronic exposures are of shorter durations than long term chronic exposures. A 90-day bioassay would be an example of an experiment that would address subchronic exposure. Physico-chemical parameters of NOAAs are expected to provide fundamental critical information to dose-response assessment. An example of this is the fact that while the well recognized unit for dose in conventional toxicological testing is mass of a material (per mass per body weight or per litre of water). Surface area and particle number are additional parameters that may be used in the dose-response assessment of an NOAA.

4.2.4 Exposure assessment

Exposure assessment is the third step in the risk assessment process and involves measuring or estimating the concentration, occurrence and duration of human or environmental exposure to a material, or estimating hypothetical exposures that might arise from the release of a material into the environment. Exposure assessment of NOAAs is expected to be complex. For example, when a material is produced in a factory, the health-related concern involves the types of exposure (exposure routes) and durations of exposures to workers. In this example, the relevant routes of exposure would include inhalation or dermal contact. However, when an NOAA is incorporated into a product and is released or enters the environment, questions arise as to what is the proper material or form of the material to be measured in order to determine the concentration and duration of exposure. Since NOAAs may change form after release to the environment, exposure assessment of the modified materials should also be considered if material modifications are reasonably anticipated.

4.2.5 Risk characterization

Risk characterization is the fourth step and is the process of assessing the probability or potential of a health effect (environmental effect) under the various conditions of human or environmental exposure described in the exposure assessment step. It is performed by combining the exposure and dose-response/concentration-effect assessments; therefore, toxicological testing is a fundamental component of risk characterization. Risk characterization conducted for NOAAs is expected to be similar to that practiced for bulk materials, such that there can be estimates of both cancer and non-cancer risks.

4.3 Physico-chemical properties of nano-objects

The extremely broad diversity of existing and future manufactured NOAAs can be partly attributed to differences in composition but will also be due to differences in shape, size, surface chemistry, degree of agglomeration, etc. for seemingly similar materials.

Simply put, physico-chemical characterization addresses three fundamental questions about an NOAA. The three questions are:

— Physical description: What does it look like?

— Chemical composition: What is it made of?

— Extrinsic properties: How does it interact with the surrounding environment/media?

The following are physico-chemical characteristics that have been proposed as the most relevant to toxicological assessment of NOAAs at this time.

For physical description, the following parameters apply:

— particle size/distribution;

— aggregation/agglomeration state in relevant media;

— shape: including length, width and aspect ratio information (ratio of longest to shortest external particle dimension) for fibres and elongated particles;

— surface area/specific surface area.

For chemical composition, the following parameters apply:

— composition;

— purity (including levels of impurities); and

— surface chemistry.

For extrinsic properties, the following parameters apply:

— surface charge, solubility; and

— dispersibility.

4.4 Purity and impurity of tested nano-objects

Before conducting any toxicological testing, it is important to comprehensively understand the material (including nano-objects) being tested by evaluating its physico-chemical characteristics including purity since the presence of impurities might be the primary cause of an adverse effect.

A material is said to be pure when its physical and chemical properties coincide with those previously established and recorded in specifications, the literature, or other defined targets. In general, purity is the fraction of the pure material within a package. Often, its content is declared by manufacturers, such as when it is packaged. In order to understand the toxicological impact of a particular material, it is important to use a pure sample of that material in order to avoid uncertainties introduced by one or more impurities. However, for regulatory toxicology, testing should be performed on materials as formulated for use in the market, which include known and sometimes unknown impurities. This concept is relevant for NOAAs.

Impurity describes an unintended constituent present in an amount of manufactured nano-objects. Where present in a sample of manufactured nano-objects, impurities could originate from the original source materials, be the result of secondary or incomplete reactions during the production process, or result from contamination before, during or after manufacture. From a toxicology point of view, impurities are relevant if they are present in sufficient quantity to have toxicological and/or eco-toxicological significance. If technically feasible, relevant impurities should be chemically identified and maximum permissible concentrations included in the material specification. However, even where a specification is given, it is important to undertake a detailed analysis in order to determine the exact composition of the sample used for toxicology testing. The scope of analysis for impurities can be informed, possibly, by the manufacturing process. For example, if a metal catalyst is used in the manufacturing process of nano-objects, then this information can inform the researcher about possible impurities in the final product. If specific additives are needed to ensure the stability of the final nano-objects, these should be described.

4.5 When to undertake physico-chemical characterization

When testing NOAA form, it will not be sufficient to rely upon a supplier's commercial characterization data as that information will be tailored to customer specifications for use in applications and further processing, rather than for toxicity testing. Separate characterization of the physico-chemical properties before toxicity testing ensures the results are related to the material presented for toxicological testing. There are important considerations regarding when to undertake physico-chemical characterization.

Several terms are used to describe when characterization can be conducted:

— 'As received' is the material removed from packaging;

— 'As administered' is the material prepared for introduction into *in vitro* or *in vivo* test systems; and

— 'After administration' refers to characterization once the material has been introduced into the toxicological test system.

The main advantage of testing ‘as received’ is that it identifies the nano-object at a common, recognized and documentable point. A combination of ‘as received’ physico-chemical characterization with a detailed and documented description of the material ‘as administered’ might provide even more valuable information. Information should be provided in any publications about the method used to draw the sample from the container, to handle the material to the point of preparation for administration, and to prepare the material for administration. The method of preparation can be critical to any alterations that occur to the NOAA; therefore, it is important to capture this information in order to understand other factors that might contribute to toxicological effects. While characterizing the material ‘as received’ has priority, it is insufficient for obtaining an understanding of those parameters, such as aggregation/agglomeration, that can undergo significant changes when the material is exposed to matrices suitable for toxicological testing. Therefore, it can also be important to carry out characterization once a sample has been prepared for testing.

A final note is that the researcher should be vigilant when opening testing material. For example, ensure that foreign materials have not been added to the product during handling and shipping. Shippers should provide the means of certification of its integrity upon arrival. Packages received open or damaged should be sealed and returned to the manufacturer.

Users of this Technical Report should be aware that there might be other relevant points in time at which to perform physico-chemical characterization. For example, evaluation of physico-chemical parameters after administration to the test system might provide valuable information on the mechanism or mode of action. For materials released into the environment, the decision regarding when characterization should be undertaken can be more complex. It is important also to consider that the physico-chemical characteristics of materials are likely to change over their lifecycle. Finally, evaluation of physical chemical parameters after storage may provide information about test material integrity over time and ensure that experiments generate meaningful observations.

Finally, users of this Technical Report should be aware that work is being done to standardize preparation procedures, such as ISO/TR 16196.

4.6 Potential problems with materials assessment

4.6.1 Confounders

Confounding involves error in the interpretation of what could be considered an accurate measurement. If an NOAA (the independent variable) is tested for the potential occurrence of an adverse effect (the dependent variable), the measured outcome is assumed to be directly related to the independent variable. However, it is important to be certain that the measured effect is clearly due to the independent variable and not to some artefact of the test system, tools, or impurities. If the measured effect is due to an artefact then there is a bias or confounding effect occurring.

