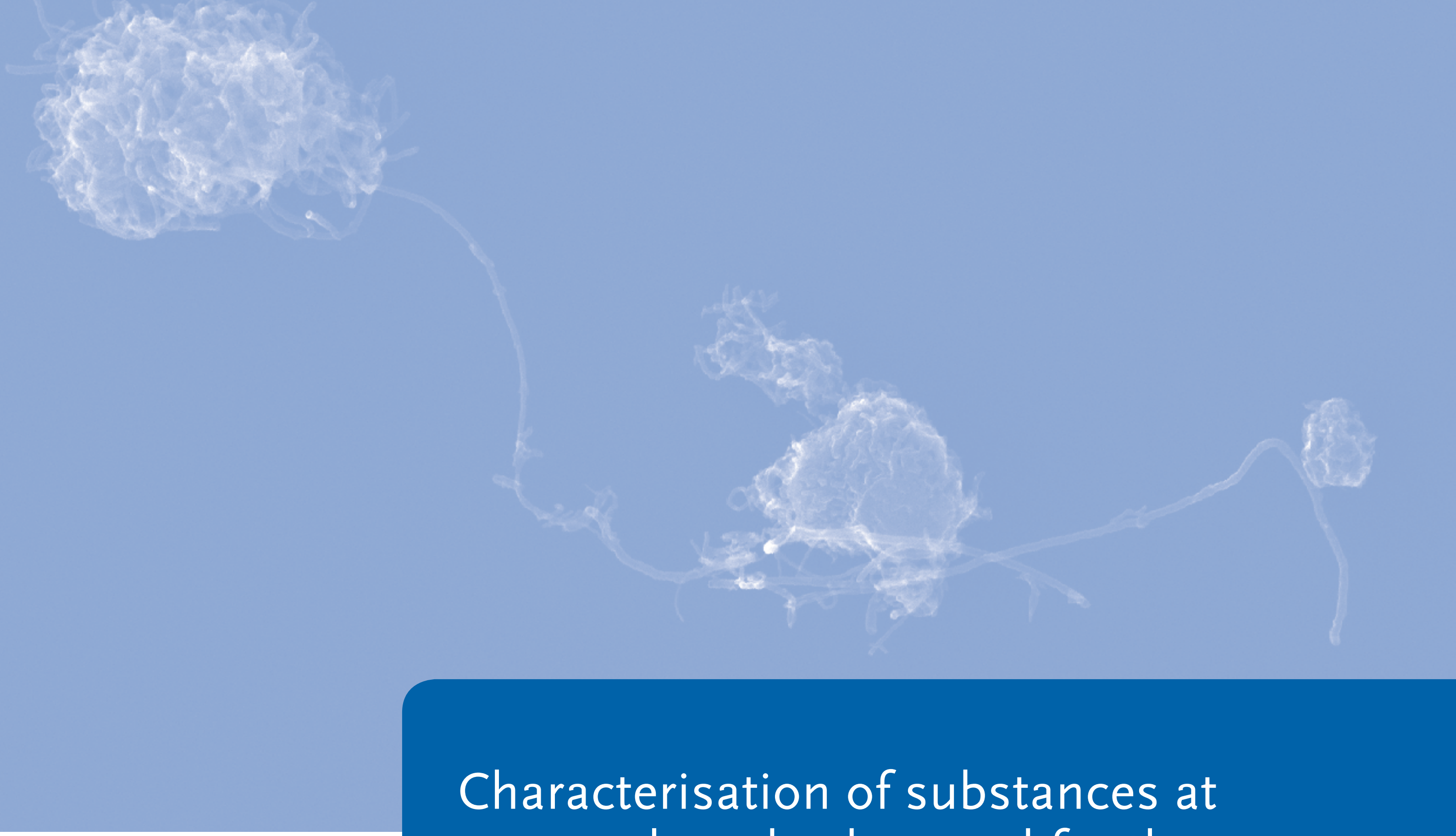

Characterisation of substances at nanoscale as background for the regulation in the framework of the regulation (EC) No. 1907/2006 (REACH)

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Bundesanstalt für Arbeitsschutz
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The responsibility for the contents of this publication lies with the authors.

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Characterisation of substances at nanoscale as background for the regulation in the framework of the regulation (EC) No. 1907/2006 (REACH)

Abstract

The presented literature documentation shows the applicability of the test methods listed in regulation EC 440/2008.

The first part explains different terms and definitions of nanomaterials and the nanomaterials' classifications.
Afterwards the physico-chemical properties of nanomaterials are described and the feasibility to characterise substances at nanoscale with the test methods listed in regulation 440/2008 is assessed.

In the last part relevant properties of nanomaterials from the perspective of international institutions are listed and possible methods for the determination are given.
Finally, it is analysed which test methods of the regulation EC 440/2008 are applicable to nanomaterials and which further properties should be investigated in the frame of the regulation of nanomaterials.

Key words:

REACH, nanomaterials, characterisation, physico-chemical data

Charakterisierung von nanoskaligen Eigenschaften chemischer Stoffe als Grundlage für die Regulierung im Rahmen der Verordnung (EG) Nr. 1907/2006 (REACH)

Kurzreferat

Die vorliegende Literaturarbeit zeigt auf, inwiefern die in der Verordnung 440/2008 EG enthaltenen Prüfnachweise für Nanomaterialien anwendbar sind.

In einem ersten Teil werden die verschiedenen verwendeten Begriffe für Nanomaterialien erklärt und die Klassifizierung der Nanomaterialien erläutert.
Daran schließen sich die Beschreibung der physikalisch chemischen Eigenschaften von Nanomaterialien und die Charakterisierbarkeit der Nanoskaligkeit mit den in der Verordnung 440/2008 genannten Prüfmethoden an.

Im letzten Teil werden die relevanten Eigenschaften von Nanomaterialien aus Sicht internationaler Institutionen beschrieben und die möglichen Bestimmungsmethoden aufgeführt.
Abschließend wird analysiert, welche vorhandenen Prüfmethoden der Verordnung 440/2008 auf Nanomaterialien anwendbar sind und welche weiteren Eigenschaften im Rahmen einer Regulierung der Nanomaterialien geprüft werden sollten.

Schlagwörter:

REACH, Nanomaterialien, Charakterisierung, physikalisch-chemische Daten

1 Introduction

1.1 Background

The prefix "nano" originates from the Greek word "νάνος" - "dwarf". Only when applied to physical units, the prefix "nano" has a clear meaning and serves as a unit scaling factor of 10^{-9}. When used in the formation of new terms like *nanomaterials* or *nanotechnology*, the meaning of the prefix “nano” has to be specified.

Nanotechnology is a key technology. By progress in the characterisation of materials at the nanoscale, materials scientists have been able to investigate chemical and morphological results of their synthesis and modification approaches. Thus, the abilities for targeted material developments at the nanometre scale have improved and gain new momentum. They have led to a large variety of organic and inorganic nanomaterials. In addition, their large surface area that is specific for nano-structured materials can be designed in a functionally diverse way and further broadens the range of new materials. Many nanomaterials have raised hopes for significantly improved material and product properties by novel and sometimes even revolutionary material features. Therefore international research and development efforts - and thus the number of new nanomaterials - will continue to increase dramatically.

As the term *nanomaterial* is at first merely a concept of lateral dimension, additional properties must be considered in order to get an impression of the magnitude of this class of materials. Nanomaterials can be of very different structure and composition. They include, in principle, not only particulate materials, but also nano-structured volume- or surface domains. Therefore, many international organisations are working on schemes for classifying nanomaterials. The most prominent include *OECD* and *ISO*. Their schemes classify nanomaterials based on their size, shape and other structural aspects as shown in Figure 2.3 [1].

Such classification schemes are also introduced to define new terms that describe the new variety of materials. Especially for legislation, such as the liability or chemicals law, reliable definitions are of key importance. *ISO* has developed so-called *Core Terms* for the nomenclature of nanomaterials [2]. There are numerous definitions of the term *nanomaterial*, which attempt to specify the peculiarities of nanomaterials.

With respect to two key characteristics by which nanomaterials are different from conventional materials, there is a high degree of consensus. Firstly, nanomaterials are *nanoscale* in at least one of the three spatial dimensions, which means that their size is small, for example, between 1 and 100 nm, which, secondly, induces new properties.

In accordance with the Core Terms of *ISO*, nanomaterials are defined as so-called *nano-objects* or *nanostructured materials*. Nano-objects are materials, which are nanoscale in one, two, or three dimensions. This definition includes particle-like, rod-like or platelet-shaped objects and their assemblages. *Assemblage* is a generic term for agglomerates and aggregates, where agglomerates are considered as weakly bound collection of interconnected particles, whereas strongly bound particles form aggregates [2]. Due to chemical bonding, for example, by sintering processes, the surface of an aggregate can be significantly smaller than the sum of the surfaces of its constituent primary particles. In assemblages of nanoscale particles, however, the interaction energy can be so high that a clear distinction between aggregates and agglomerates becomes difficult. In addition, agglomerates of flexible nanotubes may exhibit such high entanglement, that they can be separated only by very high energy even though individual tubes are only weakly bound to each other. That is why both terms are used inconsistently in the literature [3].

In order to describe the objectives of the present study, the following working definition will be applied:

> Nanomaterials are manufactured substances that are nanoscale in the range of 1-100 nm in at least one spatial dimension, including derived structures up to a maximum diameter of 10 µm.[1]

Alternative definitions will be presented in section 2.1.

The new term *nano-object* was introduced by *ISO* as a new umbrella term for all nanoscale objects. This step was taken because in the current scientific literature, the term *nanoparticle* is used to describe quite different objects, such as rods, tetrahedrons, spheres. With the new generic term nano-object, the term nano*particle* is restricted to *quasi* spherical nano-objects that exhibit three nanoscale spatial directions [4].

Many different types of nano-objects have already found a wide variety of applications and product uses. Thus, a steady increase of nano-objects in consumer products will result [5]. Such an increase can enhance the exposure probability of humans and the environment to nanoscale materials. A risk assessment requires a scientific assessment of the toxicity of nano-objects used and development of regulatory concepts [6], [7].

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[1] Specification of BAuA; Anlage 1, Leistungsbeschreibung.

It is currently debated on the European level whether the regulatory framework REACH is a suitable legal framework for nanomaterials, or how it needs to be adapted [8]. REACH concerns the **R**egistration, **E**valuation, **A**uthorisation and restriction of **Ch**emical substances. It serves the European unification of chemicals legislation and aims to ensure high levels of protection of human health and the environment from potential chemical-related risks, the promotion of alternative test methods, the cross-border trade of chemical substances and enhancing competitiveness and innovation. As part of the information required for REACH registration of substances, the industry is responsible for assessing risks from chemicals and for limiting possible risks by providing appropriate safety information. If necessary, European Union can take additional measures for highly hazardous substances [9]. Since REACH addresses regulation of chemical *substances*, the discussion on its extension to nanomaterials will use the term *nanoscale substances* as a synonym of nanomaterials hereafter. This term will, however, require precise definition by the legislator in case of a future extension of REACH.

1.2 Information requirements of REACH

For a REACH registration dossier, the level of detail of the required data increases with the amount of substance produced or imported. Annex VI of the Regulation (EC) 1907/2006 (REACH) requires the following information for all substances to be registered

- **Information for substance identification (Annex VI)**
  - 2.1. Name or other identifier of the substance
    - 2.1.1. IUPAC name
    - 2.1.2. Other names
    - 2.1.3. EINECS or ELINCS number
    - 2.1.4. CAS name and number
    - 2.1.5. Other identity code
  - 2.2. Information related to molecular and structural Formula
    - 2.2.1. Molecular and structural formula
    - 2.2.2. Optical activity and typical ratio of (stereo) isomers
    - 2.2.3. Molecular weight range
  - 2.3. Composition
    - 2.3.1. Degree of purity
    - 2.3.2. Name of impurity
    - 2.3.3. Percentage of main impurities
    - 2.3.4. Nature and order of magnitudes of any additives
    - 2.3.5. Spectral data
    - 2.3.6. HPLC, GC
    - 2.3.7. Description of the analytical methods or the appropriate bibliographical references fro the identification of the substance and, where appropriate, for the identification of impurities and additives. This information shall be sufficient to allow the methods to be reproduced.

Additional substance information requirements scale with the annual production or import volume according to Annexes VII, VIII, IX and X of the regulation

- **Physico-chemical properties (Annex VII)**, acc. 440/2008 and 761/2009
  - A.1. Melting / freezing point
  - A.2. Boiling point
  - A.3. Relative density
  - A.4. Vapour pressure
  - A.5. Surface tension
  - A.6. Water solubility
  - A.8. Partition coefficient
  - A.9. Flash-point
  - A.10. Flammability (solids)
  - A.11. Flammability (gases)
  - A.12. Flammability (contact with water)
  - A.13. Pyrophoric properties of solids and liquids
  - A.14. Explosive properties
  - A.15. Auto-ignition temperature (liquids and gases)
  - A.16. Relative self-ignition temperature for solids
  - A.17. Oxidising properties (solids)
  - A.18. Number – average molecular weight and molecular weight distribution of Polymers
  - A.19. Low molecular weight content of polymers
  - A.20. Solution/extraction behaviour of polymers in water
  - A.21. Oxidising properties (liquids)
  - A.22. Length-weighted mean diameter of fibres

- **Toxicological properties**

- **Ecotoxicological properties**.

The physical-chemical data of Annex VII, which are in the focus of the present study, must be provided as a complete record already at an annual volume between 1 and 10 t/a[2]. Additional information requirements arise for more than 1000 t/a according to Annex IX.

The testing methods for the physico-chemical data required in Annex VII, the so-called A-methods, are defined in regulation (EC) 440/2008 and its adaptation to the technical progress of regulation (EC) 761/2009 [10], [11]. For information required in Annex VI for substance identification, however, that necessitate the use of testing methods, no specific procedures are set forth in the regulation. It only requires a description of the analysis methods that were used by the registrant that allows reproducing the methods.

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[2] physico-chemical data of Phase-In substances are required if the substances comply with the criteria of annex III.

The system of EC legislation to classify substance information into “information for identification” and "physico-chemical properties" was obviously developed for *chemical* substances. While “information for identification” comprises atomistic data on the chemical (stereo)-structure, detailed spectroscopic data and information on substance purity, the “physico-chemical properties” - with the exception of A.18, A.19, A.22 – describe *phenomenological* substance properties, i.e. properties that are determinable even without knowledge of the atomic/molecular structure of the substance.

1.3 Objectives of this study

The regulation of nanoscale substances by the REACH legal framework aims at safe handling of such materials and increasing the knowledge on hazards and risks [8]. According to the project tender, this study will focus on nano-objects as a sub-class of nanomaterials. The first part of this study provides an overview on the properties of nanoscale materials and methods of their characterisation. It reviews literature findings on the applicability of test methods, which were established for substance regulation, to nano-objects. Also aspects will be discussed of standardisability of methods developed for nanomaterials. Next, the present study discusses the applicability of test methods of the Annex VII of Regulation (EC) 440/2008 [10] and amendment (EC) 761/2009 [11], the so-called „standard information requirements“, to nanoscale substances. This discussion addresses several aspects. Firstly, issues are discussed of practical feasibility of the prescribed test methods for nanoscale materials and of possible changes to the test specification. Secondly, it studies whether the application of a standard test to nano-objects may result in unexpected deviations of the measured properties compared to that of conventional micro- or macroscopic substances, and whether such deviations may indicate a nanoscale character of the tested substance. Thirdly, the study suggests additional characteristics of nanoscale substances for possible future information requirements of the REACH Regulation.

2 Fundamental aspects of nanoscale substances

Numerous studies have shown that nanoscale materials and particles exhibit properties that differ fundamentally from those of microscale and macroscale materials. AUFFAN et al. give examples of observations of such chemical and physical characteristics of inorganic nanoparticles [12]. For many nanoscale materials, these differences in properties were found to increase with decreasing particle size. For particles below 40 nm size, the number of reports in the literature on such deviations is significantly increased. Their origin is the relative number of surface atoms that increases in inverse proportion to the particle size. The chemical potential of such surface atoms is increased according to GIBBS, resulting in higher reactivity, diffusivity and phase transition probabilities. The latter can, for example, explain an altered melting behaviour, as is observed for example for nanoscale metal particles. For particles of 10 nm diameter, melting point depression in the order of 10 or 100 K has been found [13], [14] see Table 3.1.

For the assessment of potential hazards of nanomaterials and nanoscale substances, particularly those properties are relevant that may induce an increased toxicity relative to the bulk material. Such structure-effect properties of nanoscale material are of eminent interest and subject of current nanotoxicological research [15].

The literature reports many examples that show how the property variability and performance of synthetic nanomaterials opens impressive perspectives for the development of customised high-performance materials. The majority of current synthetic nanoparticles, however, is still only available in form of particles *mixtures* with very broad property distributions. This increases the complexity of any study of their impact and dissemination mechanisms in a critical manner. Due to the huge number of existing and currently developed types of nanomaterials, which exceeds by far the number of testable materials, a better understanding is sought of structure-effect principles. It should allow predictions on the toxicity of a particle type on the basis of material and structural data. For such a common understanding, uniform particle fractions appear to be necessary. However, already highly uniform synthesized nano-objects exhibit a variety of relevant material characteristics. Their determination requires combining and evaluating the results of different analytical methods. Inhomogeneous particle mixtures thus further complicate the interpretation of the material characterisation and toxicological tests.

Another aim of this study is to give a compilation of literature findings on the properties of nano-objects and of methods for their characterisation. Whenever possible, literature findings will be reported on the applicability of test methods established in the regulation of nano-objects. Of interest is also the standardisability of characterisation methods specially developed for nanomaterials.

2.1 Definitions

In the following, literature findings are reported on the definition and identifiability of this studies' objectives nanotechnology, nanomaterials and nano-object. Statements are then collected on toxicologically relevant aspects of nanomaterials and an overview is given on classification schemes for nanomaterials under discussion. A compilation of currently circulating definitions of the term *nanomaterial* is found for example in [16]. The particulate nanomaterials are a subclass of nanomaterials with many different definitions. For example KREUTER defined in ANTON et al. nanoparticles as solid particles with a size of 10 nm to 1000 nm [17]. For a more precise use of language, *ISO* recommends the concept of *nano-objects*. Its hierarchical definition scheme is given in Figure 2.1.

According to HUND-RINKE [1], nano-objects are put by *OECD* in the context of nanomaterials, where the reference is being laid on *manufactured* nanomaterials, i.e. nano-materials that were produced to achieve specific properties. They are also known as *intentionally synthesized nanomaterials*. Intentional production and biopersistence are next to size central aspects of the European cosmetics directive that was revised in 2009 and will come into effect in 2013:

> „Nanomaterial“: insoluble or biopersistant and intentionally manufactured material with one or more external dimensions, or an internal structure, on the scale of 1 to 100 nm” [18].

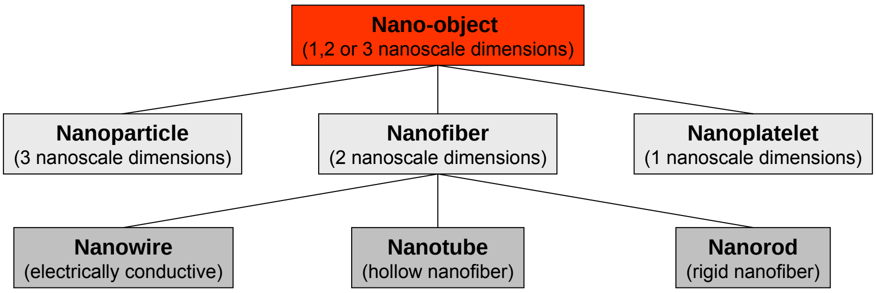

Fig. 2.1 Nanotechnological terms and their hierarchical classification [19].

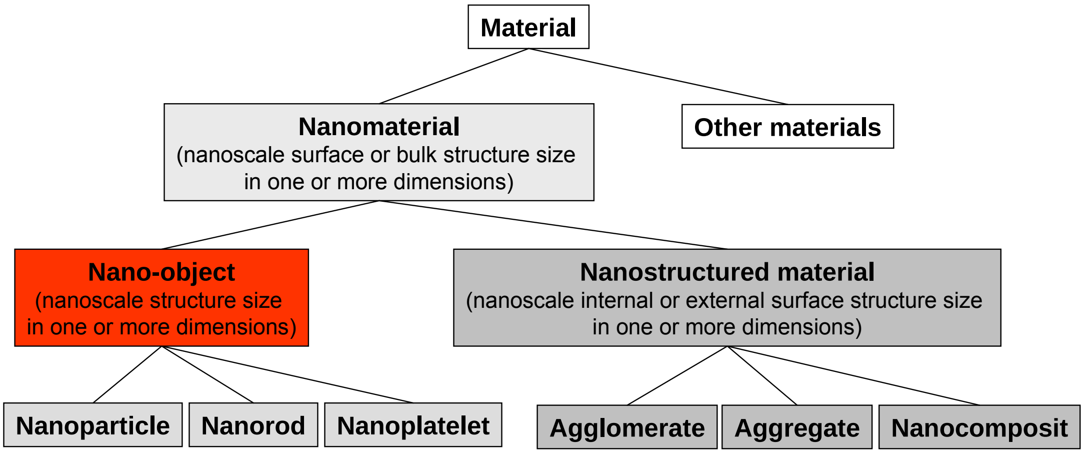

Fig. 2.2 *OECD* suggestion for a nanomaterial classification [1].