Confounders can be either positive or negative, resulting in the measured value of the dependent variable being higher or lower than its true value. One such example is provided in the comparison of results from *in vitro* assays where CNTs were tested and found to exhibit different levels of an identical measured outcome due to the presence of residual metals in the CNT from the production process[14][15][16][17]. In this example, if potential confounding was not considered and addressed, then the observed results would have been incorrectly ascribed to the pure CNTs. On the other hand, it is easy to imagine that NOAAs in a controlled environment such as that of an *in vitro* test could agglomerate and lose some of their characteristic transport properties, where in practical circumstances these NOAAs could have collected organic or other molecules on their surface which would prevent agglomeration. Many other confounders can exit. Thus, conducting a thorough physico-chemical characterization of NOAAs should help to identify potential confounders.

4.6.2 Batch-to-batch variability

Ensuring batch-to-batch consistency of NOAA formulations is one of the challenges in product development. Nano-object based formulations may be complex and even highly purified nano-object samples are typically polydisperse in size and, possibly, composition, often containing a complex mixture of chemical species. In many instances, the specific active part of the NOAA cannot be identified or characterized individually, as it could exist alongside other components that affect its characteristics. Due to this potential compositional complexity, NOAAs are likely to exhibit a high degree of batch-to-batch variability if not produced according to

ISO 9000 or GMP processes. This variability could cause significant differences in the results of *in vitro* and *in vivo* experiments conducted on different batches of materials. Seemingly small variations in the surface chemistry, coating, synthesis, or formulation of an NOAA based material can significantly affect safety and toxicity. Independent characterization of the physico-chemical properties will identify whether or not there is consistency between batches of materials. Thus, it is recommended that different batches should be characterized and tested.

5 Parameters for the physico-chemical characterization of manufactured nano-objects prior to toxicological assessment

5.1 General information

For each of the physico-chemical parameters referred to in this Clause, a descriptor, clarification, relevance and measurand is provided.

— Descriptor is a word or short phrase used to identify the parameter. Also, a reference to a definition is made.

— Clarification provides further explanation of the parameter.

— Relevance refers to the toxicological significance of the physico-chemical parameter, based on the current state of knowledge which is likely to change over time. At the time this Technical Report was developed, the toxicological relevance has been stated based on scientific judgment. However the toxicological relevance was not able to be referenced in some cases.

— Measurand describes what is measured to quantitatively assess the physico-chemical parameter.

In Annex A, a diagram is provided to guide the user in the selection of the physico-chemical parameters that are relevant to material identification and that might help in the interpretation of the results of toxicity testing.

In Annex B, examples of methods are provided for the benefit of the user so they are aware of a number of the currently available methods to assess the relevant measurand.

5.2 Particle size and particle size distribution

5.2.1 Descriptor

The physical dimensions of a particle and, for collections of particles, the distribution of the sizes of the particles, determined by specified measurement conditions. See definitions 2.24 and 2.25.

5.2.2 Clarification

Size distribution refers to a group of particles of differing sizes. Most manufactured NOAAs are not monodispersed, rather they are a mixture of sizes. Some processes produce particles with little difference in size so the distribution of sizes is narrow. Other processes produce particles in which the size of individual particles varies greatly so the size distribution is broad. When a group of particles are of differing sizes, they are best described by a particle size distribution. Note that some of these methods tend to be prone to artefact generation due to sample preparation which can also influence particle size and/or size distribution.

5.2.3 Relevance

The conditions to which a material is subjected could affect the size of the discrete form of a material. The conditions might affect agglomeration/deagglomeration, particle growth, particle dissolution, etc.[1)] Particle size is typically measured by using one or a number of physical phenomena whose magnitude depends on the size of the particle being examined. Examples of such physical phenomena underlying different measurement methods are diffusion velocity (in liquids) or electrophoretic mobility (in liquids or in gases). Any given particle

1) The sizes of certain types of nanoparticles may be altered by dehydration in vacuum when measured by electron microscopy.

will interact with its environment according to its specific physical and chemical structure. In many fields, it has been the custom to define particle size ranges with common behaviour. This means that the size of a particle determined by one technique might not be the same as the size determined with another technique. Note, however, that size of an individual NOAA can be measured using microscopy techniques and these methods are not dependent on physical phenomena in the same ways as light scattering or sedimentation techniques.

The evaluations of EHS impacts necessarily involve biological systems which are themselves complex and could also introduce complications into the evaluation of material properties. Indeed, relating change in size to changes in other properties might lead to predictable mathematical relationships. Biological systems generally include water which might result in an increase in particle size, but might also include biological surfactants[2)] which can result in greater particle dispersal. Dissolved materials in biosystems could adsorb on or be absorbed by material potentially affecting particle size and corresponding biological responses. So, for toxicological testing, measurements of parameters should be made in the same conditions as the tested NOAA. The size-specificity with regard to the toxicity of material has been discussed in relation to its surface area[20][21][22][23].

In order to assess the influence of particle size on observed toxicological effects, it is important to establish the characteristics of the particle size distribution.

5.2.4 Measurand(s)

Equivalent spherical diameter, for particles displaying a regular geometry, unit [m]; length of one or several specific aspects of the particle geometry, unit [m]; the particle size distribution, the number of peaks and their width are a set of values, often displayed as a histogram, which for each of a number of defined size classes shows the quantity of particles, being either the number of particles, or the cumulative length, area, or volume of these particles or the signal intensity they produce.

5.3 Aggregation/agglomeration state in relevant media

5.3.1 Aggregate

5.3.1.1 Descriptor

Strongly bonded or fused particles where the resulting external specific surface area might be significantly smaller than the sum of known specific surface areas of primary particles. See definition 2.1

5.3.1.2 Clarification

The forces holding an aggregate together are strong forces, for example covalent bonds, or those resulting from sintering or complex physical entanglement. Aggregates are also termed “secondary particles” and the original source particles are termed “primary particles”.

5.3.1.3 Relevance

The current understanding is that the secondary particles which form as a result of agglomeration or aggregation, are larger, which might affect exposure. Thus, if nano-objects aggregate, the size of the aggregate would be the particle size relevant to potential exposure rather than the size of the primary particle or nano-object[24]. The aggregate would then be the particle of toxicological interest and the size of the aggregate would affect ADME behaviour.

5.3.1.4 Measurand

Particle size (see 5.2.4), unit [m]; number of aggregate particles in comparison to the total number of primary particles, unit [number/number]; number of primary particles in the aggregate, unit [number/number]; distribution of number of primary particles per aggregate.

NOTE Different measurands for this parameter are not always equivalent.

2) Compound that reduces surface tension when dissolved in water or water solutions, or which reduces inter-facial tension between two liquids or between a liquid and a solid[19].

5.3.2 Agglomerate

5.3.2.1 Descriptor

Collection of weakly or loosely bound particles or aggregates or mixtures of the two in which the resulting external specific surface area is similar to the sum of the specific surface areas of the individual components. See definition 2.2.

5.3.2.2 Clarification

The forces holding an agglomerate together are weak forces, for example, van der Waals forces[3)], or simple physical entanglement. The agglomeration state is a description of the agglomerate at a given moment in time; changes of the agglomeration state are indicative of the dynamic equilibrium which depends on time and the agglomerate environment, which can change the number of primary particles that adhere.

5.3.2.3 Relevance

Agglomerates are also termed “secondary particles” and the original source particles are termed “primary particles” or “secondary particles”, depending upon the aggregation state of the source particles. The relevance to toxicity testing is the effect of laboratory and biological manipulations on particle size. Agglomerates, being weakly bonded are more likely to fragment than aggregates being strongly bonded, which means the particle of toxicological interest may differ greatly from the ‘as received’ sample. Agglomerates when they do fragment may form aggregates, smaller agglomerates and possibly discrete primary particles. Against this background, it is important that particle size be determined in the biologically relevant media, such as blood serum or tissue culture media, in order to have an ‘as administered’ value to compare to ‘as received.’ There are instances of the ‘as administered’ size being larger which is a topic addressed in 5.9.2 dispersibility. There is general supposition that particle size reduction can also occur after administration, which is a factor in discussions of dose-metrics in inhalation studies. There is a recent report[25] of bacteria-mediated size and mass alterations of titanium dioxide (TiO2) agglomerates involving engulfment and subsequent environmental transport by the bacterium. In general, the investigator should measure particle size to ascertain if they are working with secondary particles. If so, then the user should examine the effect of shear[4)], by sonication[5)] and/or manipulation, on particle size in order to anticipate interactions of the ‘as administered’ sized particle with potential biological compartment sizes, fenestration[6)] effects and engulfment processes.

5.3.2.4 Measurand(s)

Number of agglomerate particles in comparison to the total number of primary particles, unit [number/number]; number of primary particles in the agglomerate, unit [number/number]; distribution of the number of primary particles per agglomerate, or size.

5.4 Shape

5.4.1 Descriptor

A description of the contour or outline of the surface of the nano-objects or collection of nano-objects, aggregates, agglomerates, that make up the material under investigation. See definition 2.27.

5.4.2 Clarification

Molecular and physical shapes are determined by how the atoms in a molecule are bonded to each other and will assume the shape that minimizes free energy and is kinetically achievable under given environmental conditions. While this might apply to bottom-up manufacture, the shape of top-down processed nano-objects, as opposed

3) Weak attractive force acting between molecules, somewhat weaker than hydrogen bonds and far weaker than interatomic valences[5].

4) Ratio between a stress (force per unit area) applied laterally to a material and the strain resulting from this force[19].

5) Disruption of bacteria by exposure or treatment with high-frequency sound waves[26].

6) Opening in the surface of a structure, as in a membrane[27].

to molecular assemblies (e.g. carbon nanotubes) may depend upon other factors, e.g. the surface tension of the liquid phase of the material. Shape characterization includes analysis of SEM or TEM or SPM images.

Manufactured nano-objects with identical composition can have a variety of shapes (including spheres, fibres and plates). Moreover, every one of these shapes might have different physical, chemical, and biological properties, because the connectivity of molecular bonds, e.g. surface exposed molecular bonds, can differ even though they are composed of the same atoms.

5.4.3 Relevance

Manufactured nano-objects with identical composition can have a variety of shapes (including spheres, fibres and plates). The effects of the shape on the toxicity of NOAAs have not been fully investigated. In relation to potential health effects, the aspect ratio might be important, for example, some high aspect ratio nanofibres have been shown to have the potential to cause an asbestos-like response in animal studies[28]. Also, the shape of NOAAs is expected to have effects on the kinetics of deposition and absorption in the body. For further information on relevance, please refer to Powers, et al. 2007[22].

5.4.4 Measurand

Size-independent descriptors of shape (examples are ratios of extensions in a different direction such as aspect ratio, unit [m/m] or fractal dimension); distribution of values of the size-independent shape descriptors.

NOTE Many descriptors of shape are available[29].

5.5 Surface area / mass-specific surface area / volume-specific surface area

5.5.1 Descriptor

Surface area is the quantity of accessible surface of a sample when exposed to either gaseous or liquid adsorbate phase. Surface area is conventionally expressed as a mass specific surface area or as volume specific surface area where the total quantity of area has been normalized either to the sample's mass or volume. See definition 2.29.

5.5.2 Clarification

Because the surface area as an extensive quantity depends on the amount of the material, a better comparable characteristic is the ratio of the surface area to the mass of a certain amount of a material. This is the so called specific surface area which is an intensive quantity and does not depend on the amount of the material.

In the case of porous materials, it is often useful to distinguish between external surface of the particle and the surface of the pores. The external surface is usually regarded as the envelope surrounding the discrete particles or agglomerates, but is difficult to define precisely because solid surfaces are rarely smooth on an atomic scale. A suggested convention is that the external surface be taken to include all the prominences and also the surface of those cracks which are wider than they are deep; the and the surface of the pore then comprises the walls of all cracks, pores and cavities which are deeper than they are wide and which are accessible to a test gas (the adsorptive). In practice, the demarcation is likely to depend on the methods of assessment and the nature of the pore size distribution. Because the accessibility of pores could depend on the size and shape of gas or liquid molecules, the area of, and the volume enclosed by, the surface of the pore as determined by gas or liquid adsorption might depend on the adsorptive molecules (molecular sieve effect) [30][31][32].

5.5.3 Relevance

Often chemical reactions take place at surfaces; therefore a sample of material with a high surface area can be expected to have a higher reactivity on a mass basis than a sample of the same material with a low surface area to volume ratio.

Surface area appears to be relevant for a number of parameters for toxicological and ecological hazard assessment. It can dictate the surface charge, for example. This in turn has direct consequences on (a) nano-

object interaction (i.e. agglomeration) with other naturally occurring particulate matter (i.e. contaminant vectors); (b) route of exposure as a function of surface ligand-biological interface (i.e. bioaccumulation[7)] pathway, bioavailability[8)]; and (c) mechanisms of toxicity (e.g. dose response curves normalized for specific surface area could indicate different results compared to results presented on a per mass basis)[16][35][36][37][38][39][40].

5.5.4 Measurand(s)

Specific surface area is defined as surface area of a substance divided by its mass, unit [m^2/g]; or surface area of a substance divided by its volume, unit [m^2/cm^3]. The research should also consider reporting results in both m2/g and m2/cm3.

5.6 Composition

5.6.1 Descriptor

Chemical information and crystal structure of the entire sample of nano-objects including: (a) composition, (b) crystalline structure[9)] including lattice parameters and space group, and (c) impurities, if any. See definition 2.8

5.6.2 Clarification

Composition characterization must include those components expected as well as those undesired such as impurities. Furthermore, the discrimination between surface chemistry and composition becomes blurred as the size of nano-objects approaches the lower range of the nanoscale. Thus, it is possible in some cases that the molecules attached to the surface could be considered under composition; however, the preference is to characterize surface chemistry separately. This point is particularly challenging as the surface molecules may exchange dynamically, for example with molecules in a suspension medium. Their arrangement, such as positioned perpendicular or parallel to the surface would likely impact toxicity.

5.6.3 Relevance

Chemical composition and crystalline structure are well established as significant toxicological determinants at the molecular level also for many nano-objects. Therefore, it is useful to provide chemical information and structure of the entire sample including: (a) composition, (b) presence or absence of crystalline structure including lattice parameters and space group, and (c) impurities, if any, in order to best understand their toxicity.