For the *ISO*'s definition, the nano-scale ranges between 1 nm and about 100 nm. For the aim of a standardised term *nanomaterial*, *ISO* developed a Technical Specification (*ISO* / TS 27687:2008) in form of a likewise hierarchically structures definition chain [2]. The Technical Specification *CEN ISO*/TS 2768 provides terminology and terms for nano-objects [20]. *CEN* adopted this specification on 24 August 2009 as a future standard for provisional application (*CENISO*/TS 27697) [21]. With respect to the nano-objects, being in the focus of the present study, it differentiates between nano platelets, fibres or particles according to the objects' spatial dimensions. These include nanoparticles, which are nanoscale in all three dimensions, i.e. between 1 and 100, nanorods or nanotubes, which are nanoscale in at least two dimensions, and nanoplates or layers, and nanoplates or sheets that need to be nanoscale in one dimension only. *ISO* propagates using the terminology introduced also for the regulatory context, where the subset of relevance may be chosen by inclusion of additional characteristics.

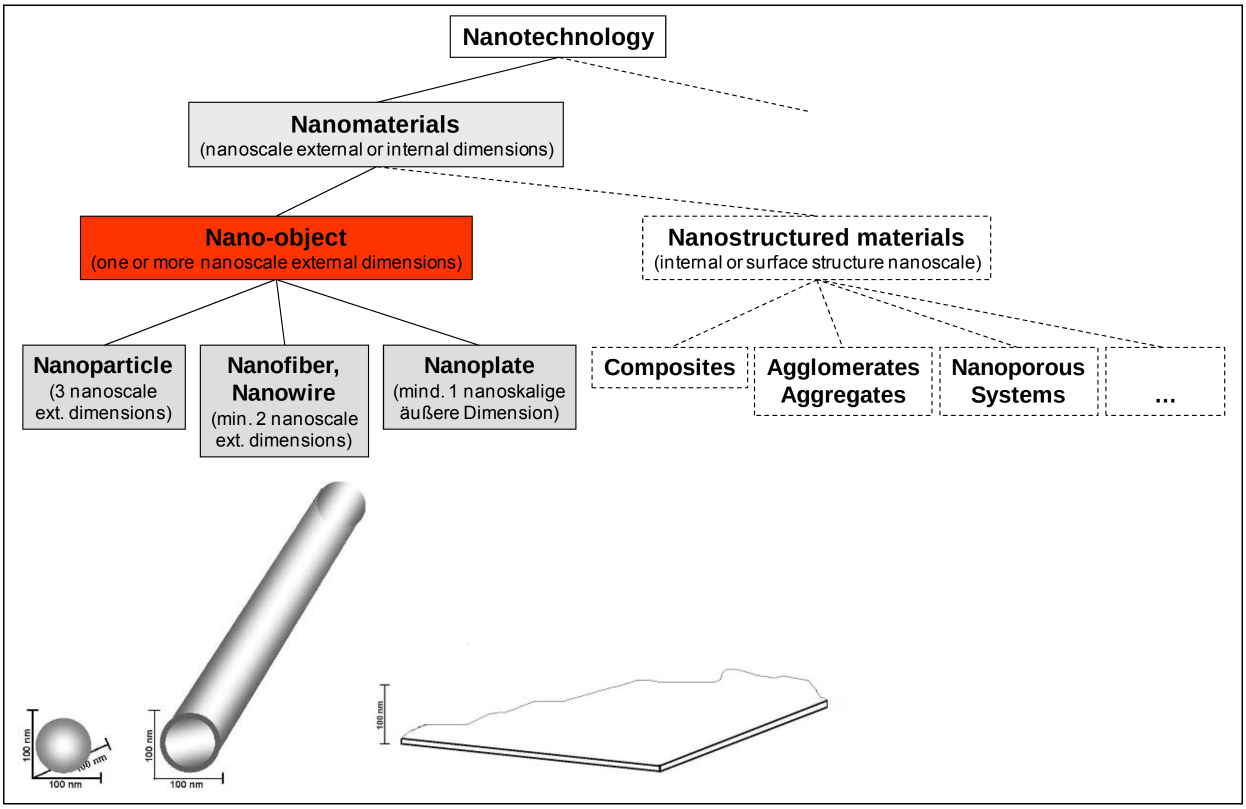

Fig. 2.3 Schematic of *ISO*'s definition of nano-objects, KRUG 2011 [22], [23].

According to KREYLING et al., in international bodies there is a *de facto* consensus in the definition of synthetic nanomaterials (manufactured nanomaterials) for regulatory purposes [16]. It comprises substances that were intentionally produced on the nanoscale (about 1 to 100 nm) and exhibit modified properties with respect to the equivalent conventional material. While the lower limit of 1 nm was chosen to discriminate nanomaterials from atoms and molecules, the upper limit of 100 nm is, in the opinion of the authors, not scientifically substantiated. In their study on a complementary definition of nanomaterials, they show that – besides the size-dependent definition – for a clear distinction from conventional substances at least one additional nano-specific parameters has to be used. They suggest using an easily accessible measurand in combination with the previously used parameters particle size and particle size distribution: the *volume specific surface area* (VSSA). According to the authors, it appears also suitable for classification and regulatory purposes. VSSA is the product of the (also for nanomaterials routinely determinable) specific BET surface area according to *ISO* 9277:2010 [24] with the material density of the corresponding bulk material. A material should therefore be regarded as nano-materials, if the value of its volume-specific surface exceeds 60 m^2/cm^3. This limit is motivated by the volume-specific surface area of spherical particles with 100 nm diameter and material density of 1 g/cm^3. The measure appears also useful to clarify the situation for mixtures of particles fractions, since it can identify the presence of unexpected ultrafine particle fractions in mixtures. At the same time, it may avoid the need for defining a *relevant* nanoscale particle fraction that classifies a material as nanomaterial.

2.2 Classification

While the *ISO* scheme on nanomaterials only serves the definition of terms, a classification of nanoparticles appears desirable on the basis of yet to be identified (probably) application specific material characteristics. The aim is to achieve a grouping of nanomaterials by physico-chemical characteristics of in a manner that allows predictions on the toxicity of a member of the class by the help of known toxicological effects of the other group members. This will require a much improved understanding of structure-effects principles of nanomaterials. In the following, examples of classification schemes are given from the literature.

SCHULTE-EBBERT et al. [25] summarise a number of classification schemes of nano-objects described in the literature. As an example, BUZEA et al. [26] differentiate nano-objects or nanomaterials according to their dimensionality, morphology, material composition and degree of uniformity and agglomeration. The authors grouped as follows to nano-objects:

Tab. 2.1 Classification scheme of BUZEA et al. [26].

Carbon structures	Fullerenes Carbon nanotubes Other carbon structures
Metal containing structures	Metal oxides Metals Quantum Dots
Dendrimers	

According to AITKEN et al. [27], particle morphology together with chemical composition and possible applications are a useful basis for the categorisation of nanoparticles:

Tab. 2.2 Classification scheme of AITKEN et al. [27].

Nano structure	**Material**
Nanotubes	Carbon
Nanowires	Metals, semiconductors, oxides, sulphides, nitrides
Nanocrystals, Quantum dots	Isolators, semiconductors, metals, magnetic materials
Other nanoparticles	Ceramic oxides, metals

In their "ICON Nanotech Survey" among 282 companies, also GERRITZEN et al. [28] classify nano-objects - albeit after a somewhat ambiguous terminology and without use of a hierarchical structure:

Tab. 2.3 Classification scheme of GERRITZEN et al. [28].

Nanopowder	Nanotubes	Nanohorns	Nanocrystals	Polymers
Fullerenes	Nanowires	Flakes	Quantum dots	Colloidal dispersions
Carbon blacks	Nanorods	Platelets	Dendrimers	Other

It must be stated that the cited schemes do not satisfy the purposes of a satisfactory classification of nanomaterials and have little value since they lack methodology and level of detail. For a mapping of nano-objects in classes of different toxicological relevance, a significantly more sophisticated classification is needed that takes into account characteristics such as solubility, reactivity, catalytic activity and (fibre) morphology. Also STONE et al. question in a summary of a 2008 *NanoImpactNet* workshop, held in Zurich [29], whether nanomaterials can in principle be assigned to specific groups. They note that so far no consensus has been reached regarding the classification of nanomaterials in categories for environmental studies, although an initial classification based on the chemical composition is regarded as a useful starting point [30]. This is also the only matching criterion for systematisation of the previously mentioned authors. The main sub-groups are: metals, metal oxides, carbon materials, organic materials and non-metals.

3 Physico-chemical properties of nanoscale substances

Nano materials have a variety of different material characteristics. The complexity of nanoscale materials can be illustrated by an example. Figure 3.1 shows an idealised and a more realistic structure of a double-walled nanotube (DWNT). The synthesis of completely uniform nanotubes (upper DWCNT in Figure 3.1) is not possible with today's methods. Already a hypothetical ensemble of structurally defect-free DWNT could show heterogeneity with respect to diameter and chirality of the inner and outer tube. CNT materials from state-of-the-art synthesis generally exhibit numerous additional characteristics: type, number, size and position of defects, including bends, branches and local diameter and chirality changes as well as other deviations from cylindrical structure (e.g. bamboo structure, horns); chemical termination or chemical functionalisation of graphene edges; type, size, composition and location of adsorbed impurities (amorphous coatings, metallic catalysts).

The term nano*material* generally implies a structural complexity. For a comprehensive characterisation of, e.g., only a single nanotube from the bottom type in Figure 3.1 in principle would require to determine all atomic coordinates and bonding states of all carbon and terminating atoms of the tube. This vast amount of information can however be reduced in case of defect-free structures by descriptive parameters for domains of (local) symmetry. Provided *relevant* structural characteristics are known, they can further reduce the amount of information necessary for the description of nanoscale materials. However, the relevance of these characteristics will depend on the type of *application* of the substance. Applications in composites would be interested, for example, in data on chemical functionalisation and dispersibility of the nanotubes; electronic applications would be interested in length and chirality; applications as conductivity additive in length and electrical conductivity; lightweight applications in length, tensile strength and chemical functionalisation; catalytic applications in chemical purity, functionalisation and defect density; sorption applications in chemical functionalisation and specific surface area, etc..

For the purpose of identification and/or characterisation of a nanoscale substance, i.e. a nanomaterial, differently large data sets are to be determined. The existing analytical methods for nanoscale materials, however, are able only to provide partial information. Therefore, appropriate combinations of methods have to be selected. Subject of ongoing discussions is what combinations of analytical techniques allow reliable statements on the nature of a nanomaterial at reasonable costs. There is general consensus that these combinations must also include complementary methods that allow considering the validity of applying a particular analysis method to a specific material. In the following, examples of such information requirements for material characteristics of nano-objects are given as well as a compilation of commonly used methods and the statistical nature of their information, see also Table 1 in Appendix 1 of this study.

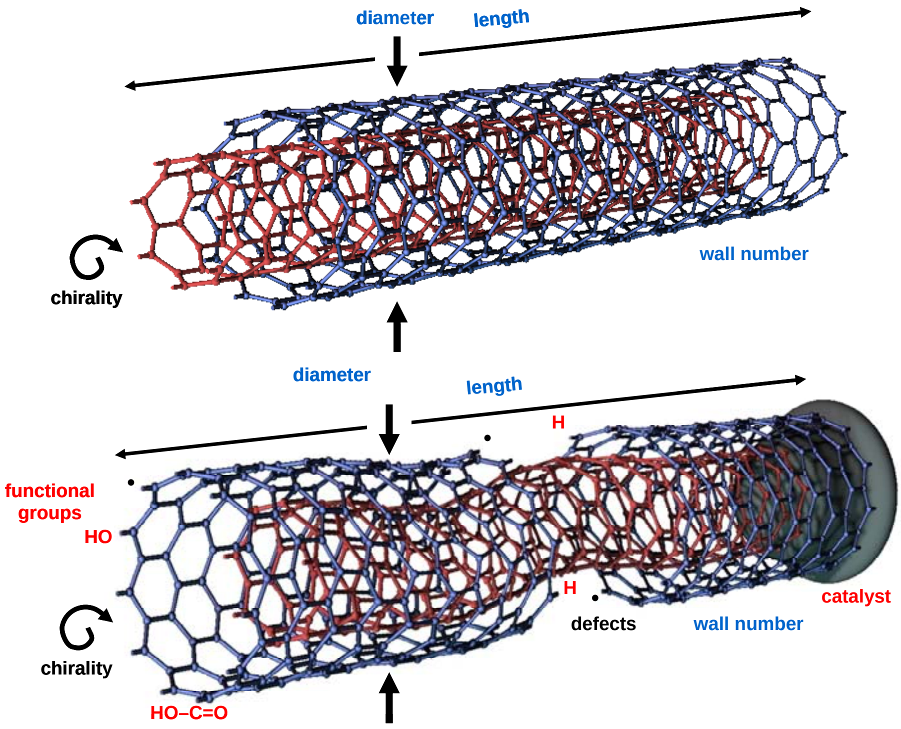

Fig. 3.1 Schematic of DWCNT. Top: highly-structured tubes according to theory. Bottom: more realistic, defect-rich structure.

3.1 Nanomaterials in the scientific literature

As the authors of a literature study in 2009 on "Environmental risks through technical nanomaterials" state [25], the number of publications on nanomaterials increased significantly within the last fifteen years, since 2003 strongly. Also on the topics of production quantities of nanomaterials and possible mechanisms of action of nano-objects as well as their physico-chemical characterisation, a large number of publications have been published. For an overview of information on relevant methods for the characterisation of nano-objects and an estimate the volume of literature, the databases *ScienceDirect*®, *ISI Web of Knowledge*® and *Google Scholar*® were used. With the more general search parameter

```
(nanoobject* OR nanoparticle* OR nanoparticulate OR
nanoscale OR nanosize OR nanomaterial*) AND (synthesis OR
syntheses OR preparation*))
```

a large number of hits were returned by the *ScienceDirect* database. The quantitative development of publications is shown in Figure 3.2. Early publications in the field date back to the 1980s. The increase was moderate up to the mid-1990s and since the mid-2000s, a significant increase is observable.

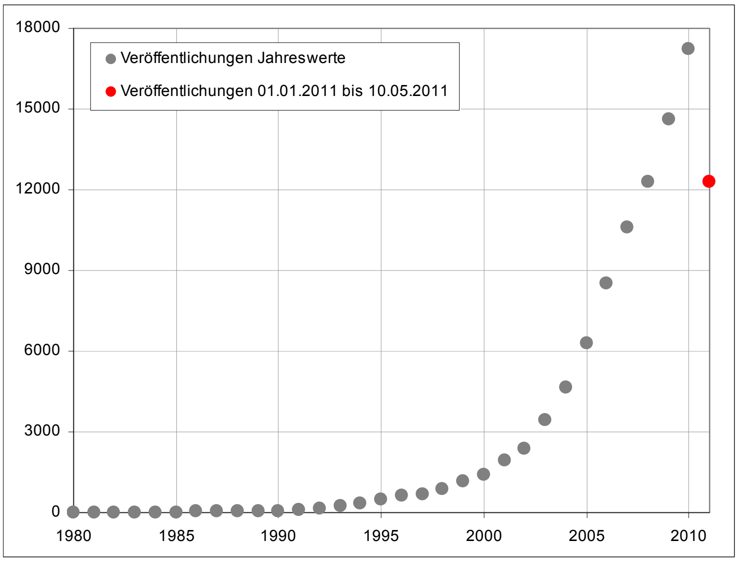

Fig. 3.2 Number of scientific articles on nanomaterials. (Source: *ScienceDirect*).[3]

Keywords related to "physico-chemical characterisation" returned a comparably high number of hits.

To begin with, review articles and those publications were evaluated, which were expected to provide an overview on testing methods under a regulatory perspective. In addition, literature on specific methods was evaluated. In the following, especially publications of international institutions are referenced that discuss the physico-chemical characterisation of nano-objects and indicate specific procedural details. Thus, documents of international bodies such as *ISO*, *OECD* or *VAMAS* are evaluated that deal with the characterisation of nanomaterials.

3.2 Statistical aspects of a characterisation of nanoscale substances

For assessing the reliability of a characterisation, basic aspects of sample preparation, the sampling and the statistical nature of the method are to be considered. For nanoscale materials, which consist generally of a particle ensemble with a broad property distribution, incomplete sample homogenisation, selective sampling and inadequate sample size will cause erroneous characterisation results. It is also important to know the statistical character of the methods used. Analysis techniques for nanoparticle ensemble can be distinguished in terms of their statistical character in two very different classes: those that can characterise properties of *individual* particles and those that determine the property distributions of *particle ensembles*.

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[3] http://www.sciencedirect.com

Individual particle analysis methods (IPA) and spatially-separated particle analysis (SSP), cf. Table 1 in Annex I, are typically based on small samples of an ensemble consisting of only a few particles. To allow for studying of isolated, well dispersed particles, these samples are relatively complicated to prepare. During the measurement, specific individual particles properties are accumulated and histogrammed to property distributions. However, such an approach results in unreliable property distributions if an excessive experimental effort prevents the characterisation of a sufficiently large number of particles. Moreover, any influence of the details of sample preparation may affect significantly the determinable property distributions.

Especially the preparation of nanoparticle suspensions requires a high degree of experimental control and care. Different mechanisms can lead to a progressive particle size fractionation of the ensemble. Firstly, larger particles or incompletely disintegrated agglomerates tend to sediment [31]. Secondly, dispersed nanoparticles may dissolve or show a strong re-agglomeration propensity. If only the supernatant of such an unstable suspension is analyzed, for example by DLS, or transferred to a TEM-grating or used for aerosol generation, artefact-prone property distributions result. The conditions for the production of a sufficiently stable suspension depend in the practice on numerous material characteristics, such as agglomeration degree, type and strength of the inter-particle interaction, solubility, wettability and surface charge [32]. Therefore, sample preparation both for single-particle-based and collective-based analysis is matter of material-specific optimisation.

Ensemble averaging analysis methods (EAA), cf. Tab. 1 in Annex I, on the other hand, allow determining material properties of large ensembles. Due to their non-microscopic nature, EAA methods may fail to detect unexpected morphological characteristics of particles or subsets of the ensemble. They can, for example, fail to distinguish large porous particles of similar-sized small particle aggregates. In order to avoid systematically incorrect ensemble averages due to unexpected particle properties or shapes, therefore supporting controls are mandatory of the particles in the ensemble by microscopic techniques. That is, the applicability of methods for the determination of ensemble averages must be verified by analysis of a representative sample of individual particles.

3.3 Particle size dependence of characteristic substance properties

The following section discusses the theoretical background and experimental results of a particle size dependence of characteristic material properties. In the context of REACH, it is of particular interest, whether the test procedures for physico-chemical properties specified in the directive are applicable to nanoscale substances, and whether the methods are in principle capable of indicating the nanoscale character of a substance if applied to a nanoscale material due to significant differences compared to the conventional material.

At the nanoscale, the ratio of particle surface to volume can become very large. Therefore surface effects can become dominant and material properties such as reactivity, the solubility and melting behaviour may change significantly compared to the macroscale material. It should therefore in principle be possible to obtain evidence for the nanoscale nature of a substance from its physico-chemical properties.

Origin of the mentioned edge or surface effects are bonding state of surface atoms that differ from those of the bulk material. If atoms of a substance are bound in three dimensions (like in the case of sp^3-hybridised carbon) the surface atoms are missing binding partners for the continuation their (tetrahedral) crystal structure. The surface atoms therefore possess open bonds (dangling bonds), unsaturated bonds, or bonds to foreign atoms. The nature of this so-called *termination* of the surface determines the amount of additional surface-energy and the chemical potential of the surface atoms. The example of sp^2-hybridised carbon shows how important considerations of the binding geometry can be. The highly conjugated planar structure of graphene sheets and their weak interaction inside the layered structure of graphite, explains why the energy of surface basal plane is not significantly enhanced compared to the graphite crystal planes. For graphitic nanoparticles, more important than the surface-to-volume ratio are the degree of curvature of the graphene plane and the length and nature of its edge termination.

3.3.1 Melting point

For conventional substances, the melting point is a characteristic intrinsic size property. At constant pressure however, solids can exhibit a melting behaviour deviating from the macroscale substance, provided their outer dimensions are in the order of a few nanometres [33]. The melting behaviour of nanoscale particles can be studied for example by means of DSC, microcalorimetry, TEM, XRD and UV/Vis absorption spectroscopy [34]. The dependence of the melting behaviour of the observed size of solid particles has been studied theoretically since the 1900s. It was predicted an altered melting behaviour [35], [36]. According to PAWLOW, the phenomenon is based on the influence of surface energy on the physico-chemical properties of small particles. Once melted, nanoparticles are entering a *supercooled* melt [37]. The melt may crystallize only in presence of a nucleus of crystallisation or some other perturbation like sound waves [38]. In practice, for molten particles supercooling has been observed in the order of 100 K [39], [40]. For particles spatially isolated by a SiO_2 coating, melting-solidification processes can be repeated several times and reproduce a hysteresis [40].