5.6.4 Measurand

The number and identity of elements alone or in molecules (can be expressed as a chemical formula with a specific stoichiometry; crystalline state; crystallographic structure; chemical state of atoms/elements; molecular structure-conformation including dextrorotatory[10)] and levorotatory[11)] (handedness[12)]); spatial distribution of the above items).

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7) Process of accumulation of a substance in organisms or parts thereof[33].

8) Stoichiometry is defined as a branch of chemistry; the relationship between the relative quantities of substances taking part in a reaction or forming a compound, typically a ratio of whole integers[34].

9) Description of the lattice structure in which atoms or molecules of an individual crystal are arranged (using lattice parameters and lattice type such as face-centred cubic, hexagonal close-packed, body-centred cubic, etc.).

10) Having the property when in solution of rotating the plane of polarized light to the right[19] description of the lattice structure in which atoms or molecules of an individual crystal are arranged (using lattice parameters and lattice type such as face-centred cubic, hexagonal close-packed, body-centred cubic, etc.)

11) Property when in solution of rotating the plane of polarized light to the left[19].

12) Asymmetric molecules that are mirror images of each other, related optically as right and left hands (chirality)[19].

5.7 Surface chemistry

5.7.1 Descriptor

Chemical nature, including composition, of the outermost layers of the NOAAs. See Definition 2.31.

5.7.2 Clarification

In some cases, surface chemistry is controlled by a single atomic species as, for example, in inorganic fullerene-like materials (e.g. MoS_2, can form nested spheres where the outer atomic layer is typically sulfur), or where specific chemical moieties have been deliberately attached to the surface with complete coverage (e.g. citrate stabilized gold). Note that nano-objects are often coated to reduce agglomeration and such coatings will dictate the surface chemistry of these particles. In cases where the nano-object has some level of porosity to the dispersing medium, the surface chemistry is more properly considered as the portion(s) of the nano-object that is in direct contact with the dispersing medium.

5.7.3 Relevance

Protein adsorption onto NOAAs alters the surface chemistry (i.e. when the surface chemistry of the NOAAs is largely defined by adsorbed proteins), and the impact on surface chemistry and activity of NOAAs has been the subject of many studies[41][42][43][44]. Early studies suggest that cellular responses to NOAAs are controlled by the surface-adsorbed molecular layer[45][46][47].

The various functional groups attached to the surface of NOAAs will lead to innumerable potential interactions and will play a key role in determining (1) entry into and distribution inside organisms; (2) fate in natural aqueous systems; (3) colloid stability; and (4) exposure to target cells or tissues. For a given functional group, this in turn will affect other physico-chemical properties, such as agglomeration, dustiness, zeta potential (see 5.8.2), specific surface area, and water solubility. Thus, it is widely hypothesized, and to some degree validated, that surface chemistry will play one of the key roles in determining the ultimate risk of any given NOAA[48].

An indicator of surface chemistry is the relative hydrophobicity or hydrophilicity of an NOAA which controls the interaction of the surface with water. In general, NOAAs for biological applications are hydrophilic. However, it has been shown that the presence of both hydrophilic and hydrophobic regions of cationic fullerene-like materials can disrupt the cell membranes of erythrocytes[13)][50].

5.7.4 Measurand

Elemental and molecular abundance unit [mole/mole], including thickness for fixed layers or [number of molecules/surface area] or [number of molecules bound/theoretical number of molecules bound with perfect reaction or perfect packing] for chemically reacted species that do not form a distinct phase; reactivity: standard chemical reaction rate concepts [mole/(dm^3 s)] preferably of a species of toxicological interest or its surrogate.

NOTE Measurement of reactivity is very specific to the measurement of the species to which it is reactive (such as reactive to water) and typically involves measuring products or by-products of that reaction.

5.8 Surface charge

5.8.1 Descriptor

Electrical charge on a surface in contact with a continuous phase. See definition 2.30.

5.8.2 Clarification

In colloidal systems (i.e. systems where the dispersed phase, NOAAs in this case, are evenly dispersed through the continuous liquid phase on the macroscale), the surface charge can be calculated by determining the zeta potential. Zeta potential is an abbreviation for electrokinetic potential in colloidal systems. From a theoretical viewpoint, zeta potential is the electric potential in the interfacial double layer (DL) at the location of the slipping

13) Non-nucleated, biconcave red blood corpuscle averaging 7,7 micrometers in diameter[49].

plane versus a point in the bulk fluid away from the interface. In other words, zeta potential is the potential difference between the dispersion medium and the stationary layer of fluid attached to the dispersed particle.

In systems where NOAAs are aerosolised, electrostatic charge can be generated by friction between surfaces, such as on walls and on macroscopic particles. From these surfaces, gas molecules can carry the charge to the NOAAs.

The presence of electrostatic charge on nanoparticles can influence the formation of agglomerates, thus affecting if and how they are formed.

5.8.3 Relevance

The significance of zeta potential is that its value can determine the rate at which certain biological systems can accumulate NOAAs in the environment, hence the likelihood of any potential toxicity manifesting itself in the biological system or organism[22][24][48][51][52][53][54]. Furthermore, it can be related to the stability of colloid dispersions. The zeta potential indicates the degree of repulsion between adjacent and similarly charged particles in a dispersion. For molecules and particles that are small enough, a high zeta potential (positive or negative) will confer dispersion stability (i.e. the dispersion will resist agglomeration). When the zeta potential is small (positive or negative), attraction exceeds repulsion and the dispersion will break and flocculate[14] through van der Waals interactions. In the case of low ionic strength (less than ~0,1 M)[48][53][56], ions with greater than unity valence[15] can bond to the surface of the nanoparticle and the addition of such ions dramatically increases its zeta potential.

In particulate toxicology, zeta potential (surface charge) plays a key role in determining (1) the degree of colloid interaction which is itself a function of the pH and ionic strength of the bulk solution, and (2) bioavailability of a compound when considering mass transport through charged membranes as related to exposure.

5.8.4 Measurand(s)

Net number of positive and negative charges per unit particle surface area, unit [Coulomb/m^2]; zeta potential, unit [V].

5.9 Solubility/dispersibility

5.9.1 Solubility

5.9.1.1 Descriptor

The degree to which a material (the solute) can be dissolved in another material (the solvent) so that a single, homogeneous, phase results. See definition 2.32.

5.9.1.2 Clarification

The concept of solubility is relevant to solids, liquids and gases as solutes, and solids and liquids as solvents (note that gases, in the form of supercritical fluids[16], are also sometimes used as solvents). Solubility typically depends on, and increases with, temperature, and might also depend on pressure and pH. Materials that are soluble at all relative proportions are said to be completely miscible, an example being alcohol and water, whereas materials that do not form a solution at any concentration are said to be completely insoluble or completely immiscible (e.g. benzene and water). Lack of solubility of one material in another is displayed by the formation of precipitates (precipitated phases) in solids, and by separation into separate phases in liquids and liquid/solid.

Because of the particle size of many nano-objects, it can be difficult to distinguish between when a nano-object is dispersed and when it is dissolved. The main difference between these terms is that dissolved requires

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14) To form larger agglomerates, to the size where precipitation occurs, or to facilitate precipitation[55].

15) Valence of an atom in relation to hydrogen, whose valency in chemistry is termed “unity”[57].

16) Substance at temperature above its critical temperature, the temperature above which a gas cannot be liquefied, no matter how much pressure is applied[58].

that molecules of a solid phase are substantially disassociated by the process, while there is no significant disassociation involved in a phase that has been dispersed into another.

5.9.1.3 Relevance

If an NOAA is soluble in biological or environmental media, then it is likely to be presented to an *in vitro* / *in vivo* test system in its molecular or ionic form and can be expected to elicit the same response as more usual bulk forms of the same substance. Note, however, that soluble materials in nano-object form will almost certainly dissolve more quickly than larger forms of the same material, and this could affect the concentration of a transient solution formed from a soluble material in nano-object form, compared to that for larger particles of the same material. If the nano-object under investigation is insoluble in biological or environmental media, then it will be presented to the test system in its original form, although its ultimate distribution can be expected to be different from that of the same material presented in larger particle form.

From a toxicological viewpoint, solubility in both oil and water will be important, as these will affect the biological and/or environmental distribution of a material[59].

5.9.1.4 Measurand(s)

Maximum mass or concentration of the solute that can be dissolved in a unit mass or volume of the solvent at specified (or standard) temperature and pressure, unit [kg/kg] or [kg/m3] or [mole/mole].

5.9.2 Dispersibility

5.9.2.1 Descriptor

The degree to which a particulate material (the dispersed phase) can be uniformly distributed in another material (the dispersing medium or continuous phase) and resulting dispersion remains stable (for example one hour or one minute). See definition 2.9.

5.9.2.2 Clarification

The concept of dispersion is relevant to solids, liquids and gases. Strictly speaking, liquid and gaseous dispersions will eventually settle out to give two separate phases. However, for very fine particles, including nano-objects, stable suspensions (colloids or aerosols) can be formed, which have indefinite stability.

5.9.2.3 Relevance

If immune or inflammatory responses of NOAAs are size dependent during a toxicity study, then dispersion of the particles becomes significant factor. Further, agglomeration or re-agglomeration of a material can hinder particle penetration of cell membranes or inhibit the ability of a macrophage[17)] to complete phagocytosis[18)]. The influence of dispersibility on toxicity results is not yet fully understood[22].

5.9.2.4 Measurand(s)

Maximum mass or concentration of the dispersed phase present in a unit mass of the dispersing medium (solvent) or in a unit volume of the dispersion (solvent plus dispersed phase) at specified (or standard) temperature and pressure, units [kg/kg], [kg/m^3], or [mole/mole].

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17) Type of motile, mononuclear, phagocytic (engulfing whole bacteria, other cells and debris), microbicidal and tumoricidal cell circulating in the blood, non-circulating, or site specific macrophages that include Kupffer cells, splenocytes, alveolar macrophages, microglia and histiocytes[49].

18) Action by phagocytes, cells which have the ability to ingest and destroy particulate substances such as bacteria, protozoa, cells and cell debris, dust particles and colloids[49].

6 Expression of measurement results and uncertainties

6.1 General

All measurements are subject to some level of uncertainty and the accuracy of a measurement result is expressed in a statement of that uncertainty. This section describes the expression of measurement results and uncertainties.

The derivation and testing of predictive models of behaviour benefit from precision in quantitative, measured values and also quality of the values. The quantity intended to be measured is referred to as the measurand, and a quantitative statement of the uncertainty must be assigned to any measured value to enable the user of the value to judge the value's reliability. The *International Vocabulary of Metrology* guide[8] provides a list of definitions and descriptors for words frequently used in the language of metrology. Aspects of repeatability and reproducibility are as important for a laboratory to establish confidence in a local set-up as they are to compare to another laboratory around the world[60]. An additional metrological requirement is traceability, meaning that the measurement result can be related to international references through an unbroken chain of comparisons. Metrological traceability provides a more profound confidence in a measurement result and improves its comparability. Global comprehension of these aspects of a measurement quantity is promoted through international guidelines[61]. For example, for physical quantities, the specification of a numerical value, the unit of measure and the measurement uncertainty can be provided. If it is written that the wavelength is $\lambda = (5{,}896 \pm 0{,}003) \times 10^{-7}$ m, then λ is the symbol for the physical quantity (wavelength), m is the symbol for the unit (metre), $5{,}896 \times 10^{-7}$ is the numerical value of the wavelength in metres and the measurement uncertainty of $\pm\ 0{,}003\ \times\ 10^{-7}$ m describes our knowledge about the accuracy of the measurement result. An explicit statement on the meaning of the stated uncertainty is helpful if provided (e.g. where the stated uncertainty refers to a 95 % confidence level).

Reliable evaluation of measurement uncertainty is pivotal in conformity assessment where decisions are made whether the output quantity conforms to a stipulated limit for manufacturing quality control, legal metrology or regulation. The *Guide to the Expression of Uncertainty in Measurement*[62] provides guidelines for evaluating measurement uncertainty so that measurements can be compared with each other. *An Introduction to the GUM*[63] is a good starting point, providing instructive explanations. A bibliography hosted on the BIPM website[63] provides links to publications of GUM-compliant worked examples with representation from all SI units and a broad spectrum of application.

6.2 Quantifying uncertainty

The measurement uncertainty in a well-defined measurand is evaluated by combining the uncertainties attributed to each of the influence parameters by adding in quadrature[19)]. The first step in the uncertainty evaluation is to describe how the measurand, or output quantity, is calculated; namely writing down the functional relationship of input quantities. The uncertainties of each influence are evaluated by either statistical evaluation from repeated measurements (Type A evaluation) or from some other knowledge such as calibration values from other measurements, scientific judgment, or the experience of the metrologist (Type B evaluation). Regardless of how it is evaluated, a *standard uncertainty* can be thought of as a standard deviation. Standard uncertainties can be attributed to factors such as: resolution, repeatability, drift, and instrument calibration. The combined standard uncertainty in the measurand is influenced by the extent to which each of the parameters influences the final result.

If the model of the output quantity y as a function of input quantities xi is given by $y = f(\mathrm{xi})$, then the sensitivity coefficients are defined by $c_i \equiv \partial \mathrm{f}/\partial \mathrm{xi}$. The sensitivity coefficients multiplying each standard uncertainty in the

19) Method of estimating combined uncertainty from independent sources by taking the square root of the sum of the squares of individual components of uncertainty (for example, coefficients of variation)[62].

quadrature sum are determined from the measurement model Formula[62], leading to the following general equation to calculate a combined measurement uncertainty:

$$u_c^2 = \sum_i \left(c_i u_i\right)^2 \quad (1)$$

where

u_i are independent standard uncertainty contributions

c_i are the sensitivity coefficients for the individual standard uncertainty contributions

u_c is the standard uncertainty.

The model, via the sensitivity coefficients, therefore has an enormous influence on the evaluation of measurement uncertainty. The expanded uncertainty is the combined standard uncertainty multiplied by a coverage factor for the purposes of interpretation. A coverage factor of $k = 2$ is common. When $k = 2$, and if the number of degrees of freedom of the combined uncertainty is sufficiently large there is approximately a 95 % probability that the true value of the measurand lies within the stated range. Other confidence levels can be used in practice, depending on the application and the intended end-use of the uncertainty value.