A particle size-depending melting behaviour is not limited to a specific material. It has been observed in metals, semiconductors and alloys. For soldering applications it has gained technological importance [41]. Therefore, the information on the nanoscale character of a substance may in principle be derived on the basis of its melting behaviour. However, impurities as well as effects of possible sintering processes on the particle size distribution are to be considered. The subsequent table 3.1 gives experimental data on the size dependence of the melting temperature of selected substances.

Tab. 3.1 Examples of experimental results for particle size dependence of melting temperature. Nanoscale materials may result in supercooled melts, see text.

		Macroscale substance	**Nanoscale substance**				**Ref.**
			20 nm	10 nm	5 nm	2 nm	
Metal	Aluminium	933,5 K	927 K	921 K			[42]
	Gold	1337,3 K	1275 K	1180 K	1150 K	650 K	[43]
	Silver	1234,9 K	1000 K	775 K			[44]
	Bismuth	544,4 K	535 K		523 K	428 K	[36]
	Tin	505,8 K	500 K		488 K	420 K	[45]
	Zinc	692,7 K		633 K			[40]
Semiconductor	Copper-I-chloride (CuCl)	699,2 K	656 K	643 K	618 K	543 K	[46]
Alloys	Tin : Silver (96,5 : 3,5)	495,8 K	491 K	481 K	467 K		[41]

However, anomalies are possible. For the glass transition temperature of semi crystalline polypropylene, for example, an increase with decreasing particle size was observed [47]. For bismuth, on the other hand, it became evident that nanoscale particles may prefer different crystalline phases than macroscale materials, resulting in an altered melting behaviour. For nanoparticles of small radius, the surface tension of the solid-liquid or liquid-gas interface generates considerable pressure on the particle. When pressurised, macro-bismuth exhibits at least six different crystalline phases and numerous liquid phases. For bismuth the surface tension pressure of a 1 nm particle was estimated to be 0.8 GPa. While macro-bismuth requires a pressure of about 2 GPa to transform from the rhombohedral structure to the cubic body-centred monoclinic structure, bismuth nanoparticles with a radius greater than 4.2 nm showed a combination of rhombic and cubic crystal structures [36]. Similar particle size-dependent transitions between different crystalline phases were likewise observed for indium, silicon, chromium and other metals. This shows that the crystal structure of very small particles may fundamentally differ from that of macroscopic objects, which in turn has a direct impact on the surface reactivity and catalytic activity of the surface. Moreover, for nanoscale solids which are in whole or in part embedded into a matrix, the matrix may affect the melting behaviour. It was found that embedded nanoparticles may overheat, i.e. melt only above the melting point of the corresponding macroscale material.

3.3.2 Sublimation point

A variety of materials exhibits no melting point at standard pressure conditions but sublimates directly into the gas phase. Examples are iodine, boron and carbon. Here again, surface effects may lower the sublimation point, so that statements on the nanoscale nature of a substance may in principle be derivable, see Figure 3.3.

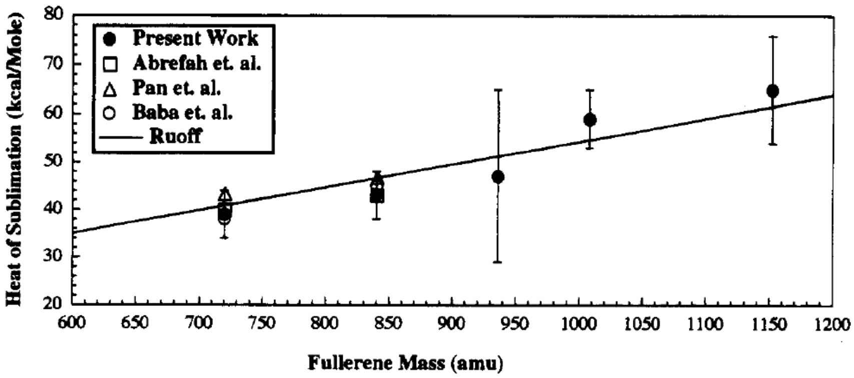

Fig. 3.3 Dependence of the heat of sublimation on the cluster size of fullerenes [48].

3.3.3 Allotropy transition

For macroscale materials that can exist in various allotropy states, the allotropy state depends on synthesis conditions, purity, pressure and temperature. A prominent example of an allotropy transition is the phase diagram of carbon. It exhibits the possibility of a transformation from the metastable diamond state to graphite above 700 °C. According to YANG and LI, this transition temperature is particle size dependent [49]. The transition temperature decreases generally with particle size. However, also a dependence was found on the type of chemical surface termination of the nanoparticles. BADZIAG et al. observed an unexpected stabilising effect of a hydrogen termination of nanoscale diamond [50].

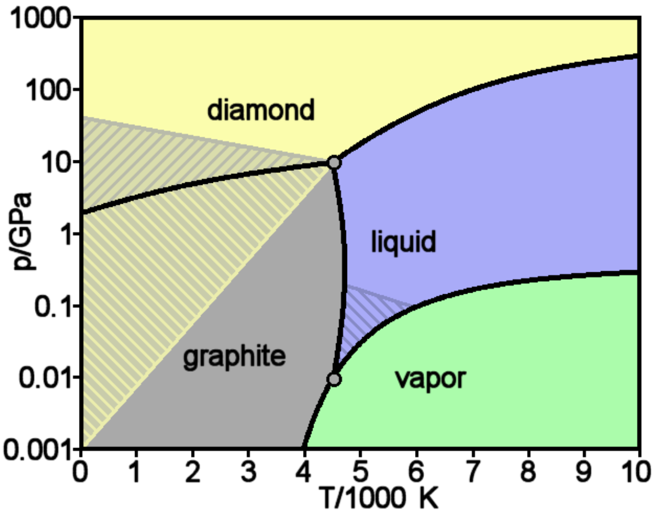

Fig. 3.4 p-T-Phase diagram of carbon [Wikimedia].

3.3.4 Thermal decomposition

In the case of thermal decomposition under inert gas, the underlying chemical processes strongly depend on the substance and its purity. These processes should not depend on the surface energy, therefore no or only a small particle size dependence is expected. For further details, a literature study would be required to evaluate results on a non-particle-size-dependent decomposition temperature of calcium carbonate [51]. However, it is expected that only for pure and well-studied substances characteristic correlations between decomposition temperature and particle size can be found that might allow deriving information on a possible nanoscale particle dimension.

3.3.5 Reactivity and solubility behaviour

The reactivity and solubility behaviour of solids are interfacial properties. For nanoscale materials with their compared to micro-particles increased volume specific surface, they can exhibit size-specific variations [52], [53]. These are caused not only by the size of the interface area, but also by changes in the bonding state of surface atoms. In the case of (partially) crystalline materials, nano-specific crystal structures can result. Their crystal boundaries have direct effects on reactivity, catalytic activity, solubility and adsorption properties of a substance. Gold nanoparticles are a prominent example of such effects [54], which also affect their cytotoxicity [55] for instance by induction of oxidative stress in biological systems [56], catalytic effects, initiation and participation in redox reactions or release of ions due to increased solubility. According to MEIER nanomaterials may exhibit enhanced interactions with biological systems [57].

In the case of a particle size-induced increased reactivity, a risk may result of unexpected chemical reactions, fires or explosions [58]. Nanoscale metals can exhibit pyrophoric properties, for instance, that may lead to spontaneous ignition on contact to the air. Flammable nanoparticles dispersed in air, similar to micro-particles, may lead to dust explosions. The minimum ignition energy of combustible nanomaterials may be partially reduced compared to microscale materials [59], [60], [61]. For an evaluation of the reactivity of nanoscale materials, it is important to know the sample age and storage conditions. Firstly, a passivation (inertisation) of the particle interface may occur during storage, which is usually associated with a reduction in reactivity. Secondly, an increase in the degree of agglomeration of the sample may result, which in turn could reduce the reactivity compared to isolated particles.

Both, particle reactivity and solubility behaviour depend on the existence and nature on a chemical interface functionalisation or surface coating. This is important to be considered since adsorbates (e.g., surfactants) are often used specifically for the stabilisation of nanoparticle suspensions [32]. Also unintentional adsorptive coating of nanoparticles with cell media components such as proteins or humic acid may occur in the case of biological test fluids [62]. Likewise, ionic multilayer coating may result in non-demineralised water. Therefore, a solubility behaviour can only be specified in relation to the nature and composition of the solvent used.

An altered solubility behaviour like increased saturation solubility and dissolution rate has been found experimentally for many uncoated nanoscale materials. For example, nanoscale zinc oxide particles of 4 nm size released within 24 h in water at pH 7.5 a 2.5- or 6-fold higher concentration of Zn^{2+} ions as 15 or 240 nm particles, respectively [62]. The increased solubility behaviour of nanoscale materials has found application for conventionally poorly water-soluble pharmaceutical substances [63], [64].

Due to the increased solubility of nanoscale materials, *supersaturation* of the solution may result, which is a metastable state that can lead to sudden precipitation if precipitation nuclei are present, a perturbation occurs (sound waves, warming), or if the precipitation pressure is too high. Purely nanoscale materials, e.g. in the form of a sediment, however, are not efficient precipitation nuclei since the increased substance solubility reduces solute adsorption. Thus, the increased solubility of nanoscale materials shifts the equilibrium between dissolved and solid nanoscale sediment.

3.3.6 Optical, electrical and magnetic properties

Nanoscale particles can exhibit modified optical, electrical or magnetic properties relative to bulk materials. One reason can be so-called quantum confinement that, if it leads to modified conduction and valence band structures, may cause entirely new, for the nanomaterial characteristic material properties. Also nanostructure related quantum effects may become possible during propagation and interaction of electron spins in nano-structured materials, which are not observed for bulk materials. Both could indicate the nanoscale nature of a material.

3.4 Indicators for a nanoscale nature of a substance

During substance testing, unexpected results may be caused by impurities, the method used or by a possible nanoscale nature of the substance. At the current level of understanding and with the standard REACH test procedures, due to the complexity of the previously discussed relationship between particle size distribution and material characteristics, probably only indications are obtainable on a potential nanoscale nature of the substance tested. Such indications could however serve to trigger a determination of the particle size distribution of the sample with microscopic methods.

To obtain such information on the nanoscale of a substance, methods capable of determining deviations of the melting or sublimation behaviour appear in principle suitable. Currently, however, such deviations appear to be detectable only for substances of known chemical composition, such as pure substances or binary compounds, whose corresponding transition temperatures of the macroscopic material are known from the literature.

Due to the dependence of the melting or sublimation behaviour on the material composition and purity, additional material data is required for assessing the significance of observed test result deviations. Similar limitations exist for the information derivable from REACH standard test procedures from the size dependence of other material characteristics such as reactivity and solubility behaviour.

To assess the usefulness of REACH standard test methods for the extraction of indications for a possible nanoscale nature of a test substance, detailed literature and experimental studies would be necessary.

3.5 Tests standards

As T.A. CAMPBELL notes, nanotechnology would be a mere Gedanken experiment without measurement technology [65]. Suitable measurement techniques can therefore be characterised as an essential foundation of nanotechnology. The current scientific knowledge on the synthesis and physico-chemical characterisation of nano-objects is extensive and complex. Literature databases show a steep increase in scientific publications on synthetic nanomaterials since the mid-2000s. Some nanomaterials have meanwhile reached a level of maturity and an application relevance that allow and require the introduction of standard procedures, reference materials and standards.

3.5.1 Pre-normative activities

The *Versailles Project on Advanced Materials and Standards* (*VAMAS*) is an international organisation devoted to pre-normative research to facilitate the trade and commercialisation of new materials and products that require advanced technologies for their production. This is achieved through international programs that have the aim of providing a basis for coordinated technical measurement methods, testing, specifications and standards. Pre-normative research activities of *VAMAS* are organised in technical committees, called Technical Working Areas (TWA). Three of the currently active 15 TWA deal with nanotechnology issues and are briefly described below.

TWA 29 - Nanomechanics applied to Scanning Probe Microscopy
TWA 29 examines the international state of the art in characterisation of nanomaterials as it relates to scanning probe microscopy (SPM) methods for measuring nanomechanical properties. It investigates protocols for the calibration of the force constants for scanning probe methods and provides recommendations for good practice on characterisation of nanomaterials using SPM. The experts of TWA 29 support the development of suitable standard reference materials for nanostructured materials [66].

TWA 33 - Polymer Nanocomposites
TWA 33 deals with technical problems in standardisation of polymer materials, containing nanoparticles, lamellar or fibrillar, natural or synthetic. It develops specific methods for characterisation of nanoparticles and is working on developing new methods or development of existing methods for the characterisation of polymer nanocomposites. Current work deals with the determination of shape, size and size distribution of nano-scale fillers and the electrical characterisation of polymer nanocomposites. In future, the application of dynamic mechanical analysis to determine mechanical properties and methods for determining the fracture mechanics of polymer nanocomposites will be evaluated [67].

TWA 34 - Nanoparticle Populations
Activities in TWA 34 advance the measurement of nanoparticle populations such as metal nano-wires, single-walled carbon nanotubes, magnetic nano-particles, nanopowders and quantum dots. The focus of the TWA is standardisation and cross-comparison of measurement techniques for determining the defining dimensional, electronic, chemical, optical or magnetic characteristics of the nano-particles.

Projects within the TWA are defined for single classes of dispersed nano-particles. Activities have been initiated with a project on refining the measurement of chirality distribution in polydisperse SWCNT samples. Other characterisations of polydisperse nanoparticle populations, such as the measurement of titanium dioxide nano-powders, magnetic nanoparticles or general measurements of particle size and dispersion state are also considered to be within the bounds of this TWA.

Project 1 : SWCNT: Chiral Vector Distribution Determination
Project 2 : Titanium Dioxide (TiO2) Nanopowder Surface Area Measurement
Project 3 : Techniques for Characterizing Morphology of Airborne Nanoparticles
Project 4 : Raman Spectroscopy of Fullerene Nanofibres.

Round-robin tests of *VAMAS*

VAMAS initiates and organizes round-robin tests that also deal with nanoscale materials. Currently active tests are [68]

TWA 33:	Determination of the shape, size and size distribution of nano-filler particles
TWA 34.3:	Techniques for characterizing the morphology of airborne nanoparticles
TWA 34.4:	Raman spectroscopy of fullerene nanofibres.

3.5.2 Standardisation activities

Especially the use of nanoscale materials in consumer-related sectors and products increasingly requires the development of standards and technical regulations that are developed specifically for new nanomaterial classes. Appropriate test procedures have mainly been developed for micro- and nanoscale materials that have been in use for a longer time, like carbon blacks or pigments, to determine the coarse or fine fraction [69], [70], [71].

It is therefore of great interest to know whether new testing standards for nanomaterials will become available in the near future, and to what extent existing standard methods can be applied. This will crucially depend, firstly, on the standardisability of characterisation methods and preparation procedures currently used in research, and, secondly, on the level of detail with regard to the nature and accuracy of determining physico-chemical properties. While the determination of the coarse fraction of a batch pigment according to *EN/ISO* 787-18:1999 [70] is a relatively simple problem, to elucidate the surface chemistry of an ensemble of nano-objects is a very demanding task and subject of ongoing research.

The status of standardisation in the field of nanomaterials also depends on the currently relevant issues. The on-going debate is dominated by issues of a release of nanomaterials from composites and open nano-toxicological aspects. Both require a comprehensive characterisation of nanomaterials with mostly not yet standardised methods. Also requirements for such a comprehensive characterisation are still under discussion.

New, currently relevant characteristics of nanoscale materials can possibly be determined using well-established analysis methods. An example is the volume specific surface area [72], mentioned before. The characteristic data required for their calculation are accessible via two standards

- *DIN ISO* 9277:2003-05 – Determination of specific surface area of solids by gas adsorption using the BET method [73],
- *ISO* 3953:1993 – Density: Table works, tap density [74].

A reliable physico-chemical characterisation requires methods that were calibrated and validated with appropriate certified reference materials. As the compilation on http://www.nano-refmat.bam.de shows, there are very few certified nano-objects. Thus the standardisation of measuring methods for nanoscale materials is difficult. The lack of agreed standards or guidelines delays the implementation of regulatory guidelines for nano-objects [75].

In the frame of this study, the question whether scientifically based methods exist for the characterisation of nanomaterials was answered positively. References were compiled to scientific literature on analytical methods and characterisation techniques that have found application for certified reference materials. In addition, the current status was evaluated of international guideline and standard development related to characterisation of nanomaterials.

Standards with relevance to nano-objects are listed in the Appendix of this study and classified as follows

- Standards developed explicitly for nanomaterials (Tab. 1)
- Standards in consultation for nanomaterials (Tab. 2)
- Standards applicable to nanomaterials (Tab. T25).

For a number of standards it remains to clarify, whether they are applicable to nanomaterials, i.e. other materials than they were developed for. The compilation of the standards in the Appendix of this study also shows that reliable, standardised measurement methods exist for many physico-chemical parameters relevant for the characterisation of nano-objects. Most of them were not originally developed for the characterisation of nanoscale materials. This means that their applicability needs to be tested for every type of nanomaterial.

The *Preliminary Review of OECD Test Guidelines for their Applicability to Manufactured Nanomaterials* [76] shows that many *OECD* test guidelines are in principle applicable to nanomaterials. A case study of the *OECD WPMN* has tested the possible approach for the creation of a REACH dossier for nanoscale silver [77]. It emphasized significant differences between silver and nano-silver and concludes that substantial material information, which is required for a chemical or toxicological risk assessment, is not available. For some of the provided data, it was not clear, whether the nano-silver was analyzed in elemental or ionic form.

The reliability of many measurement methods for the characterisation of nano-objects cannot be assessed exhaustively by the present study. Many measurement methods that are applied in the scientific literature as state-of-the-art to nanomaterials do not have appropriate test guidelines and standards. Suitable nanoscale reference materials and reproducible sample preparation methods are main obstacles for establishing test standards.

3.5.3 Reference materials

The existence of nanoscale reference materials is an essential prerequisite for the development of validated analytical methods for nanomaterials. They are needed for example for a calibration of the length or size scale of a method. Different size measurement techniques like TEM and DLS that base on different measurement principles generally lead to different results, which depend also on particle type, preparation and measurement conditions. It is therefore very important to have materials whose "true" structure size is known. Only this way the origin of discrepancies can be found and the reliability of a method be quantified. For the regulation of nanomaterials, reference materials are of eminent importance for the calibration of a method in different laboratories to ensure the comparability of results.

BAM provides a compilation of available nanoscale reference materials with links to their suppliers [78]. Currently about 65 nano-scale reference materials are listed of different categories (levels, nano-objects, porosity, ...). They also include *certified* reference materials of the highest quality level, which are usually offered by national metrological institutes (*PTB/BAM*, *IRMM*, *NIST*, *AIST*, ...). The compilation is updated regularly. Currently, however, there are very few certified nano-objects. An example is nanoscale gold particles in suspension. These were certified at the *NIST* (National Institute of Standards and Technology), an agency of the U.S. Department of Commerce, as reference materials for metrology and pre-clinical biomedical research in various particle size classes [79]. The materials have been characterised extensively with respect to their mean particle size, particle size distribution, chemical and electrochemical properties, optical absorption coefficient and their biological properties. Such certified nanomaterials provide a good insight into the state of metrology of nanomaterials and are essential for their further development. Currently, at *NIST* more certified nano-objects are in the pipeline, namely single-walled CNT, chirality selected and unpurified.

The Joint Research Centre of the European Commission (*JRC*) at the Institute for Health and Consumer Protection (*IHCP*), http://ihcp.jrc.ec.europa.eu, recently established Europe’s first repository for nanomaterials. Batches of industrially available nanomaterials are portioned, homogenized and stored under inert conditions. Among them are also sponsorship programme materials of the *OECD WPMN*. The materials can be ordered for inter-laboratory comparisons, e.g. on the toxicology of the materials. Also their physico-chemical characteristics are being determined, however, not yet at the level of a certified reference material.

4 Applicability of test procedures to nanoscale substances

The physico-chemical test methods established in Regulation (EC) 440/2008 and its adaptation to technical progress (EC) 761/2009 to nanoscale materials is discussed in two aspects in the following. First, whether their application to nano-objects may result to deviations of the measured material characteristics compared to the conventional micro- or macroscopic material. Second, whether such deviations allow deriving information on a nanoscale nature of the tested substance. Third, issues of practical feasibility of the tests procedures for nanoscale materials are discussed and possible changes suggested.

The following REACH test procedures are considered as not applicable or not relevant to the testing of nanoscale materials since they relate to liquid or gaseous substances, or the differently regulated polymers. They are explicitly "only for liquids" or "only for oily substances", etc. and include
A.5. Surface tension
A.8. Partition coefficient
A.9. Flash-point
A.11. Flammability (gases)
A.15. Auto-ignition temperature (liquids and gases)
A.18. Number – average molecular weight and molecular weight distribution of Polymers
A.19. Low molecular weight content of polymers
A.20. Solution/extraction behaviour of polymers in water
A.21. Oxidising properties (liquids).