6.3 Application of uncertainty to nano-objects

There is a wide diversity of manufactured NOAAs which means that measurements to characterize them can be expected to be likewise varied. The identification of the specific measurand will be key to evaluating the measurement uncertainty. In any measurement, the end-use of the measurement is most important in determining what will be measured, and precisely how it will be measured (what instrument, conditions, end-points, etc.). For example, a particle diameter measurement could have different meaning depending on the boundary conditions of the application – manufacturing process control, chemical characterization or identification, and so on. The evaluation of the measurement uncertainty is entirely dependent on the specifics of the reported measurement. Sometimes measurement uncertainty evaluation can be a straightforward exercise, for example when the measurement model is well understood; however it always involves in-depth knowledge of all aspects of those parameters that influence the measurement. For that reason, GUM-compliant uncertainty evaluations for nanotechnologies are in development, mirrored with the development of accurate measurements used to characterize NOAAs. In the example of nanoscale size measurement, guidance for measurements by SPM have appeared[64][65], and contributions to the scientific literature continue to increase as our understanding of accuracy, traceability and uncertainty evaluation improves.

Not only the measurement instrument and method, but also the quality of reference materials/calibration materials makes a significant contribution to measurement uncertainty. Appropriate reference materials are important for validation exercises in confirming our understanding and to obtain lowest measurement uncertainties[66]. Development of best quality reference materials continues based on anticipated needs. Standard methods and protocols outlined in the normative documentary standards are important to defining the measurement model. Measurement procedures, expertise of the operator/metrologist and the surrounding laboratory environment are also significant contributors to measurement uncertainty. All of these aspects are discussed, to the extent that they are known, in the documentary standards outlining the particular measurement and measurand. Such aspects might include an outline of the required information for quantitative characterization; namely, measurement procedures, descriptions and restrictions, avenues for SI-traceability[20)], measurement units, measurement tools and instrumentation, validation and quality control, peer-review publications, and proposed or suggested uncertainty parameters or an uncertainty budget.

6.4 Importance of validation

Measurement results and uncertainty evaluations should both be validated, either through an in-house validation study using appropriate reference materials, or through interlaboratory comparison exercises, namely, when several laboratories perform a prescribed measurement on the same reference material or sample and the

20) Property of the result of a measurement or the value of a measurement standard whereby it can be related to stated references, usually national or international measurement standards (SI - international system of units), through an unbroken chain of comparisons, all having stated uncertainty[8].

results are compared with each other. Comparison data are analysed to verify that all laboratories obtain the same results within the boundaries of their reported measurement uncertainties as expected. This can be achieved via simple analysis of the plotted data or by statistical methods and employing international protocols[67][68][69]. In the case of data that is suspected of being discrepant, statistical methods[70] and tools[71] are available for more thorough analysis. Identifying the technical reasons behind discrepant or outlier data can make important contributions to our understanding of measurement methods and the sample.

7 Reporting

The physico-chemical data should be reported in a format which enables their correct interpretation and use by the customer, and preferably also enables the data to be entered into a material properties database. Many factors can determine the correctness and reliability of test data. Some of these include: human factors, laboratory environment, measurement method and method validation, equipment, measurement traceability, sampling and sample handling and preparation. Detailed discussion of these influences and guidelines for best practice are outlined in *General requirements for the competence of testing and calibration laboratories*[72].

Test reports promote comprehension and comparability when they clearly state the purpose of the characterization that has been performed and, when appropriate, should include the following:

— material description and details using the appropriate physico-chemical characteristics noted herein;

— sample preparation including identification of standard protocols applied;

— laboratory environment conditions where they influence the quality of the results; and

— standard analytical methods and extraction conditions.

The reference to the standard method provides scientific support for the results observed. When developing and validating new methods, the testing performed should contain at least the following information:

- appropriate identification of test laboratory and measurement method;
  - scope (in terms of materials, measurement range and concentrations, where appropriate);
  - description of the type of item to be tested or calibrated;
  - parameters or quantities and ranges/intervals to be determined;
  - quantitative data generated, including measurement uncertainty and traceability statement;
  - reference standards and reference materials required;
  - environmental conditions required and any stabilization period needed;
  - description of the procedure, including:
    - affixing of identification marks, handling, transporting, storing and preparation of items;
    - checks to be made before the work is started;
    - checks that the equipment is working properly and, where required, calibration and adjustment of the equipment before each use;
    - the method of recording the observations and results;
    - any safety measures to be observed;
  - criteria and/or requirements for approval/rejection;
  - data to be recorded and method of analysis and presentation;

  - the uncertainty or the procedure for estimating uncertainty;

- equipment / instruments used;
- measurement traceability – calibration and reference standards / materials;
- qualitative data, opinions and interpretations generated, always as a section clearly separated from the sections reporting the observations; and,
- quantitative data generated (including measurement uncertainty and traceability statement).

Annex A
(informative)

Diagram illustrating the use of physico-chemical characterization in toxicological testing