For the characterisation of nanoscale materials only those test methods are relevant that were developed for the characterisation of solids. Of the properties listed in the regulation (EC) 440/2008 and (EC) 761/2009, following are considered relevant
A.1. Melting / freezing point
A.2. Boiling point
A.3. Relative density
A.4. Vapour pressure
A.6. Water solubility
A.10. Flammability (solids)
A.12. Flammability (contact with water)
A.13. Pyrophoric properties of solids and liquids
A.14. Explosive properties
A.16. Relative self-ignition temperature for solids
A.17. Oxidising properties (solids)
A.22. Length-weighted mean diameter of fibres.

These test methods are discussed in terms of their applicability to nano-objects. The practical test procedure is examined in detail with respect to potential problems.

For all methods, it has to be considered that storage-dependent chemical alterations of the nano-objects may affect test results. Due to their very large specific surface such alterations can induce lead significant deviations of results depending on storage duration and conditions. An example is the surface oxidation of metal powders, which can, depending on the age of the sample, lead to formation of a surface passivation layer and thus affect testing of their oxidisability.

Whether the REACH methods that are applicable to nanoscale materials might be suitable for a distinction between macroscopic and nanoscale materials, has in part already been discussed in Chapter 3 and will be deepened here.

Since REACH testing procedures in part are based on *OECD* test guidelines, results of their applicability are briefly summarised below.

4.1 Applicability of Technical Guidelines of OECD

Also the *WPMN* of *OECD* evaluates the applicability of *OECD* Technical Guidelines (TG) to nanomaterial [80]. Many TGs were already assessed and divided into three categories. The first category includes such TGs, which are known to be directly applicable to manufactured nanomaterial.

These are the guidelines

- TG102 - Melting Point/Melting Range
- TG109 - Density of Liquids and Solids
- TG113 - Screening Test for Thermal Stability and Stability in Air
- TG116 - Fat Solubility of Solid and Liquid Substances.

The second category comprises *OECD* test guidelines that are applicable under certain circumstances to manufactured nanomaterials or to some classes of materials.

These are the test guidelines

- TG101 - UV/Vis Absorption Spectra
- TG105 - Water Solubility
- TG106 - Adsorption - Desorption Using a Batch Equilibrium Method
- TG107 - Partition Coefficient (*n*-octanol/water): Shake Flask Method
- TG108 - Complex Formation Ability in Water (Polarographic Method)
- TG111 - Hydrolysis as a function of pH
- TG112 - Dissociation Constants in Water
- TG115 - Surface Tension of Aqueous Solutions
- TG117 - Partition coefficient (*n*-octal/water) - HPLC method
- TG121 - Estimation of the Adsorption Coefficient on Soil and on Sewage Sludge using High Performance Liquid Chromatography (HPLC)
- TG123 - Partition Coefficient (*1*-Octanol/Water): Slow-Stirring Method.

For these test guidelines applicable to solutions, is not known how the results are influenced by the presence of colloidal suspensions, which are produced by the incomplete dissolution of nano-objects.

Testing guidelines of category three are not applicable to manufactured nanomaterials or, when applied, provide no meaningful results. This category contains the following two test guidelines

- TG103 - Boiling Point
- TG114 - Viscosity of Liquids.

While for TG114, the applicability to suspensions or colloidal solutions was not tested, TG103 does not provide a relevant characteristic property of a *nano*material since the phase transition from solid to liquid state irreversibly destroys the nanostructure. The boiling point would thus correspond to that of the conventional material. Thus it is applicable but does not give nano-related information. In the case of nano-emulsions, heating to the boiling point modifies (and ultimately destroys) the structure of the nanomaterial. In addition, due to the multi-phase nature of nano-emulsions, not a discrete boiling point, but a boiling range can be expected [80].

4.2 Applicability of REACH test procedures

A detailed description of the practical application of the REACH methods was published in Ref. [10] and [11]. The review of scientific literature performed for the present study showed that test methods for physico-chemical characteristics of nanoscale substances require nano-specific considerations. There is a variety of particle size-dependent material properties, which are capable of significantly influencing the test results. However, little information specifically on the applicability of REACH test procedures was obtained from the scientific literature and discussions with experts. The following discussion on expected problems is thus partially based on general considerations.

4.2.1 Occupational health aspects

One of the main concerns regarding the use of nanoscale materials are potential toxic effects. Other potential hazards especially from nano-objects, such as increased explosiveness, have received little attention so far [81]. Such risk aspects are, however, of fundamental importance for occupational safety during material testing.

Nano-objects can be subjected to the relevant test methods for physico-chemical characteristics of solids. For nanoscale substances, however, a number of special aspects have to be considered. During all phases of the handling of nanoscale substances, i.e. during the preparation, execution and subsequent cleaning of the experimental setup, the release of ultrafine particles may occur. Due to the lack of a toxicological classification of most nano-objects, special occupational health and safety measures have to be established [82, 83]. The uptake of nanomaterials by inhalation in the workplace poses a health risk for laboratory workers. Therefore, the exposure of humans and the environment to nano-objects has to be minimised according to the precautionary principle. This is especially true for the load of the executive tester and the contamination of the laboratories used.

For this reason, the steps for sample preparation such as powder pouring, grinding, sifting, weighing, compiling, compressing, etc. require qualified handling [84]. To be able of performing powder transfers by pouring, as they are needed for many tests, requires the investigated nanoscale substances to exhibit a sufficient sedimentation propensity [85]. This condition may not be fulfilled for non-agglomerating nanoparticles. Electrostatic repulsion and/or morphological factors, such as a small number of contact points between neighbouring particles, can reduce the agglomeration of nanoparticles to such a degree that they do not form (spontaneously) sedimenting aerosols. Examples are thermally exfoliated graphenes or rigid, non-oriented MWCNTs. Both can exhibit extremely low solid bulk densities. Such materials may, however, be handled by filtration of an aerosol or a suspension to a compressed filter cake. Otherwise, draughty laboratory air or process gas flows, temperature gradient-induced gas convection, ignition or explosion processes can lead to powder losses, aerosol formation and dissemination of nano-objects in the test room and the air. Therefore, for the testing of nanomaterials, methods should be preferred that use closed apparatuses. Moreover, concepts have to be developed to clean test equipment in a qualified way from nanoscale residues. If this is not possible, special protective measures have to be applied [86]. When working with dry particles, personal protective equipment should be used together with exhaust ventilation or glove boxes. The exposition of the executive tester and the effectiveness of the protection measures are to be verified at periodic intervals [87].

According to the EU project NANOSAFE2 and the Swiss Institute of Safety and Security, Basel, few basic principles of occupational safely allow to achieved a high degree of protection for the persons involved in testing of potentially toxic and possibly highly dusty nano-scale substances [86]

- Open handling of the powders is limited to the transfer of the powder from the shipping container (e.g. the powder flask) either to
    - the container, in which the test is carried out later or
    - an intermediate container, from which the sample can be transferred into the test apparatus in an entirely closed way.
- The remaining open handling is carried out in working stations equipped with laminar flow chambers.
- The personnel wear adapted personal protection equipment. A risk analysis has to be performed to assess if the measures are sufficient also in case of deviations form the normal operating conditions.

After the test, the residual material is either

- destroyed and washed out by a "cleaning in place" installation
- disposed directly in the (closed) test container
- removed from the testing equipment inside the special room under laminar flow.

For the implementation of these principles, it may be necessary to redesign certain test methods and/or equipments.

4.2.2 A.1 Determination of meting point

The test methods A.1 serve a determination of the melting temperature of test substances. Depending on the procedure, very small - a few milligrams - to moderate amounts of test substance are needed.

Due to their intermediate size range between atomic and continuous macroscopic structures, many physical and chemical properties which are independent of size in the macroscopic world start to depend on particle size for nanoscale substances. Particulate substances can therefore exhibit a size-dependent melting behaviour. An exact transition range between the micro- and nanoscale world cannot be specified since the deviations depend on the material, its crystalline structure and external conditions such as temperature and pressure [88]. An overview of the particle dependence of characteristic material properties was given in Section 3.3. There, also details of the theoretical background and examples of experimental data on particle size-dependent melting behaviour were presented. For many substances a significant reduction in the melting temperature was observed, with decreasing particle size. It can lead to significant deviations for nano-objects relative to the melting behaviour of microparticles and solid materials. Melting nanoparticles may enter a supercooled state [37]. It sometimes freezes only in presence of crystallisation nuclei, perturbations like sound waves, or at significantly lower temperature [38]. In practice, for molten nanoparticles, supercoolings of up to 200 K were observed [39].

In Regulation (EC) 440/2008, different procedures are defined to determine the melting temperature: Capillary, hot stage and so-called thermal analysis methods. The applicability of these methods to nanoscale materials and other aspects are discussed below.

In order to perform the required powder transfer, pouring processes and particle packings, the nanoscale substances to be tested must have a sufficient sedimentation propensity [85], see 4.2.1. Since nanoparticles, in addition, may also exhibit a significantly reduced thermal conductivity, it has to be check whether a reduction of the heating rate compared to those specified in the test procedure is required. Such a reduced thermal conductivity of nanoparticle packings, for example, was founded by PRASHER by theoretical analysis [89] and demonstrated by HU et al. [90] using the example of aluminium nanoparticles.

The methods of thermal analysis (differential thermal analysis, DTA, and differential scanning calorimetry, DSC) are particularly well suited for small substance quantities. They should be should preferably be used for nanoscale substances, provided non-permeable crucibles are [81]. The typically investigated temperature range spreads from -150 to 700 °C [91], special equipment allows studying melting points of up to 1500 °C. Several publications have already studied the melting behaviour of nanoscale substances. For example ZOU et al. [92] investigated the particle size-dependent melting properties of tin oxide nanoparticles with diameters of 34 to 81 nm and XU et al. that of indium nanowires with diameters of 20 to 80 nm [93].

Table 4.1 shows that the test procedures A.1 are in principle applicable to nanoscale substances provided powder losses caused by convection do not affect the test result. In particular, thermo-analytical methods that work with closed sample containers are well applicable.

Tab. 4.1 Applicability of test procedures for melting temperature determination to nanoscale substances.

A.1 Melting temperature	**Applicable**	**Justification for applicability or reasons for non-applicability**	**Nano-specific Information derivable?**	**Ref.**
Capillary methods with temperature-based determination	Yes, provided	powder losses can be avoided during measurement, e.g. by closing the capillary with a membrane stopper.	Yes, provided that the substance shows supercooling when melting such that its optical properties change significantly.	
Capillary methods with photocell determination	Yes, provided	powder losses can be avoided during measurement, e.g. by closing the capillary with a membrane stopper.	Yes, provided that the substance shows supercooling when melting such that its optical properties change significantly.	
Hot stage methods	Yes, provided	powder losses by convection processes of the air above the hot stage do not change the powder packing significantly.	Yes, provided that the substance shows supercooling when melting such that its optical properties change significantly.	[94]
Thermal analysis (DTA,DSC)	Yes, provided	powder losses can be avoided during measurement, e.g. by closing the measurement cell.	Yes, endothermy at lower temperature, broader peaks and supercooling were observed for nanoparticles.	[39], [91], [95]
Optimisation necessities for nanoscale substances				
Powder losses	Closed measurement chambers or crucibles should be used. DSC should be preferred.			
Melting behaviour	Due to a possibly complex melting behaviour, additional information should be acquired, e.g., a possibly broadened melting transition range. By analysis of a heating-cooling-heating cycle, supercooling-induced hysteresis effects should become identifiable in order to obtain information on a possible nanoscale nature of the substance.			
Aging condition	For nanoscale solids, the sample age and storage conditions must be known. Otherwise, the inertisation state of the substance must be determined analytically.			

4.2.3 A.2 Boiling point of low-melting solids

The test procedures serve to determine the boiling point A.2 of test substances and are relevant for low-melting solid substances. The required amounts of substance correspond to those required for melting temperature determination.

Since it the boiling temperature is determined for the phase transition from liquid to gaseous form, no deviations are to be expected for nanoscale substances. The reason for this is that for liquid substances, a possible former nanoscale structure is lost and is not expected to be regenerated by the cooling process. The method therefore provides no nano-specific information. For this reason it is expected that the method, as well as the *OECD* TG 103 (Boiling Point), is suitable for determining the boiling point of nanoscale materials [3]. However, in the case of a significantly increased sublimation rate of nanoscale materials, it is possible that due to the temperature rise during the measurement a direct phase transition from solid to gaseous occurs, so that depending on test procedure and unlike the analogue microscale material, no boiling point may be observable. If, as in the implementation of the dynamic method and the distillation method, a recondensation temperature is determined, a possible reduction of the recondensation temperature may occur by condensation of vapour on convecting nanoparticles, which is, however, considered a negligible effect.

Tab. 4.2 Applicability of test procedures for boiling point determination to nanoscale substances.

A.2 Boiling Point	Applicable	Justification for applicability or reasons for non-applicability	Ref.
Static and dynamic method	Yes. Without	limitations since the recondensation behaviour of vapours is studied.	
Distillation method	Yes. Without	limitations since the recondensation behaviour of vapours is studied.	
Siwoloboff in heat bath	Yes. Without	limitations since melting is studied in closed sample tubes.	
Siwoboloff in metal block with photocell detection	Yes. Without	limitations since melting is studied in closed sample tubes.	
Thermal analysis (DTA, DSC)	Yes, provided	powder losses during the melting can be avoided, e.g., in closed measurement cells.	[91], [95]
Optimisation necessities for nanoscale substances			
Powder losses	Closed measurement chambers or crucibles should be used. DSC should be preferred.		

If the characteristics of nanoparticles are considered, the test procedures for A.2 using closed test equipment appear suitable for nanoscale substances provided convection-caused powder losses do not affect the test results.

4.2.4 A.3 Relative density

The test procedures A.3 serve to determine the relative density of a substance. The substance density is an important parameter of nano-objects, which affects particle properties like dry sedimentation or deposition of particles in the lung [96].

The inherent density of inert nanoparticles should only be insignificantly affected by density differences of particle size-dependent crystal conformations. However, studies of PARK et al. on diesel soot particles have shown that the practical density determination in the case of nanoparticles smaller than 100 nm can be significantly impeded by surface adsorbed substances or recondensed liquids [52]. For reactive substances, drying processes that were investigated for the removal of such adsorbates, could lead particle size-dependent thermal oxidation behaviour. For instance, in case of aluminium nanoparticles drastic changes in particle density resulted. The density changed depending on size by surface oxidation due to the density differences between aluminium (2.70 g/cm³) and alumina (3.97 g/cm³). With decreasing feature size, not only the reactivity but also the volume-specific surface is generally enhanced so that chemical surface differences and the thickness of the surface boundary layer of a nanoscale substance may become significant for the material density. This underlines the importance of knowledge on the aging conditions and the inertisation state of a nanoscale material. According to M. HOSOKAWA [97], the density of powders consisting of nano-objects can be determined by means of fluid compression and gas displacement pycnometer methods.

Pycnometer methods

Pycnometer methods are described as simple and reliable [98]. They have been successfully used for measuring the density of nanoscale ceramic powders with primary particle sizes of 15 to 30 nm [99], [100]. The density of porous MWCNT samples was determined using helium pycnometry [101]: The density of MWCNT agglomerates with a particle size distribution of 20, 200, 560 µm (d10, d50, d90), a BET surface of 195 m²/g and embedded nanotubes with diameters of 15 to 20 nm was 2.0 to 2.1 g/cm³. In the case of hollow nanostructures, closed pores or poorly wetting test media [102] may cause a deformation of the nanostructure by the gas or fluid pressure. Thus, pressure and test medium dependent variations in the determined density may result. Especially mercury pressures of up to one thousand atmospheres are regarded as problematic [103], [104]. In the case of nanoporous materials and open nanotubes, also pore size and pore infiltrability of the test medium can affect the measurement result. For this reason, mercury and helium-based methods may provide different results. Thus, for carbon nanotubes, which are closed with fullerene end caps, a significantly lower density of 1.4 g/cm³ was found with helium pycnometry than the 1.9 to 2.0 g/cm³ found for oxidatively end cap-opened CNT.[4] This is caused by the high pore infiltrability of helium, so that for complete gas infiltration the nanotube density corresponds to that of graphite (2.1 to 2.3 g/cm³).

Air comparison pycnometer

The density of the substance is calculated from the difference in weight between the full and empty pycnometer and its known volume. The volume of a substance is measured in the air or in an inert gas in a cylinder of variable, calibrated volume. To calculate the density after completion of volume measurement, weighing is performed.

The test procedures A.3 are in principle suitable also for nanoscale substances, provided porous materials are examined, which micro-and nano-pores are easily infiltratable by the test medium. In the case of closed pore-systems or poorly wetting test media, deviations may result according to the mechanical stability of the sample and the applied hydrostatic test pressure. Densities differing from conventional materials may indicate a nanoscale structure, for example, nanoporosity or nanoparticle agglomerates.

Tab. 4.3 Applicability of test procedures for relative density determination to nanoscale substances.

A.3 **Relative Density**	**Applicable**	**Justification for applicability or reasons for non-applicability**	**Ref.**
Pycnometer methods, Air comparison pycnometry	Yes, provided	that porous materials are examined, whose micro-and nanopores are easily accessible for the test medium. In the case of closed-pore systems or poorly wetting test media, different results may result depending on the degree of compressibility of the sample.	[104]
Optimisation necessities for nanoscale substances			
Density definition	The term *density* should be specified more clearly for nanoporous materials: Is the apparent or true material density to be determined? Strategies for their determination should be developed.		
Nanoporosity	Since nanoporosity is an indication of nanoscale, e.g. in the case of aggregates of nanoparticles, the density determination should be performed with combinations of methods that are capable to detect nanoporosity.		

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[4] A. Dresel, Ohm-Hochschule, private communication.

4.2.5 A.4 Vapour pressure

The test procedures A.4 serve to determine the vapour pressure of a test substance, and are mainly relevant for volatile substances. The test procedures for vapour pressure determination are specified in Directive 67/548/EEC, Annex 5, and in *OECD* TG 104 [105] in detail. More information on standardised methods for determining the vapour pressure can be found in *ASTM* D 2879-86, *NFT* 20-048 (5) and *NFT* 20-047 (6).

The isoteniscope is a special case of the so-called static method, as described below. The regulation (EC) 761/2009 contains procedure changes [11] that admit the "Effusion method: isothermal thermogravimetry" as an additional method that is particularly suitable for substances with very low vapour pressures - up to a minimum pressure of 10^{-10} Pa [106]. The other test methods are re-evaluated in terms of their application range. The selection of a suitable measuring method depends on the type of sample and its estimated vapour pressure [107].

Methods of vapour pressure measurement that can be applied to volatile substances, which are solid at room temperature, are also relevant for nanoscale substances, as was shown by ABREFAH et al. for BUCKMINISTER fullerenes [108]. The vapour pressure of nanoscale substances is, however, according to the GIBBS-THOMSON relation, which is based on a model of spherical particles, increased relative to that of microscale materials. This was shown for example by measurements of NANDA et al. for PbS particles [109], [110]. For the determination of the vapour pressure of nano-objects, however, no differences are expected if the substance is melted prior to the measurement, i.e. becomes liquid such that its nanoscale structure is lost. Nano-specific deviations in vapour pressure can be expected, however, if the rate of sublimation of a nanoscale solid is significantly increased compared to that of a microscale material. In this case, nanoscale substances sublimate and resublime without melting. This in principle allows obtaining information on their nanoscale structure from the resulting vapour pressure curve. Also aerosol formation during the measurement, for example by convection above the particle bed, could result in nano-specific deviations.

4.2.5.1 Static and dynamic method

Static method

For the static method, the vapour pressure of a substance is determined at the thermodynamic equilibrium of a closed system for a given temperature. Due to their large specific surface area not yet melted nanoscale substances can in principle show higher sublimation propensity than macroscale materials, from which information may be derivable on the nanoscale nature of a substance.

Dynamic method

For the dynamic method of vapour pressure measurement, a boiling vessel with attached cooler and a device for measuring and controlling temperature and pressure is used. The boiling temperature of a substance is measured at several given pressures between 10^2 and 10^5 Pa by varying the temperature. A total of 5 to 10 different measurements at successively increasing pressures are performed. For validation, the measurements must be repeated with decreasing pressure. For an application of the method to nanoscale substances, deviations between the measurements for increasing and decreasing pressure are possible if the nanoscale structure of the sample is lost during melting only above one of the studied pressures. Such deviations could help to identify a substance as nanoscale.

4.2.5.2 Gas saturation method

A stream of inert carrier gas is passed over the substance in such a way that it becomes saturated with its vapour. The amount of material transported by a known amount of carrier gas is measurable either by collection in a suitable trap or by an inline analytical technique. It is then used to calculate the vapour pressure at a given temperature. The gas saturation method is suitable for solid and liquid substances. Solid samples are placed in glass tubes with an inner diameter of 5 mm between glass wool plugs. During application of the method to samples containing nano-objects, nano-objects that are entrained by the gas stream may not be held back by the glass wool plug. Thus, the vapour pressure of the substance would be overestimated.

4.2.5.3 Spinning rotor method

The spinning rotor technique can be carried out using a spinning rotor viscosity gauge, which consists of a small steel ball, suspended without contact in a magnetic field, rotated by rotating electromagnetic fields in an evacuated measuring chamber [111]. The gas pressure in the chamber is determined via the de-acceleration of the ball caused by the gas friction. If applied for determining the vapour pressure of nanoscale substances, a spinning rotor viscometer, the measurement may overestimate the vapour pressure in the case of particle adsorption on the surface of the ball. For this reason, nanoscale substances containing solid fractions must be melted before the measurement in a separate apparatus under inert conditions. Therefore, the spinning rotor method is not applicable to nanoscale substances without modifications.

4.2.5.4 Effusion methods

The methods are based on a determination of the mass per unit time of a test substance that escapes from a heated sample cell as a vapour through an orifice of known size into a detection chamber. Vacuum conditions ensure that a return of substance into the measurement cell can be neglected.

Problems of an application the effusion methods to nanoscale substances are expected if nano-objects are entrained through the orifice by the forming evaporation gas flow. This could happen if a bed of nanoparticles vaporises that is only partially melted, which is possible in case of a low thermal conductivity of the bed or in inhomogeneous mixtures that contain higher-melting particle fractions. The amount of substance (vapour plus particle) leaving the cell per unit time could thereby be increased. A related contamination of the cell chamber and the attached detection chamber with nanoparticles could provide condensation nuclei, which might cause the vapour to be detected to condensate in unexpected locations of the chambers. For this reason, nanoscale substances containing solid fractions must be completely melted under inter conditions in a separate apparatus before measurement.

Effusion method: Vapour pressure balance

The escaping vapour is directed onto a balance pan of a highly sensitive balance. The momentum of the vapour jet acts as a force on the balance. When applied to samples containing nano-objects, nano-objects entrained by the vapour stream may exert an unexpectedly high momentum transfer on pan and the derived vapour pressure may be overestimated.

Effusion method: Knudsen cell

In a Knudsen cell [112], the vapour escapes through a micro-orifice. The mass of effused vapour can be obtained either by determining the loss of mass of the cell or by condensing the vapour at low temperature and determining the amount of volatilised substance using chromatography [113]. The method is recommended for vapour pressures in the range of 10^{-10} to 1 Pa. When applied to samples containing nano-objects, nano-objects entrained by the vapour stream would lead to an overestimation of the mass loss in case of gravimetric quantification. For chromatographic quantification, possible effects of nanoparticles would require detailed investigation.

Effusion method: Isothermal thermogravimetry

The method is based on the determination of accelerated evaporation rates for the test substance at elevated temperatures and ambient pressure in inert atmosphere using thermogravimetry. The test substance is applied to the surface of a roughened glass plate as a homogeneous layer. In the case of solids, the plate is wetted uniformly by a solution of the substance in a suitable solvent and dried in an inert atmosphere. When applied to soluble nanoscale substances, the nanoscale character would be lost, so that no nano-specific features are expected. For (partially) insoluble nano-objects, nano-objects may be entrained by the inert gas stream and disturb the method since mass loss by evaporation would be overestimated.

Tab. 4.4 Applicability of test procedures for vapour pressure determination to nanoscale substances.

A.4 **Vapour Pressure**	**Applicable**	**Justification for applicability or reasons for non-applicability**
Static or Dynamic method	Yes.	
Gas saturation method	Yes, provided	nanoscale sample fractions are not dispersed by or filtered off the measurement gas flow.
Spinning rotor method	No, since	nanoscale substances would need to be melted under inert conditions before the test in an external setup.
Effusion methods	Yes, provided	nanoscale sample fractions are not dispersed and removed by the forming vapour gas flow. Therefore, the sample must be melted under inert conditions before the test in an external setup.
Optimisation necessities for nanoscale substances		
Controlled melting	Solids should be melted in a controlled way prior to vapour pressure determination, i.e. fully melted in a crucible, such that their nanoscale nature is lost reliably.	
Indications of nanoscale nature	An enhanced sublimation pressure of nanoscale solids could allow deriving information on its nanoscale nature. This would require alternative testing methods.	
Aging condition	For nanoscale solids, the sample age and storage conditions must be known. Otherwise, the inertisation state of the substance must be determined analytically.	
Filtration	Filters capable of filtering nanoparticles should be used.	

The test procedures A.4 are in principle applicable to nanoscale substances, provided powder losses by aerosol formation can be avoided, for instance by preceding melting or dissolution of the test substance.

4.2.6 A.6 Water solubility

The test methods A.6 serve the determination of water solubility of solid test substances. The saturation solubility is a substance-specific property that depends on the temperature. However, the solubility behaviour may also depend on the particle size [62]. Especially nanoscale solids can exhibit a significantly increased dissolution rate. This can be explained by the increased volume specific surface area of nanoparticles compared to microparticles and modified bonding states of surface atom groups, see discussion and examples in Section 3.3. As a consequence, the altered behaviour of nanoscale substances finds application for traditionally poorly water-soluble pharmaceutical substances [64].

Column elution method

This method is based on the elution of a test substance with water from a micro-column which is charged with an inert support material, such as glass beads or sand, coated with an excess of test substance. The water solubility is determined when the mass concentration of the eluate is constant. This is shown by a concentration plateau as a function of time.

Since the test substance is first mixed with a volatile solvent during sample preparation, the nanoscale nature of soluble sample fractions is lost. Insoluble nanoscale components can, however, form a suspension with the dissolved fraction.

If this suspension is not freed from solid constituents by appropriate filtration before the transfer to the micro-column, solid particles may eluate from the column and fake an increased solubility. If the micro-column exhibits high filtration efficiency for nanoparticles, only small deviations have to be expected.

Shake-flask method

The test substance is dissolved in water and the mass concentration of test substance in a solution containing no undissolved particles is determined by appropriate analytical methods [10].

This test method appears recommendable if a complete removal of undissolved nanoscale particles by suitable ultrafiltration methods, e.g. nano-filtration membranes [114] is ensured. Using the shake-flask method, for example the solubility behaviour of nanoscale drug particles was investigated, which were synthesized to increase the water solubility of poorly soluble drugs [115]. Due to the increased solubility of nanoscale materials, an oversaturation of the solution may occur corresponding to a metastable state. In the absence of precipitation nuclei and in relatively undisturbed systems, high degrees of supersaturation may occur. For such supersaturated solutions, the saturation solubility is overestimated. Provided that the saturation solubility and not the precipitation limit are determined, such a supersaturation or an increased solubilisation rate could provide indications on the nanoscale nature of a substance, see Section 3.3.5.

According to the summary in Table 4.5, the test procedures A.6 are applicable in principle to nanoscale substances, however, the possibility of existing insoluble nanoscale fractions and a supersaturation of the solution have to be considered.

New methods should be developed to facilitate deriving information on the nanoscale nature of a substance via its solubility behaviour. This should be done with low-perturbance solubility tests that may help to prevent premature precipitation of a supersaturated solution. Also an increased dissolution rate could serve as evidence.

Tab. 4.5 Applicability of test procedures for water solubility determination to nanoscale substances.

A.6 Water Solubility	**Applicable**	**Justification for applicability or reasons for non-applicability**	**Nano-specific Information derivable?**	**Ref.**
Column eluation method	Yes, provided	it is ensured that the eluate does not contain non-soluble nano-scale components of the substance or its impurities.	No, the column packing would cause the precipitation of a supersaturated solution.	
Shake-flask method	Yes, provided	if not soluble nanoscale components are removed by appropriate filtration process from the solution.	Yes, if the disturbance of the supersaturated solution by shaking the flask does not lead to precipitation.	[114]
Optimisation necessities for nanoscale substances				
Filtration	Filters capable of filtering nanoparticles have to be used.			
Indications of nanoscale nature	Solubility tests should be performed perturbation-free to avoid early precipitation of supersaturated solutions. The solubilisation rate should be measured.			
Aging condition	For nanoscale solids, the sample age and storage conditions must be known. Otherwise, the inertisation state of the substance must be determined analytically.			

4.2.7 A.10 Flammability (solid substances)

The test methods A.10 serve to determine the flammability of powdered or paste-like substances. These are judged to be highly flammable if they completely burn in one of the prescribed tests in a period shorter than 45 seconds, or, in the case of metals or metal alloys, if they are flammable and the reaction zone propagates within 10 minutes over the entire sample.

There is little experimental data on fire and explosion properties of nano-objects [116]. Basically, the reactivity of nano-objects may be changed depending on size so that an individual assessment needs to be performed [53].

Due to the previously described tendency of nano-objects to agglomerate and to undergo surface passivation reactions, there is a possibility of false negative test results when testing the flammability of nanoparticles of unspecified age and storage conditions. An oxidised interface layer forming on metallic nanomaterials may exhibit the character of an protective oxide layer that reduces the reactivity of a tested materials [7], [117], [118]. Carbon-based nanoparticles, on the other hand, can develop a reduced thermal resistance due to oxidative reactions of carbon during storage. Such oxidation processes generally increase the polarity of a surface and thus change its wettability. This can increase the amount humidity adsorbing to the surface of nanoscale materials and may lead to delayed or modified flammability. Therefore, adsorbate water should be quantified, for instance by thermogravimetry, and must be removed prior to testing by suitable drying procedures that are capable of avoiding oxidation [119].

These considerations show how important the history of a nanomaterial may be for the outcome of a test method. The individual history must therefore be considered for property comparisons of nanoscale materials and the interpretation of experimental results. If the storage history of a substance is unknown, it might help to analysis the chemical composition of its surface and volume in order to understand the oxidation state of the particle interface. However, it again depends on the particle size, whether analytical methods that are classically considered as surface sensitive techniques are able to obtain actual *surface* information. An example is XPS. Despite a very low information depth of less than 10 nm, when applied to particles smaller than 20 nm, XPS essentially determines the atomic composition of the volume material.

The project NANOSAFE2 [60] studied potential explosion risks of nanoparticles. Based on the fire and explosion properties of selected nanoparticles it was shown that the standard testing equipment and techniques used for conventional substances are only applicable agglomerated nanoparticles, while they are inappropriate for non-agglomerated ones. During the storage of nanoscale samples, not only the described inertisation but also agglomeration may occur. Both are usually accompanied with a decrease in reactivity. Therefore, in the absence of knowledge of the inertisation and agglomeration state, the unmindful application of test procedures may lead to an underestimation of the security risk of nanoscale substances.

The test procedure A.10 appears in principle applicable for nanoscale solids if the special requirements of nanoscale substances are considered appropriately with respect to the substance identity, its history and surface oxidation degree.

Tab. 4.6 Applicability of test procedures for the flammability of solids to nanoscale substances.

A.10 Flammability of Solids	**Applicable**	**Justification for applicability or reasons for non-applicability**	**Ref.**
Powder train ignition test	Yes, provided	the test substance exhibits sufficient sedimentation propensity, its surface inertisation state and residual moisture are known, and the powder losses caused by convection do not affect the test result.	[117], [118], [119]
Burning rate test	Yes, provided	the test substance exhibits sufficient sedimentation propensity, its surface inertisation state and residual moisture are known, and the powder losses caused by convection do not affect the test result.	
Optimisation necessities for nanoscale substances			
Aging condition	For nanoscale solids, the sample age and storage conditions must be known. Otherwise, the inertisation state of the substance must be determined analytically.		
Residual moisture	For nanoscale solids, the residual moisture must be determined and - if necessary - reduced. However, uncontrolled surface inertisation during drying must be avoided.		

4.2.8 A.12 Flammability (contact with water)

The test procedure A.12 serves a determination of the flammability of a substance on contact with water. The tendency of the materials is tested to develop dangerous amounts of highly flammable gases by reaction with water or damp air. The method can be applied to both solid and liquid substances, but not for pyrophoric substances that spontaneously ignite when exposed to the air. The substance is tested according to a step-by-step sequence. If ignition occurs at any step, no further testing is necessary. If it is known that the substance does not react violently with water then the 4th step can be directly tested.

In case of application of the method to nanomaterials, as before, there is the possibility of varying results depending on the particle size [53] and the degree of surface oxidation or passivation inducing the risk of false negative conclusions [7], [117]. Therefore, sufficient knowledge is required about the history of the substance to be tested that must be considered for any interpretation of test results.

Tab. 4.7 Applicability of test procedures for flammability in contact with water to nanoscale substances.

A.12 Flammability (Water contact)	**Applicable**	**Justification for applicability or reasons for non-applicability**	**Ref.**
Step 1 – Powder into water	Yes, provided	the test substance exhibits sufficient sedimentation propensity and its surface inertisation state is known.	[117], [118]
Step 2 – Powder on filter paper on water	Yes, provided	the test substance exhibits sufficient sedimentation propensity and its surface inertisation state is known.	[117], [118]
Step 3 – Water droplet on powder	Yes, provided	the test substance exhibits sufficient sedimentation propensity, its surface inertisation state is known and the powder losses caused by convection do not affect the test result.	
Step 4 – Gas formation of a powder water mixture	Yes, provided	the test substance exhibits sufficient sedimentation propensity and its surface inertisation state is known.	
Optimisation necessities for nanoscale substances			
Aging condition	For nanoscale solids, the sample age and storage conditions must be known. Otherwise, the inertisation state of the substance must be determined analytically.		
Powder losses	Test procedures should be developed that allow avoiding powder losses.		

The test procedure A.11 appears in principle applicable for nanoscale solids if the special requirements of nanoscale substances are considered appropriately with respect to the substance identity, its history and surface oxidation degree etc., see Table 4.7. The procedures appear well performable in closed test equipment that would allow minimising exposure risks.

4.2.9 A.13 Pyrophoric properties of solids and liquids

The substance, whether solid or liquid, is added to an inert carrier and brought into contact with air at ambient temperature. The time span to self ignition is measured.

When applying this test method to nanoscale substances, a significant dependence on the particle size, storage history and surface passivation degree is expected. A high degree of care is therefore required for the characterisation and documentation of sample age and surface inertisation state. One of the tests also requires enhanced air contact of the sample to be tested by dropping 1 to 2 cm³ of the powdery substance from about 1 m height onto a non flammable surface. It is to be observed, if it ignites. For an application of this test to nanoscale substances, closed test equipment should be developed with external tilt mechanism for dropping the sample under controlled oxygen partial pressure.

The test procedure A.13 appears in principle applicable for nanoscale solids if the special requirements of nanoscale substances are considered appropriately with respect to the substance identity, its history and surface oxidation degree etc., see Table 4.8. The procedures appear well performable in closed test equipment.

Tab. 4.8 Applicability of test procedures for pyrophoric properties to nanoscale substances.

A.13 **Pyrophoric Properties**	**Applicable**	**Justification for applicability** **or reasons for non-applicability**
Powder pouring test	Yes, provided	the test substance exhibits sufficient sedimentation propensity, its surface inertisation state and residual moisture are known, and the powder losses caused by convection do not affect the test result.
Optimisation necessities for nanoscale substances		
Aging condition	For nanoscale solids, the sample age and storage conditions must be known. Otherwise, the inertisation state of the substance must be determined analytically.	
Residual moisture	For nanoscale solids, the residual moisture must be determined and - if necessary - reduced. However, uncontrolled surface inertisation during drying must be avoided.	
Powder losses	Test procedures should be developed that allow avoiding powder losses.	

4.2.10 A.14 Explosive properties

The method provides a scheme of testing to determine whether a solid or a pasty substance presents a danger of explosion when submitted to the effect of a flame (thermal sensitivity), or to shock or friction (sensitivity to mechanical stimuli), and whether a liquid substance presents a danger of explosion when submitted to the effect of a flame or shock.

Due to the possibility of surface passivation reactions during storage of the substances not under an inert atmosphere, also this method can lead to false negative test results if applied to nanoscale substances without knowing the history of the materials.

Tab. 4.9 Applicability of test procedures for explosive properties to nanoscale substances.

A.14 **Explosive properties**	**Applicable**	**Justification for applicability** **or reasons for non-applicability**	**Ref.**
Thermal sensitivity	Yes, provided	the surface inertisation state and residual moisture are known and the amount of nanoscale test substance that might escape the test tube does not change the test result.	[120]
Mechanical sensitivity	Yes, provided	the surface inertisation state and residual moisture are known.	[86], [121]
Frictional sensitivity	Yes, provided	the surface inertisation state and residual moisture are known and it is ensured that a nanoscale sample is not dispersed to an aerosol by the movement of the pestle. For housed test setups, possible crackles/detonations sounds must remain audible.	
Optimisation necessities for nanoscale substances			
Aging condition	For nanoscale solids, the sample age and storage conditions must be known. Otherwise, the inertisation state of the substance must be determined analytically.		
Residual moisture	For nanoscale solids, the residual moisture must be determined and - if necessary - reduced. However, uncontrolled surface inertisation during drying must be avoided.		
Powder losses	Test procedures should be prefered that allow avoiding powder losses.		

The test procedure A.14 appears in principle applicable for nanoscale solids if the special requirements of nanoscale substances are considered appropriately with respect to the substance identity, its history and surface oxidation degree etc., see Table 4.9.

4.2.11 A.16 Relative self-ignition temperature for solids

For the determination of the self-ignition temperature of solids at elevated temperature according to test method, a wire mesh cube with mesh size of 0.045 mm is used to hold the test substance. It is heated in a furnace at a rate of 0.5 K/min with natural air circulation to 400 °C or to the melting point of the sample. The temperature is measured inside the sample.

The test procedure A.16 appears in principle suitable for nanoscale substances, provided that possible powder losses do not affect the test result. Therefore, it has to be tested whether wire mesh cube is capable of holding the nanoscale substance. For nano-objects of sufficient agglomerate size, such problems are not expected [81]. Since meshes of the cube are relatively large, nanoscale substances may be entrained by convecting hot air and disseminated inside the furnace. In the case of significant material losses, the contact between the temperature probe and the sample may be lost.

As part of the EU project NANOSAFE2 [122], LERENA et al. [86] developed new test methods that were adapted to nanoscale substances in order to avoid disadvantages of conventional test methods, see Section 4.2.13. Instead of the wire mesh cube, a method for determining the self-ignition temperature has been developed based on dynamic DSC. Only a few milligrams of test substance are needed to be filled in a DSC crucible which is sealed under an atmosphere of 5 bar pure oxygen. The high oxygen pressure ensures a sufficiently high sensitivity to an oxidation propensity of the material. It was shown that the onset data can be correlated with those of the conventional tests [120].

In this way, a particle size dependence of the temperature of the onset oxidation temperature of aluminium powders was found. For nanoscale aluminium particles, this oxidation temperature was lowered by up to 200 to 300 °C. In addition, the burning of aluminium nanoparticles was found to start already below the melting temperature of microscale aluminium powders. For aluminium nanoparticles of 80 nm size a combustion temperature of 400 °C was observed, while the melting point of microscale aluminium was 660 °C. It was further found that the mechanisms of combustion of nanoscale microscale aluminium particles may differ significantly. Therefore, storage of such particles in industrial quantities requires special precautions and protective measures [121].

For multi-walled carbon nanotubes (MWCNT), the kinetics of combustion and self-ignition were investigated in the project NANOSAFE2 using two alternative procedures, TGA and DSC, as well as a standard method of self-ignition of solids according to *NF EN* 15288 (*CEN* 2006) [102]. The relative self-ignition temperature of MWCNTs was determined, for agglomerates with diameters of ~ 200 µm with embedded MWCNT with diameters in the order of 15 to 20 nm and a BET surface area of 195 g/cm³. Their density according to helium pycnometry was 2.1 g/cm³.

Compared to a cube of wheat flour of 125 cm³ volume, which ignited at 192 °C, a similar volume of MWNCT ignited at 372 °C.

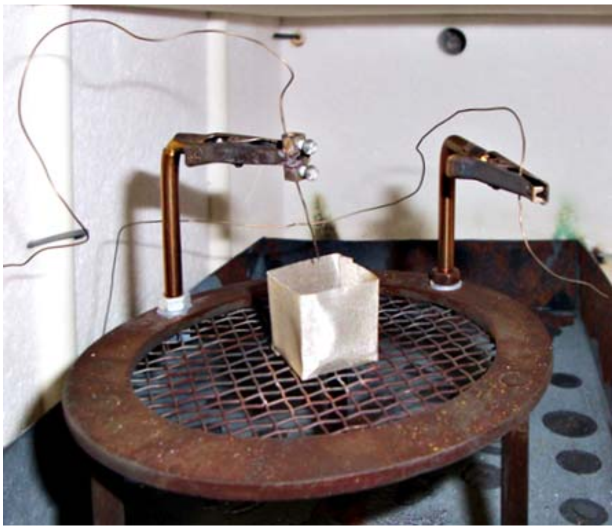

Fig. 4.1 Sample wire mesh cube in furnace.

Tab. 4.10 Applicability of test procedures for relative self-ignition temperature determination to nanoscale substances.