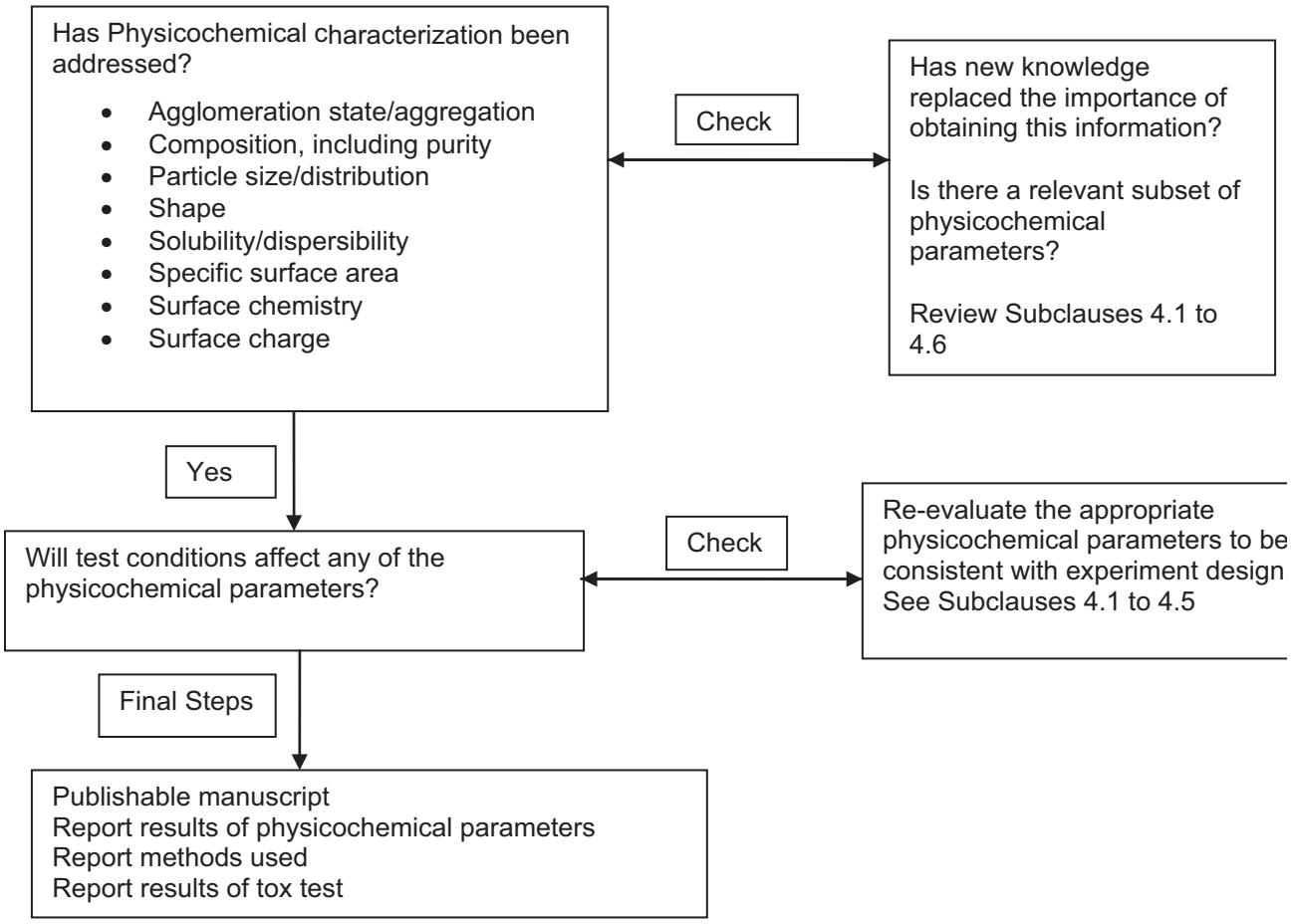

Figure A.1 — Diagram illustrating the use of physico-chemical characterization in toxicological testing

Annex B
(informative)

Example measurement methods and standards

Examples of measurement methods are provided for the benefit of the user so he/she is aware of at least a number of currently available methods. The user is cautioned that these methods have not necessarily been validated for use in characterizing multiple types of NOAAs. Due to the diversity of NOAAs, most of the listed methods will be applicable only for a minority of the NOAAs, and only for part of the possible concentrations in which the NOAAs are presented in the toxicological tests. Thus, there is an urgent need for development and validation of (additional) measuring methods for these parameters.

Similar to the list of methods is the list of current standards. Standardisation of new, relevant, validated methods is currently being undertaken by ISO/TC 229 and other relevant ISO technical committees such as ISO/TC 201, *Surface chemical analysis*, and ISO/TC 24, *Particle characterization* (information available at http://www.iso.org). Other measurement methods and procedures are being used or recommended by some international organizations (e.g. OECD, ASTM International) and host nation organizations (e.g. EPA Harmonized Test Guidelines, NCI Nanotechnology Characterization Laboratory), and the interested reader can consult those organizations as well.

NOTE ISO continually updates documents that might supplant the information presented.

Table B.1 — Measurement methods and relevant standards

Parameter	Measurement methods	Relevant standards
Particle size	Dynamic light scattering (also known as Photon correlation spectroscopy)	- ISO 9276-1:1998, *Representation of results of particle size analysis – Part 1: Graphical representation* - ISO 9276-1:1998/Cor 1:2004, *Representation of results of particle size analysis – Part 1: Graphical representation – Technical Corrigendum 1* - ISO 9276-2:2001, *Representation of results of particle size analysis – Part 2: Calculation of average particle sizes/ diameters and moments from particle size distributions* - ISO 9276-3:2008, *Representation of results of particle size analysis – Part 3: Adjustment of an experimental curve to a reference model* - ISO 9276-4:2001, *Representation of results of particle size analysis – Part 4: Characterization of a classification process* -ISO 9276-5:2005, *Representation of results of particle size analysis – Part 5: Methods of calculation relating to particle size analyses using logarithmic normal probability distribution* -ISO 9276-6:2008, *Representation of results of particle size analysis – Part 6: Descriptive and quantitative representation of particle shape and morphology*
	Small angle X-ray scattering	
	Size exclusion chromatography	
	Analysis of SEM or TEM or SPM images	
	Differential mobility analysis	
	Separation techniques such as: - Centrifugal liquid sedimentation - Nanoparticle tracking analysis	
	Raman spectroscopy (nanotube diameter measurement)	
	Laser-induced incandescence (LII)	

Table B.1 *(continued)*

Parameter	Measurement methods	Relevant standards
		-ISO 9277:2010, *Determination of the specific surface area of solids by gas adsorption – BET method*
		-ISO 13318-1:2001, *Determination of particle size distribution by centrifugal liquid sedimentation methods – Part 1: General principles and guidelines*
		-ISO 13318-2:2007, *Determination of particle size distribution by centrifugal liquid sedimentation methods – Part 2: Photocentrifuge method*
		-ISO 13318-3:2004, *Determination of particle size distribution by centrifugal liquid sedimentation methods – Part 3: Centrifugal X-ray method*
		-ISO 13320:2009, *Particle size analysis – Laser diffraction methods*
		-ISO 13321:1996, *Particle size analysis – Photon correlation spectroscopy*
		-ISO 13322-1:2004, *Particle size analysis – Image analysis methods – Part 1: Static image analysis methods*
		-ISO 13322-2, *Particle size analysis – Image analysis methods – Part 2: Dynamic image analysis methods*
		-ISO/TS 13762:2001, *Particle size analysis– Small angle X-ray scattering method*
		-ISO 14488:2007, *Particulate materials – Sampling and sample splitting for the determination of particulate properties*
		-ISO 14887:2000, *Sample preparation – Dispersing procedures for powders in liquids*
		-ISO 15900:2009, *Determination of particle size distribution – Differential electrical mobility analysis for aerosol particles*
		-ISO 20998-1:2006, *Measurement and characterization of particles by acoustic methods – Part 1: Concepts and procedures in ultrasonic attenuation spectroscopy*
		-ISO 21501-1:2009, *Determination of particle size distribution – Single particle light interaction methods – Part 1: Light scattering aerosol spectrometer*
		-ISO 21501-2:2007, *Determination of particle size distribution – Single particle light interaction methods – Part 2: Light scattering liquid-borne particle counter*
		-ISO 22412:2008, *Particle size analysis – Dynamic light scattering (DLS)*
		-ASTM E2490-09, *Standard Guide for Measurement of Particle Size Distribution of Nanomaterials in Suspension by Photon Correlation Spectroscopy (PCS)*
		ISO 16700:2004, *Microbeam analysis – Scanning electron microscopy –Guidelines for calibrating image magnification*

Table B.1 *(continued)*

Parameter	Measurement methods	Relevant standards
Aggregation / Agglomeration State	Analysis of cryo-SEM or cryo-TEM image.	See section 'Particle Size' (above) for measurement methods ISO/TC 24, *Particle Characterization*, has started the development of ISO/TR 13097, *Guide for the characterization of dispersion stability* ISO/DIS 12025 *Nanotechnologies, developed a general framework for determining the nano-object release from powders* ISO 13322-1:2004, *Particle size analysis – Image analysis method – Part 1: static image analysis methods*
	Angle dependent scattering at different wavelengths	
	Static light scattering	
	Small angle X-ray scattering	
	X-ray diffraction	
	Small angle neutron scattering	
	Rheology methods	
	Centrifugal liquid sedimentation	
	Laser diffraction	
	Nanoparticle tracking analysis	
Shape	Analysis of SEM or TEM or SPM images	ISO 16700:2004, *Microbeam analysis – Scanning electron microscopy –Guidelines for calibrating image magnification* ISO 13322-1:2004, *Particle size analysis – Image analysis method – Part 1: static image analysis methods*
	Scattering techniques	
Surface area	Methods based on gas or liquid adsorption isotherms	ISO 15901-1:2005, *Pore size distribution and porosity of solid materials by mercury porosimetry and gas adsorption – Part 1: Mercury porosimetry* ISO 15901-2:2005, *Pore size distribution and porosity of solid materials by mercury porosimetry and gas adsorption – Part 2: Analysis of mesopores and macropores by gas adsorption* ISO 15901-3:2005, *Pore size distribution and porosity of solid materials by mercury porosimetry and gas adsorption – Part 3: Analysis of micropores by gas adsorption* ISO 18757:2003, *Fine ceramics (advanced ceramics, advanced technical ceramics – Determination of specific surface area of ceramic powders by gas adsorption using the BET method* ISO 13322-1:2004, *Particle size analysis – Image analysis methods – Part 1: Static image analysis methods*
	Liquid porosimetry	
	Image analysis	
	Laser-induced incandescence (LII)	

Table B.1 *(continued)*

Parameter	Measurement methods	Relevant standards
Composition	X-ray fluorescence – chemical purity	- ISO 22309:2006, *Microbeam analysis – quantitative analysis using energy-dispersive spectrometry (EDS)* - ISO 22489:2006, *Microbeam analysis – electron probe microanalysis – quantitative point analysis for bulk specimens using wavelength-dispersive X-ray spectroscopy* - ISO 24173:2009, *Microbeam analysis – Guidelines for orientation measurement using Electron Backscatter Diffraction* - ISO 13084:2011, *Surface chemical analysis – Secondary-ion mass spectrometry – Calibration of the mass scale for a time-of-flight secondary-ion mass spectrometer* - ISO 18114:2003, *Surface chemical analysis – Secondary-ion mass spectrometry – Determination of relative sensitivity factors from ion-implanted reference materials*
	X-ray photoelectron spectroscopy – (surface) chemical purity	
	Auger electron spectroscopy – (surface) chemical purity	
	X-ray diffraction – crystallinity, relative amount of different crystal phases, purity	
	Raman and other molecular spectroscopies	
	ThermoGravimetric Analysis-purity	
	Ultra Violet/Visible spectrometry	
	Scanning Electron Microscopy	
	Nuclear magnetic resonance (NMR)	
	Inductively Coupled Plasma-Optical Emission Spectrometer (ICP-OES)	
	Inductively Coupled Plasma-Mass Spectrometer (ICP-MS)	
Surface Chemistry	Auger electron spectroscopy (AES) Scanning Auger Electron Microscopy	Under development: ISO/DTR 14187, *Surface chemical analysis – Characterization of nanostructured materials* ISO 18115:2001, *Surface chemical analysis – Vocabulary* ISO 24236:2005, *Surface chemical analysis – Auger electrons pectroscopy – Repeatability and constancy of intensity scale* ISO 15471:2004, *Surface chemical analysis – Auger electron spectroscopy – Description of selected instrumental performance parameters* ISO/TR 19319:2003, *Surface chemical analysis – Auger electron spectroscopy and X-ray photoelectron spectroscopy – Determination of lateral resolution, analysis area and sample area viewed by the analyser* ISO 17973:2002, *Surface chemical analysis – Medium resolution Auger electron spectrometers – Calibration of energy scales for elemental analysis* ISO 18118:2004, *Surface chemical analysis – Auger electron spectroscopy and X-ray photoelectron spectroscopy – Guide to the use of experimentally determined relative sensitivity factors for the quantitative analysis of homogeneous materials*
	X-Ray photoelectron spectroscopy (XPS)	
	Secondary ion mass spectrometry (SIMS)	
	3D atom probe tomography	
	Energy Dispersive X-Ray Spectrometry	
	Electron Energy Loss Spectroscopy (EELS)	
	Low Energy Ion Spectroscopy	
	Raman and other molecular spectroscopies	

Table B.1 *(continued)*

Parameter	Measurement methods	Relevant standards
		ISO 20903:2006, *Surface chemical analysis – Auger electron spectroscopy and X-ray photoelectron spectroscopy – Methods used to determine peak intensities and information required when reporting results* ISO/TR 18394:2006, *Surface chemical analysis – Auger electron spectroscopy – Derivation of chemical information* ISO 23830:2008, *Surface chemical analysis – Secondary-ion mass spectrometry – Repeatability and constancy of the relative intensity scale in static secondary ion mass spectrometry* ISO 17560:2002, *Surface chemical analysis – Secondary-ion mass spectrometry - Method for depth profiling of boron in silicon* ISO 18114:2003, *Surface chemical analysis – Secondary-ion mass spectrometry – Determination of relative sensitivity factors from ionimplanted reference materials* ISO 20341: 2003, *Surface chemical analysis – Secondary-ion mass spectrometry – Method for estimating depth resolution parameters with multiple delta-layer reference materials* ISO 15472: 2002, *Surface chemical analysis – X-ray photoelectron spectrometers – Calibration of energy scales* ISO 21270:2004, *Surface chemical analysis – X-ray photoelectron and Auger electron spectrometers – Linearity of intensity scale* ISO 24237:2005, *Surface chemical analysis – X-ray photoelectron spectroscopy - Repeatability and constancy of intensity scale* ISO 15470:2004, *Surface chemical analysis – X-ray photoelectron spectroscopy – Description of selected instrumental performance parameters* ISO 19318:2004, *Surface chemical analysis – X-ray photoelectron spectroscopy – Reporting of methods used for charge control and charge correction* ISO/TR 18392:2005, *Surface chemical analysis – X-ray photoelectron spectroscopy – Procedures for determining backgrounds* ISO 18516:2006, *Surface chemical analysis – Auger electron spectroscopy and X-ray photoelectron spectroscopy – Determination of lateral resolution* ISO 18117:2009, *Surface chemical analysis – Handling of specimens prior to analysis* ISO 23812:2009, *Surface chemical analysis – Secondary-ion mass spectrometry – Method for depth calibration for silicon using multiple delta-layer reference materials*
Surface charge	Isoelectric point	ISO 20998-1:2006, *Measurement and characterization of particles by acoustic methods – Part 1: Concepts and procedures in ultrasonic attenuation spectroscopy*
	Electrophoretic light scattering	
	Electrophoresis	
	Electrosmosis	
	Electric sonic amplitude	
	Colloidal vibration current	

Table B.1 *(continued)*

Parameter	Measurement methods	Relevant standards
Solubility	There are no specific methods for the assessment of the solubility of nano-objects, however, consider reporting equilibrium dialysis, Inductively coupled plasma mass spectrometry (ICP-MS) or inductively coupled plasma optical emission spectroscopy (ICP-OES) as possible measurement methods.	No standards available
Dispersibility	Most common methods to assess the dispensability of nano-objects are based on particle size measurements (see above)	ISO/TC 24 new work item on dispersion, ISO/TR 13097, *Guidelines for the characterization of dispersion stability*

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