A.16 Relative Self-ignition Temperature	**Applicable**	**Justification for applicability or reasons for non-applicability**
Test cube in furnace	Yes, provided	that during heating of the sample no significant quantities of nanoscale material escape from the openings of the wire mesh cube, and that the heating rate chosen does not lead to a passivation layer on the particle surface, which may delay ignition.
Optimisation necessities for nanoscale substances		
Aging condition	For nanoscale solids, the sample age and storage conditions must be known. Otherwise, the inertisation state of the substance must be determined analytically.	
Heating rate vs. Inertisation	The relationship between heating rate and the inertisation of the particle surface must be understood so that surface passivation that may take place during a low heating rate does not lead to unexpected delayed ignition. If necessary, different heating rates must be compared.	
Residual moisture	For nanoscale solids, the residual moisture must be determined and - if necessary - reduced. However, uncontrolled surface inertisation during drying must be avoided.	
Powder losses	Test procedures should be developed that allow avoiding powder losses.	

It should be pointed out that the oxidation resistance and flammability of carbon-based nanoparticles will show a very large dependence of their morphology and presence of impurities such as amorphous coatings, catalyst residues and functional groups. Both can even vary from batch to batch and depending on sample age and storing conditions. Adsorbed water can also lead to a significantly modified or delayed reactivity and flammability. Prior to testing, it has to be removed by suitable drying methods [9], [119]. Therefore, the substance identity including its nanoscale morphology must be known for any risk assessment of a material. Membership to a specific class of nanomaterials alone will generally not be sufficient for safety assessment of the substance by extrapolation of earlier test results of other class members to the new substance.

4.2.12 A.17 Oxidising properties (solids)

For an assessment of the oxidizing properties of test substances, a water-free test substance is mixed with oxidisable material in definite proportions and ignited with the flame of a gas burner. A substance is oxidising if it can lead to highly exothermic reactions in contact with other, particularly flammable substances. Oxidizing agents support combustion, but are not themselves flammable.

Mixtures of oxidizers and cellulose or sawdust must be regarded as potentially explosive and handled with great care. Due to their large specific surface area, catalytically active nanoparticles can be highly oxidizing. However, due to possible particle surface passivation, false negative conclusions may result if applied to nanoscale substances of unknown age and storing conditions. Generally, the reactivity of nano-objects can vary size-dependently, so that nanoscale substances of similar composition but different particle size distribution must be subjected to individual tests [53]. Also, possibly present adsorbed water has to be removed by suitable drying methods [9], [119]. For nanoscale substances, which show little propensity of sedimentation, a mixture with micro-scale cellulose or sawdust will facilitate the preparation of powder trains.

Tab. 4.11 Applicability of test procedures for oxidising properties to nanoscale substances

A.17 **Oxidising properties**	**Applicable**	**Justification for applicability** **or reasons for non-applicability**
Oxidising Properties	Yes, provided	the inertisation state and residual moisture of the substance are known.
Optimisation necessities for nanoscale substances		
Aging condition	For nanoscale solids, the sample age and storage conditions must be known. Otherwise, the inertisation state of the substance must be determined analytically.	
Residual moisture	For nanoscale solids, the residual moisture must be determined and - if necessary - reduced. However, uncontrolled initiation of surface inertisation processes must be avoided.	

The test procedure A.17 appears in principle applicable for nanoscale solids if the special requirements of nanoscale substances are considered appropriately, see Table 4.11.

4.2.13 New methods for flammability testing of nanoscale substances

A major criticism of established test procedures is that their execution usually assumes open handling of the test substance. This may lead to an exposure of the tester to nano-objects. For safe testing of the explosivity, flammability, ignitability and thermal stability, the EU project NANOSAFE2 [86], [122] therefore suggested new test methods for nanoscale substances that avoid disadvantages of conventional tests. The main focus was put on avoiding open contact with the test substance. The methods are briefly described in the following.

Hartmann tube

The preliminary assessment of the risk of electrostatic dust explosions is usually performed using a Hartmann tube in which the ignitability of a cloud of powder in air is tested by a continuous spark. The explosion is detected by means of a sensor that is mounted on a movable lid [123]. During this standard test, obviously part of the test substance can escape and contaminate the laboratory. The modified Hartmann tube test method for testing of nanoscale powders [121] uses a closed stainless-steel pipe instead of the glass tube. The substance sample is weighed under a fume hood into a capsule. The capsule is sealed and attached to the tube. A valve which connects the capsule is opened and the sample drops into the tube. By injecting a controlled amount of gas, a cloud is generated inside the tube. Ignition by a spark is detected via the resulting overpressure. After the test, the tube is cleaned by incineration of the residues in a propane explosion and subsequent water-rinsing with a circular nozzle.

Minimum ignition energy

Also for the standard test apparatus for determining the minimum ignition energy, MIKE-3, in which, like for the Hartmann tube, an open test vessel is used. It was found that the use a closed test vessel is possible [124]. This way, the minimum ignition energy and explosion strength of different carbon blacks, aluminium nanoparticles of different sizes and of CNT samples were studied by BOUILLARD et al. according to the standard procedures *ASTM* E1226 or *VDI* 3673(1995) [121].
It was found that

- CNT materials show explosion strengths in the order of magnitude of different carbon materials, nano-structured carbon blacks or wheat flour
- metal nanopowders may be protect by an oxide layer, which reduce their explosivity
- nanoscale powders, which show a tendency to agglomerate, show explosive strengths of a magnitude equal to those micro-scale powders of the same substance.

Sensitivity to impact

Mechanical impact on a solid can cause an explosion. The standard test procedures for impact sensitivity usually enable the tester to detect an explosion either acoustically or visually. In practice, the impact sensitivity of a solid is tested by means of a hammer. Samples of about 100 mg are packed into aluminium foil and are then hit by a dropping hammer. If during repeated testing always an explosion takes place, the experiment is repeated without the aluminium foil, as it may act as a sensitizer. Explosions are detected by a microphone or acoustic perception. A detonation is defined via a noise peak of 5 dB above the noise that is generated by an inert sample. During the test, the sample material and its reaction products can be distributed around the impact point and into the laboratory, so that the test has to be considered inapplicable to toxic (and unclassified) nanoscale substances. To test the impact sensitivity of nano-powders BOUILLARD et al. developed an improved, closed test apparatus which is equipped with pressure or temperature sensors and allows computerised data acquisition [121].

4.2.14 A.22 Length-weighted mean diameter of fibres

Important characteristics of nano-objects are their spatial dimension and aspect ratio. The test method A.22 for the study of mineral fibres, which determines the average fibre diameter by microscopic techniques such as scanning electron microscopy (SEM) and transmission electron microscopy (TEM), is also relevant for nano-objects [125]. It was developed for manufactured mineral fibres with diameters in the micrometer range but is in principle also applicable for nanoscale substances. The *OECD* Test Guideline TG 110 [126], which is recommended in the test description, mentions two measurement methods. While the first method is not applicable to nanoscale materials, the second could – with modifications such as the inclusion of fibres with a length less than 5 µm and a thickness smaller than 100 nm – be applied to nanoparticles as well as nanotubes and nanofibres [80]. Particles with average diameters less than 0.5 µm are explicitly mentioned and for the measurement transmission electron microscopy (TEM) is recommended. However, the test method does not describe the procedures for sample preparation and sample analysis of such small particles [11].

Since the measurement of length and diameter is both a tedious and time consuming process, the standard method measures the diameter of only those fibres that touch an imaginary line on a SEM or TEM field-of-view. Since the probability of selecting a particular fibre in this way is proportional to its length, a so-called length-weighted diameter is obtained. The length of the fibres themselves process is not determined.

For nanoscale substances, the test specification would need to be adapted with regard to nano-specific requirements. For dispersion, water is used standardly, which may be suitable for mineral fibres to obtain sufficiently stable dispersions. For nanoscale fibres of various possible compositions especially carbon nanofibres, water may not be an appropriate solvent but non-polar organic solvents or dispersing agents must be used to wet the large surface of nanoscale materials and obtain sufficiently stable dispersions. Samples taken from the dispersion are standardly filtered with polycarbonate filters. These are not suitable for many organic solvents. In addition, the specified pore size of 0.2 µm is not sufficient for retention of short fibre-like or not fibrous nano-objects. For a reliable detection of nano-objects, a sufficient spatial resolution of the SEM must be ensured. Nanofibres with diameters in the range of ten of nanometres would analysis by TEM. According to the present state of knowledge, no nano-specific problems are to be expected during the examination of such samples, since electron microscopy is frequently applied to nanomaterials [127]. However, a calibration of the length scale of an electron microscope to the nanometre requires a high degree of expertise and care.

The following guidelines and literature to determine the length-weighted average fibre diameter can be specified:

- *OECD*, Paris, 1981, Test Guideline 110, Particle Size Distribution/Fibre Length and Diameter Distributions"; Method A: Particle Size Distribution (effective hydrodynamic radius), Method B: Fibre Length and Diameter Distributions [126]
- Guidance Document on the Determination of Particle Size Distribution, Fibre Length and Diameter Distribution of Chemical Substances [128]
- G. BURDETT and G. REVELL. Development of a standard method to measure the length-weighted geometric mean fibre diameter: Results of the Second inter-laboratory exchange. IR/L/MF/94/07. Project R42.75 HPD. Health and Safety Executive, Research and Laboratory Services Division, 1994 [129].

Tab. 4.12 Applicability of test procedures for the length-weighted mean diameter to nanoscale substances.

A.22 **Length-weighted mean diameter of fibres**	**Applicable**	**Justification for applicability or reasons for non-applicability**
Length-weighted mean diameter of fibres	Yes, provided	the pore size of the filter is adapted and sufficient microscopy resolution for nanoscale substances is guaranteed. For nanofibres with diameters in the tens of nanometre range, TEM must be applied.
Optimisation necessities for nanoscale substances		
Length information	For nanoscale fibres also the fibre length should be determined.	

4.3 Applicability of the test procedures of Annex VII

The central question of this study was whether the test procedures specified by Annex VII of the Regulation in (EC) 440/2008 and (EC) 761/2009 are applicable to nanoscale substances. In the previous sections of this chapter, details of the test methods were compiled and evaluated with respect to experimental handling and implementation aspects of nanoscale materials. The sections each contain a summarizing paragraph and a table. The following issues were addressed:

- Are the prescribed test procedures applicable to nanoscale substances?
- What changes to the test procedures are necessary in order to apply them to nanoscale substances?
- Can the application of the tests to nano-objects lead to deviations of the measured material characteristics compared to conventional micro- or macroscopic material, from which information is derivable on the nanoscale nature of the tested substance?
- Are there modifications to the test possible that allow deriving more specific or more reliable information on the nanoscale nature of a substance?

Especially test procedures that determine physical properties, which tend to be modified by a large specific surface area of nano-objects, can be expected to measure deviating results. These can in some cases be used as an indication of a nanoscale test substance. More detailed information, e.g., on particle size will only be possible in exceptional cases, due to a still imperfect understanding of the underlying relationships between size and measurement result.

It was found that most test procedures are applicable to nanoscale substances with only minor restrictions in regard to some nano-specific requirements.

The following test procedures in principle allow deriving information on a nanoscale nature of the test substance

A.1. Melting / freezing point
A.3. Relative density
A.6. Water solubility.

This, however, is only possible in direct comparison with the conventional substance or data from the literature. Also, all ignition and explosion tests A.10 to A.17 are generally sensitive to a possibly increased reactivity of nanoscale substances. However, if - prior to the test - such an increased reactivity should initiate surface reactions on the nanoscale test substance during the period between production and testing, flammability and explosivity may change dynamically and complicate risk assessment and interpretation of indications for a nanoscale substance.

For the majority of tests specified for chemical safety assessment under the REACH regulation, the inertisation or oxidation state of a nanoscale material will be of decisive importance. Therefore, a detailed documentation of age and storage conditions of a nanoscale sample will be necessary to assess the validity and transferability of test results on chemical safety. Alternatively, in the absence of information on storage conditions and sample age, the inertisation state of a sample, i.e. its surface chemistry, has to be characterised.

For further details, please refer to Table 4.1 to 4.10 and the corresponding sections.

5 Relevant properties of nanoscale substances

Unlike bulk materials, inhomogeneous ensembles of nanoparticulate materials are in practice fully characterisable only in few special cases, see Section 3. This is partly due to the particle size and morphology dependence of their physico-chemical (surface) properties. For this reason, a selection of *relevant* substance characteristics has to be made. Such a selection can be motivated by a materials science, a nanotoxicological or a regulatory perspective. The materials science point-of-view aims at the most complete description of the physico-chemical state of the substance. For physico-chemical characterisation of toxicological relevance, additionally to basic physico-chemical data also interaction effects of the nanoscale substance in selected media are of relevance. These include the solubility behaviour, the radical activity or the redox potential. From the regulatory perspective, substance registration would require physico-chemical characteristics for substance identification, whereas risk assessment in a regulatory context would require toxicologically relevant material data.

The materials science approach to select relevant material properties, as is pursued e.g. by *NIST* or *BAM* for the characterisation of nanoscale reference materials, is also reflected in the *ISO's* definition of nanomaterials. *ISO* has taken a hierarchically structured approach to a most comprehensive description of the large variety of nanostructured materials [2]. While the certification of nanoscale reference materials requires a comprehensive physico-chemical characterisation, the assignment of a material to its appropriate *ISO* nanomaterial class would require corresponding physical-morphological structure data. The nanotoxicological perspective to select relevant physico-chemical characteristics is taken for example, by *OECD* and U.S. *EPA*, see also Table 5.1. However, the requirements for a sufficiently complete characterisation are still under discussion in the nanotoxicological literature.

5.1 Positions of international experts and organisations

Several international institutions have proposed sets of relevant physico-chemical characteristics of nanoscale materials and associated measurement methods, partly also of detailed procedures. In this section, the following sources will be evaluated with respect to such information: *NIST*, *NanoImpactNet, WPMN* of *OECD, EPA,* and *ISO*. In order to discuss the extent of agreement, the proposed relevant material characteristics of nanomaterials are compiled in Table 5.1. It turns out that the more material scientific aims of reference materials development of *NIST*, appear to require less material characteristics than nanotoxicologically motivated perspectives of the other institutions.

In the following, some exemplary positions in the nano-toxicological literature on the requirements for a comprehensive characterisation are briefly summarised. According to LANDSIEDEL et al. [130], a comprehensive characterisation of nano-objects should comprise in addition to the determination of the BET specific surface area [131], the size and shape of primary particles by TEM, the chemical composition, the degree of crystallinity and crystal modification, possible surface modifications and the iso-electric point in water. The materials should also be tested for contaminants. Primary particles in a suspension or a powder, are generally surface modified and/or agglomerated. Therefore, their dispersion state needs to be characterised. According to PARK et al. [132], a complete characterisation of nanoscale silver requires the particle size distribution, particle shape and other morphological properties together with the chemistry of the material, its solubility, specific surface area, degree of dispersion, surface chemistry, and other physico-chemical properties. SCHWEINBERGER et al. make recommendations for relevant characteristics of CNTs for nano-toxicological investigations [133]. Due to their high aspect ratio, CNTs require length and diameter distributions. Also the high relevance of possible surface contaminants is underlined. According to KREYLING et al., as mentioned in Section 2.1, another nano-specific parameter, the volume specific surface area, must supplement the previously mentioned material properties [16].

Tab. 5.1 Information considered relevant for nanoscale substances by international institutes and organisations.

Parameter	**Institution**
Size or particle size distribution	*NIST* [79], NanoImpactNet [30], *ISO* [134], *OECD* [76], *EPA* [135]
Aggregation, agglomeration, dispersibility	*NIST*, NanoImpactNet, *ISO*, *OECD*, *EPA*
Zeta potential (surface charge)	*NIST*, NanoImpactNet, *ISO*, *OECD*, *EPA*
Chemical composition, surface chemistry, elemental composition	*NIST*, NanoImpactNet, *ISO*, *OECD*, *EPA*
Solubility orwater solubility or dispersibility	NanoImpactNet, *ISO*, *OECD*, *EPA*
Specific surface area	NanoImpactNet, *ISO*, *OECD*, *EPA*
Shape or representative SEM image	*ISO*, *OECD*, *EPA*
Octanol-water partition coefficient	*OECD*, *EPA*
Optical absorption, electrolytic conductivity, pH value of suspension	*NIST*
Crystalline phase, crystallite size, dustiness, photo-catalytic activity, tap density, porosity, redox potential, radical formation potential, flammability, explosivity, incompatibility	*OECD*
Specific electrical conductivity, structure, melting point, boiling point, vapour pressure, reactivity, stability	*EPA*

5.2 Recommendations for nanoscale substance information requirements

While the previous section presented characteristics that are being discussed as relevant for nanomaterials by international experts and institutes, the following section proposes specific extensions to the information requirement for a hypothetical regulation of nanoscale substances.

It is of fundamental importance to take into account that nanoscale materials have - compared to conventional materials - a significantly larger number of characteristic material properties, cf. Section 3. In addition, these exhibit more or less broad property distributions, depending on the homogeneity of a sample. This leads to huge set of material data, as has been relevant so far for conventional materials only for polymers, i.e., molecular mass, copolymer units, end/side group functionality. Accordingly, the effort and cost of generating such sets of characteristic data will drastically increase for nanoscale substances. For this reason, a balance has to be found between necessary and reasonable expenditure. In most cases, for real-world nanoscale materials, a complete characterisation will not be possible. Therefore, the requirements need to be defined for a *sufficiently complete* record for the registration.

In contrast to the predominantly phenomenological physico-chemical substance information specified in Annex VII of the Regulation, the information specified in Annex VI relates to atomic/molecular and detailed spectroscopic material data. According to the complexity of nanoscale substances, the previously requested information will not be determinable for most nanoscale substances or does not have sufficient information content for substance identification. It is therefore proposed to define data requirements for nanoscale substances data that are *equivalent* to those specified in Annex VI of the Regulation. An unambiguous material identification of nanoscale substances would thus require additional data. For example, paragraph 2.2. of Annex VI of the Regulation specifies the requirement of a chemical formula. In case of *chemical* substances, this provides valuable information. For nanoscale substances, however, additional morphological information must be provided. Moreover, for nanoscale substances it seems necessary to establish conditions that allow identifying material and structural changes caused by post-synthesis storage. An example is the transformation of nanoscale metal particles into nanoscale metal oxide particles by storage under non-inert conditions. As a consequence, additional physico-chemical data of nanoscale substances have neither validity nor reliability without specifying a minimum set of relevant material features, especially aging conditions and particle size distribution. Therefore, the central question of this study related to additionally required physico-chemical data for nanoscale substances cannot be answered without specifying supplementary nano-specific information that serves to identify a substance.

The first part of Table 5.2 therefore proposes a comprehensive set of material characteristics for nanoscale substances. Due to the large amount of such nano-specific information, it appears to be desirable to reduce this maximum requirement for technical and economic reasons. A possible prioritisation of the information request is: *1* - important, *2* - desirable, *3* - optional information. A reduced priority would be justified by the present experimental effort rather than by the relevance of information. For a definition of the information requirements for regulation, feasibility, cost and reproducibility of the experimental methods were to be assessed in detail.

The proposed set of material data could achieve a reliable identification of nanoscale materials and lay the foundations of a future risk assessment. The expenditure for the provision of this record, however, will be very high for the present maturity level of characterisation techniques.

Tab. 5.2 Recommendations for nanoscale substance information requirements with prioritisation (1 – important, 2 – desirable, 3 – optional).

I. Additional information requirements for nanoscale substance characterisation		**Priority**
Batch identification and sample age	LOT and production date	1
	Storage conditions	1
Physical and morphological aspects	Primary particles: Shape	1
	Aspect ratio	1
	Porosity	2
	Agglomerates: Shape	1
	Pore size distribution	2
	Agglomeration or aggregation	2
	Crystallinity & crystalline phases	1&2
	Chirality	2
	Primary particle size distribution	1
	Length distribution	1
	Diameter distribution	1
	Agglomerate-/aggregate size distribution	1
Aspects of chemical structure	Binding state and chemical structure	1
	Type & size of ordered structure domains	1&3
	Crystallinity, crystalline phases	1,2
	Wall number, chirality	1,2
	Type & frequency of structure defects	1&3
	Surface chemical aspects	1
	Inertisation state	2
	Surface termination	2
	Covalent functionalisation	2
Aspects of composition and purity	Degyee of purity	1
	Synthesis related catalysts and adsorbates	1
	Inertisation state	2
	Age and storage conditions	2
	Storage-related structure and surface modifications	3
	Catalysts, adsorbates	1
	Additives and dispersants	1
	Type of storage-related modifications	1
	Content of relevant impurities	2
	Kinetics of storage-related modifications	2
	Dispersants	1
	Coating state	1
	Adhesion strength of coating	3

... Table 5.2 continuation.

II. Additional physico-chemical property data requirements for nanoscale substances		**Priority**
Physical and morphological aspects	Representative electron microscopy image Mass specific surface area Volume specific surface Fibre stiffness	1 1 1 2
Dispersion and agglomeration aspects	Dustiness Sedimentation propensity	1 2
	Dispersibility Re-agglomeration propensity	1 2
Adsorption and Reactivity aspects	Zeta potential Adsorption-desorption properties	1 2
	Redox potential Radical formation potential (Photo)-catalytic activity	2 2 2

The second part of Table 5.2 proposes additional physico-chemical property data requirements for nanoscale substances. Knowledge of these properties would provide important information for chemical safety assessment. The proposed prioritisation is again based on the information content and the effort for the determination of the characteristic. The *phenomenological* character of the properties in the second part of Table 5.2 becomes apparent, e.g., for the zeta potential and adsorption-desorption properties. They describe *system properties* that do not result from the nanoscale substance alone but *only* in combination with a definable test medium. The complexity of the interaction phenomena between the material surface and the test medium impede any understanding of the role of the microscopic material properties if only phenomenological test procedures are used. Also properties like radical formation potential, dispersibility, dustiness and sedimentation do not describe intrinsic material properties but properties that crucially dependent on the experimental conditions of the test procedure. For example, dustiness is no uniquely definable quantity, but depends on the applied agglomerate dispersion energy and the classification limits of particle size fractions as *dust*.

6 Methods for characterising relevant properties of nanoscale substances

First, a compilation is given of methods that are considered relevant by international committees and experts for the determination of relevant properties of nanoscale substances. Subsequently, methods are discussed for properties considered relevant by the present study.

6.1 Positions of international experts and organisations

Below, a compilation is given of the current state of method recommendations of the institutions *NIST, OECD, EPA* and *ISO* for the characterisation of relevant properties of nano-objects. References to the literature for the specified methods can be found in the acronym Table 1 in the Appendix of this study. It should be noted that the choice of a suitable method will crucially depend on the type of surface material and its preparation state.

The Australian experts in the "Committee of Experts" of the GHS suggest the following strategy for the choice of additional test methods for safety data sheets:

- Standard techniques for particles in general that may be relevant to nanomaterials
- Standards specifically applicable to nanomaterials that are currently under development
- Experimental techniques used by researchers.

The document „Information relating to Nanomaterials for inclusion on the guidance on the preparation of Safety Data Sheets“ [136] contains in its Annex a compilation of possible measurement techniques for additional physico-chemical parameters and a table of relevant standards.

Also the nano-toxicological literature provides information on essential methods. According to LANDSIEDEL et al. [130], the BET method, TEM, XRD, XPS and zeta potential determination with titration belong to the most important characterisation methods. KUMARA et al. describe XRD, TEM and SAXS as essential for characterizing the crystal structures, spatial distribution and dispersion of nanoparticles [137]. SUH at al. describe the application of analytical techniques such as XRD, NMR, SEM and TEM, as well as optical, fluorescence and confocal microscopy for the characterisation of nanoparticle structures [138]. SCHWEINBERGER et al. consider TEM/EDX, SEM/EDX, Raman, TGA, BET, XPS and UV/Vis/NIR spectroscopy as relevant methods for nano-toxicological studies of CNTs and emphasise methods that provide quantitative data [133].

Tab. 6.1 Methods for relevant properties as proposed by *NIST, OECD, EPA* and *ISO*.

Size or particle size distribution	***NIST***	***OECD***	***EPA***	***ISO***
Differential Mobility Analysis (DMA)	✓			✓
Transmission Electron Microscopy (TEM)	✓		✓	
Dynamic Light Scattering (DLS)	✓		✓	✓
Flow field-flow fractionation (A4F)	✓		✓	✓
Atomic Force Microscopy (AFM)	✓		✓	✓
Scanning Electron Microscopy (SEM)	✓		✓	✓
Small-Angle X-ray Scattering (SAXS)	✓			✓
Mass Spectrometry (MS)			✓	
Gel Permeation Chromatography (GPC)			✓	✓
Raman Spectroscopy (SWCNT diameter)				✓
MALDI-ToF-MS				✓
ESI-ToF-MS				✓
Separation techniques like Sedimentation-FFF (SDFFF)				✓
Aerosol-based Methods (SMPS)				✓
Standards, see Appendix 2				✓
OECD TG 110 (Particle size, fibre length and diameter distributions)		✓		

Aggregation, agglomeration, dispersibility	***NIST***	***OECD***	***EPA***	***ISO***
Neutron Small Angle Scattering (SANS)		✓		✓
A4F				✓
MALDI-ToF-MS				✓
Rheological methods				✓
Analysis of SEM- or TEM images				✓
ESI-ToF-MS				✓
Static Light Scattering (SLS)				✓
Optical Tweezers (OT)				✓
Angle-dependent scattering at different angles [139]				✓
X-Ray Diffraction (XRD)				✓
Standards, see Appendix 2				✓

Surface charge and zeta potential	***NIST***	***OECD***	***EPA***	***ISO***
Dynamic Light Scattering (DLS)	✓			
Static Light Scattering (SLS)		✓		
Electro osmosis				✓
Iso-Electric Point (IEP)				✓
Colloidal oscillations current				✓
ElectroPhoretic Mobility (EPM)		✓		✓
Electrophoretic Light Scattering [140]				✓
Electric sound amplitude [141]				✓
Acoustic Methods *ISO* 20998-1:2006 [142].				✓

Solubility und dispersibility	***NIST***	***OECD***	***EPA***	***ISO***
Water solubility: *OECD* TG 105 [143]		✓		
Fatty acid solubility: *OECD* TG 116 [144]		✓		
Solubility: There are no nano-specific *ISO* standards				✓
Dispersibility: Possible future standards may be derived from *ISO* TC 24/SC 4 [145] and TR 13097 [146]				✓

Specific surface area	***NIST***	***OECD***	***EPA***	***ISO***
Liquid intrusion porosimetry [147]				✓
B922-02 Standard test for metal powders: *CEN EN ISO* 18757:2003 [148]		✓		
Image analysis and geometric estimation [149]				✓
Gas adsorption isotherms (BET)				✓
Standards, see Appendix 2				

Shape, form factor or representative EM image	***NIST***	***OECD***	***EPA***	***ISO***
Electron microscopy: (TEM and SEM)		✓	✓	✓
Atomic Force Microscopy (AFM)				✓
UV/Vis imaging microscopy [150]				✓
Small-Angle X-ray Scattering (SAXS)				✓
Standards, see Appendix 2				✓

***n*-Octanol-water partition coefficient**	***NIST***	***OECD***	***EPA***	***ISO***
OECD TG 107 – Shake-flask method [151]		✓		
OECD TG 117 – HPLC Method [152]		✓		
OECD TG 123 – Slow-stirring method [153]		✓		

6.2 Availability and application aspects of relevant methods

The information content, application relevance and availability is discussed for the characterisation methods proposed in Table 5.2.

6.2.1 Physical and morphological aspects

For the **representative electron microscopy images**, SEM or TEM will be required, depending on the resolution necessary. Artefacts of sampling and preparation may affect the result, as noted in Section 3.2. For example, not successfully dispersed particles may agglomerate and sediment and may therefore not be sampled. Or, if particles re-agglomeration during preparation, they cannot be measured by TEM due to excessive thickness. Especially, difficult to individualize nano-objects require high dispersion energies and tend to re-agglomerate easily. Appropriate preparation steps are therefore to be developed substance-dependently.

Such characteristic electron microscopic *single particle analysis* (IPA), alternatively by AFM and confocal optical microscopy (COM), allow determining the ***primary particle and agglomerate shape***. Whether a particle assemblage is ***agglomerated or aggregated***, may not always be clarified easily, since no sharp distinction criteria and procedures exist. In addition, many nanoscale substances are mixtures of both types. While an agglomerate is considered as a collection of weakly interconnected particles, aggregates consist of strongly interacting particles. Detailed knowledge on the synthesis process can help to clarify the situation if it is evident that sintering processes or formation of chemical bonds occurred in the course of the synthesis. Electron microscopic analysis may detect sintered structural features through which the surface of an aggregate can become significantly smaller than the sum of the geometric surfaces of its primary particles, which might be detectable by BET measurements. However, agglomerates of flexible nanotubes may have reached such a high degree of entanglement that they can be separated only by very high energy via fibre fractures. Thus, they may exhibit characteristics specific of aggregates.

Information on ***particle- and agglomerate pore-size distribution*** can be obtained with gas (BJH) and mercury porosimetry or USAXS. For compressible materials, but also depending wettability, there may be deviations between the results of the different methods, cf. Section 4.2.4. The order of pore size magnitude should, if possible, verified with a single particle methods like TEM and SEM. TEM-based tomography is a promising method for such a single particle pore size analysis.

State-of-the-art microscopic single-particle methods limit the statistical reliability of ***primary particle and agglomerate size distributions*** by a restricted sample size. However, such microscopic methods are essential for assessing the applicability of ensemble-based methods, that average over larger samples. Whenever ensemble-averaging analysis methods are used for determining a particle size distribution, it has to be verified whether the assumptions of the method are satisfied in order to avoid misinterpretation. For this, a successful individualisation and sufficiently stable suspensions have to be assured. For methods that derive the particle diameter from the assumption of a quasi-spherical particle, the validity of this assumption has to be tested. Moreover, these methods determine particle diameters that may differ systematically due to the underlying measurement principle: aerodynamic (DMA, SMPS) or hydrodynamic (DLS, A4F, centrifugation) diameters, or the particle mass (mass spectrometry and NTA).

The determination ***of length and diameter distributions*** of fibrous nano-objects with high ***aspect ratios*** inevitably requires microscopic methods as are specified in the test method "length weighted average fibre diameter" A.22. A main difficulty in the determination of length distributions is an excessive entanglement of the fibres, so that individual fibres can not be detected and measured in a microscope. Highly entangled or agglomerated nano-fibre ensembles are therefore to be thinned out and dispersed. However, to quantify the fibre-shortening effect of the applied de-agglomeration energy will be very difficult. Furthermore, for very thin and very long nanofibres, limitations of the size of the microscope’s field of view is a practical problem, as several high-magnification images must be stitched to an overview image. The adjustment of the position of the focal plane can be time-consuming. A simplification is to be expected of successful approaches for automatic image analysis [154], [155].

Diameter distributions of nanofibres are directly determinable only with microscopic techniques (TEM, SEM, AFM, COM). For single and double-walled CNTs, diameters are indirectly determinable with Raman or photoluminescence-spectroscopy. Since the quotient of independently derived length and diameter distributions will result in very broad ***aspect ratio*** distributions, length and diameter of individual tubes are to be determined pair-wise. Primary particle or agglomerate ensembles of not too high aspect ratio are characterisable with SAXS as EAA method. For defective or specially structured nanofibres, for example with conical or so-called bamboo structure, fibre diameter variations *along* the fibre are possible, that would require specifying additional material parameters.

In the case of nanoscale fibrous materials, further differentiation appears necessary with respect to their mechanical properties, which also depend on the fibre diameter. In contrast to microscale fibres, nanoscale fibres can be highly flexible. In this case, radii of curvature of a few nanometres are possible. They can entangle to dense agglomerates that may lose their fibrous character. It is expected that highly flexible nanofibres may show different toxicological effects than long, rigid fibres. For sufficiently long and rigid carbon nanofibres, asbestos-like inflammation of tissues were observed that were not found for thin, flexible carbon nanotubes [156].

Therefore, the test method A.22, which determines a weighted average fibre diameter but not a length distribution, is not sufficient for an assessment of fibre-related risks of nanoscale materials. Length, diameter and wall number distributions should be combined with information on material properties (chemical composition and structure, and reference materials), to estimate the ***mechanical stiffness of nanofibres***. Therefore, in addition to diameter and length, bending radii of fibres should be evaluated statistically on SEM, TEM or AFM images, since small bending radii indicate flexibility. A direct measurement of fibre flexibility (Young's modulus) is possible, for example, with AFM-based force-distance or resonance frequency measurements of nanofibres on nuclear track membranes [157]. It is complicated but could serve to provide reference material data. The authors of the cited work found for polypyrrole nanotubes that their elasticity strongly increases for diameters below 50 nm.

As explained in Section 2.1, the ***volume specific surface area*** (VSSA) is discussed as a relatively easy to perform method for the identification of significant nanoscale fractions in a material sample [16]. As product of the ***mass-specific BET surface*** and ***material density*** A.3, it is easily accessible for nanomaterials. Nano-specific aspects of test procedure A.3 are discussed in Chapter 4. In approximation, the volume specific surface area of a substance can also be estimated microscopically via its shape and particle size distribution, by calculating by the ratio of geometric surface area and volume. This approach, however, requires that the substance exhibits no significant porosity, which could contribute to surface portions and are difficult to estimate geometrically.

6.2.2 Aspects of chemical structure

The elucidation of the chemical structure of a substance requires the use of analytical methods for determining the chemical composition and bonding. Knowledge of details of the synthesis process may be important in this context. In the case of nanoscale materials of large surface to volume ratio, the chemical structure of the volume and the surface must be differentiated, see the following section. The information depth of the analysis methods used have to be considered accordingly. Information on the chemical bonding properties of a substance also includes details of crystallinity, crystalline phases, the size of ordered domains, and the type and frequency of structural defects. They are not only responsible for the reactivity, but also for the morphological structure of nanoscale materials. Important ***chemical structure information*** about the composition of the particle bulk is obtainable with spectroscopic techniques such as NMR, FTIR, UV/Vis. Also EDX analysis, TEM or SEM based, allow determining element concentrations of heavy atoms with an analysis depth in the micrometer range. They therefore permit only for very thin samples or thin sections both high vertical *and* lateral spatial resolution. Analytical TEM provides access to a variety of structural information. Sufficiently aberration-corrected TEMs are capable of imaging atomic positions and contrasts, so that the atomic ordering and defect structures become visualisable. TEM/EELS can provide information on the chemical bonding, so that, e.g., graphitic, diamond-like and amorphous carbon structures are characterisable in detail. Transmission electron diffraction (TED) opens the possibility to generate ***crystallinity information***.

As a particle ensemble method without spatial resolution, also XRD can serve to study ***crystalline phase, structural order and domain size***. The interpretation of the diffraction spectra, however, can be complicated by the fact that nanoscale substances - due to increased surface energy - may develop different preferential crystal faces than conventional solids.

In addition to AFM for single-walled carbon nanotubes, Raman and photoluminescence spectroscopy for single and double-walled CNT, TED is becoming an important technique for ***chirality determination*** of multi-walled CNT [158]. Chirality indices of MWCNT with up to 11 walls can be determined by TED, being in the wall number range of commercial MWCNT. TEM also is the method of choice for determining the number of walls of nanotubes. For statements about a few walls, Raman and photoluminescence spectroscopy are suitable. The problem of heating and/or damage caused by the energy of a Raman excitation laser was discussed at a workshop at *NIST* [159]. For information on individual CNT, issues of fibre individualisation are the focus of practical problems to be solved.

6.2.3 Surface-chemical aspect

In contrast to conventional materials, due to the high surface-to-volume-ratio of nanoscale objects it is essential for substance identification to characterise the ***chemical*** and physical ***properties of the particle surface***. ***Chemical termination, inertisation and functionalisation state*** determine the reactivity in a decisive manner. However, the elucidation of the chemical surface properties of nanoscale materials is one of the most challenging analytical problems. A variety of chemical analysis methods, including XPS, AES, SIMS, TEM/EDX, SEM/EDX, FTIR, Raman, are considered surface-sensitive. However, this classification applies mostly for the micrometric length scale of conventional materials. For the analysis of nanoscale materials, their information depth is still too large. For AES and XPS it is only of a few nanometres, but already in the magnitude of the nanoscale substance to be characterised. Therefore, the analysis result may be dominated by the composition of the particle bulk. A purely surface-sensitive method is atomic force microscopy with chemically functionalised tips (AFM/FDC). It investigates chemical interactions between an AFM tip and the surface via force-distance measurements.

Also, analyses of particle boundaries with TEM/EDX or TEM/EELS with an appropriate choice of the focal plane are in principle able to achieve surface-chemical analysis on the nanoscale. However, they lack, as well as many other methods such as XPS and UV/Vis spectroscopy, the ability to differentiate ***chemical functionalities***. XPS, which in addition of the element composition can also provide information about the chemical bonding, for example, the oxidation state and can be used for a quantitative determination of chemical surface groups, especially after chemical derivatisation. The latter labels reactive surface groups such as amino, hydroxyl, carboxyl, etc. with marker atoms. Also for fluorescence spectroscopic detection, fluorescent dyes are frequently coupled derivatively. However, such coupling reactions raise questions of conversion efficiency and cross sensitivity, which in many cases have not been satisfactorily answered. They may be affected also by gas-phase or solvent-dependent swelling processes of the nanoscale material or its surface, which are able to influence the labelling and characterisation depth. Such swelling effects are also relevant for analysis techniques like potentiometric titration, dynamic vapour sorption (DVS) or inverse gas chromatography (IGC), which can quantify acidic or basic surface groups, surface energies and/or polarities. Such a quantification of acidic or basic surface groups by titration is, however, critically impeded in presence of physisorbed fragments due to dynamic adsorption-desorption processes [160]. Thermally induced decomposition and subsequent desorption of fragments is used for coupled thermo-gravimetric methods such as TG/MS and TG/IR for the detection and quantitative estimation of surface groups. Also ion bombardment can generate fragments of surface groups that may allow chemical surface characterisation by SIMS although the resulting fragment spectra are often difficult to interpret.

Due to the complexity of the problem described here, no universal analytical strategy for an elucidation of the surface chemistry can be specified. The knowledge of the manufacturer on the nature and objective of the synthesis of functionalised nanoscale materials should be used whenever possible.

6.2.4 Aspects of composition and purity

For nanoscale substances, the terms *composition* and *purity* comprise more aspects than for conventional materials. Apart from a purely chemical ***composition and purity***, as can be determined with chemical elemental analysis (AAS, ICP-MS/OES, CHNS analysis, XFA etc.), also the nature and structure of nano-specific impurities are to be characterised. A prominent example is contamination of CNTs by nanoscale catalyst particles during synthesis. Not only their concentration but also the type of embedding or encapsulation into or through the nanoscale material can affect the toxicological relevance of the impurities. For such a characterisation, nano-analytical techniques such as STEM/EDX are particularly suitable. ***Adsorbates and dispersants*** can be detectable by titration, extraction and chromatography or thermal desorption processes, but also by high-resolution microscopic methods. Quantification by TGA, if applicable, may be relatively easy to implement.

In contrast to conventional materials, the chemical and structural surface state of nanoscale materials can change according to storage conditions. Since they lead to a (partial) conversion of the initially produced substance, such changes can be considered as sources of nano-specific contaminants which need to be investigated with respect to their ***dependence on the sample age***. An important characteristic in this context is the ***inertisation state*** of a nanoscale substance. For its investigation, TGA or DSC, see test A.16, may be relevant in presence of oxygen. Freshly prepared metallic nanoparticles, for example, tend to be drastically less inert than surface-oxidised ones after storage, which shows the relevance of this substance property for safety assessments.

Especially for surface-coated nano-objects, issues of adhesion, reactivity and solubility of the coating are of great importance. Unfortunately, no methods are established that allow assessing adhesion strength of coatings on nanoparticles. Information may be obtainable from the rheology of polymer nanocomposites [161]. Therefore, synthesis specific information of the manufacturer will be necessary.

6.2.5 Dispersion and agglomeration aspects

Toxicologically relevant aspects of nanoscale substances of their individualisability and release are closely related to the stability or instability of their primary particles and agglomerates, i.e. the preservation or loss of the nano-scale nature of a substance. Such dispersion and agglomeration phenomena usually show a complex dependence on many material and system properties. They are generally not traceable to single physico-chemical properties of the particles. Of central importance are especially type, strength and range of the interaction among particles and between particles and the surrounding medium. Depending on the material composition and surface functionalisation, interaction forces between particles can be quite different: dispersive, polar, ionic, covalent and combinations thereof. Together with the number of the interacting contact points and the area of the interacting particles surface, they determine the magnitude of the interaction energy and thus the tendency to agglomeration or de-agglomeration. In addition, also mechanical properties of a substance (chemical structure, domain size, etc.), the nature of inter-particle bonds of an agglomerate and geometric form factors (entanglement, cross-linking degree, leverage) influence the stability of an agglomerate.

Methods for determination of the ***dispersibility*** generally base on agglomerate size measurements after application of a defined dispersion energy. For this, depending on substance, suitable particle measuring techniques are to be used. Only in case of successful individualisation, relatively narrow particle size distributions are obtained for uniform materials. DLS is therefore not necessarily the method of choice because it is not suited for *multimodal* distributions. In the case of air-dispersed particles, aerosol measurement technology (SMPS) is used. For an estimation of the dispersibility, the de-agglomeration is monitored via a decrease in agglomerate size or increase of the fine fraction. This can be done *in situ* with EAA or SSP-measuring methods, made such as NTA, or *ex situ* with IPA-microscopic method, see standards in Table 1 to T25. This way - depending on substance, test medium and dispersion technique - information is obtained on a *system*-specific, not *substance*-specific ***de-agglomeration propensity***. There are is variety of different dispersion techniques to be chosen substance-specifically for gas or liquid phase. Also quasi spontaneous de-agglomeration was observed for special material-medium combinations or very dusty nanoparticles [155]. For further developments, please refer to TR 13097 of *ISO* TC 24/SC 4.

An assessment of the ***re-agglomeration propensity*** can be achieved via the stability of a previously generated dispersion by temporal monitoring of the agglomerate size distribution. This likewise will require appropriate particle size measurement techniques.

Dispersibility and re-agglomeration propensity, but also substance density and particle size distribution are crucial factors for the ***dustiness*** of a material. By classification of air-dispersed particles by their size as inhalable (<30 µm, suspended particulate matter), thoracic (<10 µm) and respirable fractions (<2.5 µm), the dustiness of a substance is assessed after quantification of the released fractions under defined dispersion conditions. Very different methods have been developed for dustiness testing [162]: rotating drums [163], powder dropping experiments with counter-gas flow (*EN* 15051:2006), shakers [164]. They are coupled with corresponding aerosol measurement technology. *EN* 15051:2006 is a standard recommended for nanomaterials.

For an assessment of the ***sedimentation propensity*** in gases or liquids, also particle size measurement techniques such as DLS or SMPS may be applicable, but also easy-to-use light scattering techniques. The sedimentation can be accelerated by centrifugation in not too viscous media. To determine the dispersion stability or sedimentation propensity in liquid dispersions, for example, a so-called Lumifuge® can be used, which allows to monitor the sedimentation in a cell during centrifugation using light scattering technique.[5]

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[5] http://www.lum-gmbh.com/produktinformation-91.html

6.2.6 Adsorption and desorption aspects

In addition to a solubility of the nanoscale substance itself, which could lead to a release of ions or low molecular weight components, there is the possibility of a significant ***adsorption-desorption activity*** of the large surface area of nanoscale materials. Their adsorption potential is dependent on the chemical functionalisation and termination of the material's surface, but possibly also by the local curvature and total surface area. This way, nanoscale substances in biological systems might act as transporter of toxic adsorbates or as trap for nutrients. Also immobilisation of proteins or nucleic acids could have toxic effects. Especially in biological systems, it remains to be clarified what influence dynamic adsorption-desorption processes of biomolecules can have on the solubility of a particle if they lead to a coating. It appears therefore necessary to evaluate the ***adsorption capacity*** of a nanoscale material. This would correspond to the inverse of the octanol-water partition coefficient determination of conventional materials, because the adsorption capacity of a nanoscale material for polar, non-polar, or other substances must be determined. It could, for example, be quantifiable by the weight gain or increase in diameter due to adsorbate layer formation. Also, the adsorption of UV/Vis absorbing substances can be quantified spectrometrically. Other approaches could apply a surface energy or surface polarity determination, as is possible with IGC, DMS or powder tensiometry.

Also, a ***zeta potential*** measurement is a variant of surface charge and polarity determination. It is used for stability analysis of dispersions. It allows, in principle, considerations on the immobilisation tendencies of particle surfaces for ions and polar substances such as biomolecules. The idea of zeta potential is based on the fact that the electric charge of a particle in suspension is compensated by ions of the surrounding solution. Counter-ions are attracted by the particle charge layer and form a charged layer, which in turn attracts counter-counter-ions, etc. In this way, the particle is surrounded several layers of oppositely charged, partially only loosely bound ions that shield the particle charge. Such an ion cloud increases the hydrodynamic radius of the particle. For a particle that moves in a suspension, the less tightly bound ions of the outer layers are sheared by fluid friction. Thus, the shielding effect of the ion cloud is reduced, so a surface-charged particle can be subjected - in spite of the shielding ions – to the force of an electric field. The resulting particle speed is a function of field strength and a measure of the zeta potential of the particle, i.e. the total carried surface charge. The zeta potential, however, depends on the viscosity, temperature, pH, ionic strength of the solution and the ionic charge, so many experimental parameters affect the reproducibility of a measurement. Most importantly, the nanoparticle suspension needs to have sufficient stability. In practice, the zeta potential is determinable by means of electrophoretic dynamic light scattering (DLS) or ultrasound (spectroscopy USS). In the case of multimodal solutions, misinterpretations are possible. For DLS, not all size fractions scatter the incident laser light equally. With increasing number of particle size fraction, the information contained in the laser scattering signal cannot be clearly attributed to the hydrodynamic radii involved in the scattering process. Consequently, not only DLS but also zeta potential measurements lose reliability for multimodal samples.

6.2.7 Reactivity aspects

The origins of an increased ***radical, catalytic or photo-catalytic activity*** of (partly) crystalline substances, although understood in principle, strongly depend on the structure of crystalline domains of a nanoparticle. Nanoparticles can show very high catalytic efficiency and selectivity, as structure, size and orientation of crystalline domains, as well as the surface curvature can be optimised in many ways [165]. Also with respect to the ***redox potential*** of a nanoscale sample, substance-specific quantification of is needed. For nanoparticles, it is not solely determined by the chemical composition, but there are particle size and surface termination dependent reactivity differences to be expected. In addition, nanoparticles can be photo-catalytically active and show high surface redox activity in case of charge separation of photon-generated electron-hole pairs (excitons) [166]. In aqueous media, such photo-catalytic particles can form reactive oxygen species (ROS), radicals and hydrogen.

In practice, effect predictions are difficult due to broad property distributions of technical nanomaterials. Therefore, catalytic, free radical and redox activity effects must be measured material and system specific. For REACH, such chemical and physical properties should preferably be determined by physico-chemical test methods and not cell assays, as is customary, for example, to quantify the ROS activity of a nanomaterial [167]. Such physico-chemical tests for the photo-catalytic or photo-redox activity of a substance, for example spectrometrically study the degradation of a dye such as Rhodamine B under defined (UV) illumination conditions. An accurate control of pH is important, therefore, is usually worked in highly de-ionized water or high-purity organic solvents of HPLC grade. Spectrometric methods appear well suited for standard test procedures. Alternatively, the photo-catalytic production of hydrogen can be quantified. A summary of such photo-catalytic reactions is given in reference [168]. By means of EPR also a direct physical quantification of photo-induced free radicals, i.e. unpaired electrons/holes can be achieved [169]. EPR can also be used for the detection of free radicals at the boundary of crystalline domains [170]. Such radicals are produced (or uncovered), for example by breaking of sintered aggregates and can then develop potentially toxic effects. The radical concentration would need to be investigated in dispersing EPR experiments. Alternatively, analytes such as 1,1-diphenyl-2-picrylhydrazyl (DPPH), which change colour upon recombination with a radical, may serve for a quantification of surfaces accessible radicals.

6.3 Conclusions

In comparison to conventional materials, nanostructured substances exhibit a significantly higher level of material complexity. For an intended substance identification, classification and safety assessment, this induces drastically increased information requirements. The present study therefore concludes that for an inclusion of nanoscale substances into the REACH process, additional requirements on characterisation data as well as physico-chemical data need to be specified. In Table 5.2, nano-specific substance properties are identified and prioritised. The material data of the first part of the table is most relevant for substance identification. Especially the particle size distribution, crystallinity, catalytically active impurities and the inertisation state of a nanoscale substance are also of crucial importance for the applicability of REACH standard tests, as the discussion in Chapter 4 shows. Supplementary physico-chemical property data are proposed in the second part of Table 5.2. The availability of test standards for these additional data is summarised in Table 6.2.

It becomes evident that the experimental effort to determine nanoscale material data relevant for substance identification is very high and can even exceed that of physico-chemical testing. If a substance to be registered was intentionally synthesized with nanoscale size, most of the identity data will in general have been determined in-house by the manufacturer for the synthesis optimisation and quality control. Should this not be the case, the large variety of substance information will need to be reduced to a practically feasible minimum data set according to the state of the art.

Tab. 6.2 Methods and relevant standards for physico-chemical properties of nanoscale substances proposed in Table 5.2 with prioritisation (1 – important, 2 – desirable, 3 – optional) .

Characteristic property	**Priority**	**Characterisation method**	**Standard or TG** **cf. Tab. 1-T25**
Morphology			
Representative EM image	1	SEM, TEM	*ISO*/DTS 10797
Shape	1	SEM, TEM, AFM, COM	*ISO*/DTS 10797, *ISO*/DTS 10798, *ISO*/DTR 10929, *ASTM* WK28561
Aspect ratio	1	SAXS, SEM, TEM, AFM, COM	*ISO*/DTS 10797
Porosity and pore size distribution	2,2	BJH, Hg-Porosimetry, USAXS, TEM, SEM	*ISO* 15901:2005
Primary particle size			
Primary particle size distribution	1	TEM, SEM, AFM, SMPS, DMA, DLS, NTA, A4F, MS, Centrifugation, SAXS	*OECD* TG 110, *ISO* 9277:2010, *ISO* 13320:2009, *ISO* 13322:2004, *ISO* 21501:2007 *ISO* 22412:2008, etc.
Length distribution	1	TEM, SEM, AFM, SPMS	*ISO*/TR 27628:2007
Diameter distribution	1		
Volume specific surface area	1	Product of material density and mass specific surface area, alternatively determined geometrically from morphology and size	
Agglomerates and agglomeration			
Agglomeration or aggregation	2	BET, SEM, TEM	*ISO*/DTS 10797
Agglomerate-/aggregate size distribution	1	TEM, SEM, AFM, SPMS	*ISO*/TR 27628:2007
Dispersibility	1	Dispersion with determination of particle size distribution (PSD)	*ISO*/TR 13097, *ASTM* B859-03
Re-agglomeration propensity	2	Dispersion with temporal observation of PSD	
Fibre persistence and entanglement			
Fibre stiffness	2	AFM, TEM, SEM	

Emission aspects			
Dustiness	1	Air dispersion (powder dropping, rotating drum, shaker) with determination of PSD	*EN* 15051:2006
Sedimentation propensity	2	Dispersion with/without centrifugation, temporal observation of PSD	

Characteristic property	**Pri-ority**	**Characterisation method**	**Standard or TG** **cf. Tab. 1-T25**
Chemical structure			
Binding state and chemical structure	1	NMR, FTIR, UV/Vis, Raman, XPS, AES, SEM/EDX, STEM/EDX, STEM/EELS	*ISO*/DTS 10797, *ISO*/DTS 10868, *ISO* 22309:2006, *ISO* 22489:2006
Type & size of ordered structure domains	1&3	XRD, SEM/EDX, STEM/EDX, STEM/EELS	*ISO*/DTS 10797, JIS R 7651:2007
Type & frequency of structure defects	1&3		
Crystallinity, crystalline phases	1,2		
Wall number, chirality	1,2	TEM, STEM/TED, Raman, PLS	*ISO*/DTS 10797, *ISO*/PRF TS 10867
Surface chemistry and reactivity			
Mass specific surface area	1	BET	*ISO* 9277:2010, *ISO* 13318:2001
Zeta potential	1	Electrophoretic DLS or USS with pH variation	*ISO*/CD 13099, *ASTM* WK21915, JIS R 1638:2000
Adsorption-desorption properties	2	Tensiometry, mass increase, UV/Vis, DVS	*OECD* TG106, *OECD* TG121
Redox potential	2	Dye decomposition	
Radical formation potential	2	EPR, DPPH	
(Photo)-catalytic activity	2	Dye decomposition, H_2 production, EPR	
Inertisation state	2	TGA, TG/MS, TG/FTIR, FTIR, UV/Vis, Raman, XPS, AES, SIMS, DSC, Titration, AFM/FDC, DVS, IGC	*ISO*/PRF 10810, *ISO*/WD TR 14187, *ISO*/TR 19319:2003, *ISO* 20341:2003
Surface termination	2		
Covalent functionalisation	2		
Substance composition and purity			
Chemical composition & purity	1&1	AAS, ICP-MS/OES, CHNS, XFA, SEM/EDX, TEM/EDX	*ISO* 22309:2006, *ISO* 22489:2006
Synthesis related catalysts and adsorbates	1	TEM, STEM/EDX, STEM/EELS	*ISO*/TR 27628:2007
Additives and dispersants	1	Titration, extraction with chromatography, TGA	
Age and storage conditions	2	Type & modification of structure, surface chemistry and chemical composition	
Type of storage-related modifications	1		
Content of relevant impurities	2	AAS, ICP-MS/OES, CHNS, XFA, SEM/EDX, TEM/EDX	*ISO* 22309:2006, *ISO* 22489:2006
Kinetics of storage-related modifications	2	Temporal dependence of storage-related modifications	
Coating state	1	SAXS, TEM	
Adhesion strength of coating	3	Synthesis know-how	

The standards for nanomaterials developed so far (Table 1) provide hardly relevant procedure guidance for substance identification based on the characteristics of Table 5.2. The standard drafts in consultation phase in Table 2 comprise relevant topics for substance identification: particle shape (CNT), primary particle size, aggregation or agglomeration state, chemical composition (CNT), and surface chemistry. However, their emphasis is put on the development of standards for the characterisation of CNT. For other nanoscale substances, the standards listed in Appendix 2, Table 3 are recommended. They include test methods for particle shape, primary particle size, particle size distributions, hydro- and aerodynamic diameter, aggregation or agglomeration state, specific surface, chemical composition, surface chemistry. However, these standards do not in any case meet the previously described nano-specific requirements. This becomes evident for standards for *surface* chemistry characterisation for the question of analysis depth, which was discussed before. Therefore, there is significant need for development of adapted test guidelines and standards for nanoscale substances.

Among the standards developed or in consultation phase explicitly for nanoscale matter in Table 1 and 2, there are none with direct applicability to the physico-chemical properties proposed in Table 6.2. However, the standard for a determination of workplace atmospheres according to *ISO*/TR 27628:2007 comprises particle measurement methods that would be required for a determination of their sedimentation behaviour in air. The standards recommended for nanoscale substance application in Appendix 2, Table 3 include test procedures for zeta potential and dustiness. For all other additional properties proposed in Table 5.2, test procedure need to be specified.

The required substance identity verification and physico-chemical property characterisation for substance registration and regulation will critically depend on the quality and level of detail of the test procedures to be specified. The current state of standardisation and technical guideline development does not meet the requirements of a REACH registration and regulation process for nanoscale substances. The legislator therefore needs to initiate test procedure specification. Otherwise, the nano-specific information requirements of REACH have to be satisfied by data generated by in-house methods of the manufacturer.

Tab. 6.3 Comparison of substance data of macro-/micro- and nanoscale zinc.

Characteristic property	**Zinc**	**Nanoscale zinc**	**Method, Ref. and remark**
Melting temperature	692,7 K	633 K (10 nm)	XRD [40], zinc oxide, which may form as oxide layer, only melts under pressure
Boiling point	1180 K		
Relative density	7,14 g/cm³	reduced in case of surface oxidation	Surface oxidation is probable
Water solubility	pH dependent		
Flammability Min. ignition energy	(15 µm) [6] > 300 mJ	(130 nm) 3 – 10 mJ	[61]
Explosivity Max. explosion pressure Equivalent K_{st} Explosion class	(15 µm) [6] 5,5 bar 150 bar m/s St1	(130 nm) 5,6 bar 101 bar m/s St1	Different Tests in 2 l sphere [61]
Self-ignition temperature	255 °C (15 µm) [6]		

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[6] http://www.zincchemicals.umicore.com/ZincMetalPigment/ZMPhse/ZMPhse_explosiChar.html.

7 Summary

Nanotechnology is considered to be one of the key technologies of the future. About 800 companies in Germany have already been working with nanomaterials [171]. However, in recent years, nanotechnology has increasingly come into focus of a critical public discussion [172]. Major concerns regarding nanomaterials are potential human or ecotoxicological effects. When evaluating these and other possible risk potentials of nanomaterials, such as an altered flammability or explosiveness, progress in the characterisation and testing of safety-relevant material properties will be of crucial importance for the social acceptance of this class of materials [81].

In light of the precautionary principle, the increasing use of nanomaterials of partially unknown risk potential, it is becoming increasingly urgent to clarify possibilities for their regulation, for example by the European chemicals law. The present study therefore examined the information requirements defined by the Regulation (EC) 1907/2006 (REACH) and their according test procedures specified in Regulation (EC) 440/2008 with respect to their applicability to nanoscale substances.

Since only a substance that is uniquely identifiable can be registered and regulated appropriately, it became apparent that the information requirements, which were originally defined for conventional substances, would need to be extended considerably, especially for nanoscale substance identification. Therefore, the present study proposed additional data requirements to form datasets of sufficient discriminatory power for the existing spectrum of nanomaterial variants. The data should be able to characterise not only morphological, structural, chemical and surface chemical aspects but also nano-specific impurities, see Table 5.2. It became clear that the additional characterisation effort already for the substance identification part can very high and may even exceed that of physico-chemical testing. Moreover, the procedures for such characterisations were found to require significant progress in method development and standardisation.

Concerning the physico-chemical procedures for materials testing, this study has shown that the procedures developed for conventional materials are in general also applicable to nanoscale substances. However, in most cases nano-specific peculiarities have to be considered for the practical implementation of testing and for the interpretation of results. In cases where nano-specific features lead to restrictions on the applicability of the method, nano-specific modifications were proposed. Particularly important is the consideration of the particle size dependence of physico-chemical properties, which can, for example, critically affect the melting and solubility behaviour as well as the reactivity of nanoscale substances. For test procedures that are sensitive to such dependencies, significant discrepancies may result between the test results of conventional and nanoscale substances, see Table 6.3. For melting point and solubility determination, such deviations may indicate a nanoscale nature of the tested substance. If the nanoscale nature of a substance was not known before, such indications should give rise to a comprehensive – nanoscale – material characterisation.

A particle size dependence of the reactivity generally leads to complications during chemical safety testing of nanoscale substances, because an increased surface reactivity will induce property changes subsequent to material synthesis. They can be pronounced very differently, depending on storage conditions and sample age. Since reliable and reproducible test results are mandatory for registration and regulation, the documentation of a material’s post-synthesis history becomes very important for nanomaterials. Alternatively, if such documentation is missing, information has to be generated on the present surface reactivity state of the material prior to substance identification and testing.

In addition to the substance tests prescribed for conventional substances, relevant physico-chemical properties of nanoscale substances were proposed and appropriate procedures were discussed. Information on the following additional phenomenological properties should be requested from a registrant of a nanoscale substance: dispersibility and dustiness, re-agglomeration and sedimentation propensity, zeta potential, adsorption-desorption properties, redox potential, free radical formation potential, (photo)-catalyzed activity and fibre stiffness, see Table 6.2.

Finally, it must be emphasized that an inclusion of nanoscale substances into the REACH process will make necessary a significant extension of the information requirements already at the level of substance identification. Currently, for substance identification, no standard characterisation procedures are established, but the analyses are to be described in a reproducible way by the manufacturer. Considering the analytical challenge of a comprehensive characterisation of nanoscale substances, there is a strong need for standardised implementation instructions.

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List of acronyms

Table list of acronyms 1: Used acronyms.

Acronyms	**Description**
F4F	Asymmetric Flow Field-Flow Fractionation
AAS	Atom Absorption Spectroscopy
AES	Auger-Elektron-Spectroscopy
AFM	Atomic Force Microscopy
AFM/FDC	Atomic Force Microscopy with Force-Distance Curve measurement
AIST	Association for Iron and Steel Technology
APS	Aerodynamic Particle Sizer
ASTM	American Society for Testing and Materials
BAFU	Federal Office for the Environment (Schweizerisches Bundesamt für Umwelt)
BAM	Federal Institute for Materials Research and Testing (Bundesanstalt für Materialforschung und –prüfung)
BAuA	Federal Institute for Occupational Safety and Health (Bundesanstalt für Arbeitsschutz und Arbeitsmedizin)
BET	Brunauer-Emmett-Teller method
BJH	Barrett-Joyner-Halenda method
CARACAL	Competent Authorities for REACH and CLP (CARACAL)
CAS	Chemical Abstracts Service
CEN	European Committee for Standardization (frz. Comité Européen de Normalisation)
CLP	Regulation on Classification, Labelling and Packaging of Substances and Mixtures (EC) 1272/2008
CNT	Carbon Nanotube
COM	Confocal Optical Microscopy
CPC	Charged Particle Counter
Cryo-TEM	TEM with cryogenic fixation
DGUV	Deutsche Gesetzliche Unfallversicherung
DIN	Deutsches Institut für Normung
DLS	Dynamic Light Scattering
DMA	Differential Mobility Analysis
DSC	Differential Scanning Calorimetry
DTA	Differential Thermo Analysis
EAA	Ensemble-Averaging Analysis
EC	European Commission
EELS	Electron Energy Loss Spectroscopy
EN	European Standard (Europäische Norm)
EPA	Environmental Protection Agency of U.S.A.
EPM	ElectroPhoretic Mobility
EPR	Electron Paramagnetic Resonance spectroscopy
ESI-ToF-MS	Electrospray-Ionization Time-of-Flight Mass Spectrometry
et al.	et alii, et aliae, et alia
FFF	Field Flow Fractionation
FTIR	Fourier-Transformation-InfraRed spectroscopy
GPC	Gel Permeation Chromatography
HPLC	High Performance Liquid Chromatography
HR-TEM	High-Resolution TEM
ICON™	International Council of Nanotechnology

ICP-MS/OES	Inductively Coupled Plasma-Mass Spectrometry or optical ICP-Emissions spectrometry
IEP	Iso-Electric Point
IGC	Inverse Gas Chromatography
IPA	Individual Particle Analysis
IRMM	Institute for Reference Materials and Measurement
ISO	International Organization for Standardization
JIS	Japan Industrial Standard
MALDI-ToF-MS	Matrix-assisted Laser-Desorption/Ionization Time-of-Flight Mass Spectrometry
MMMF	Man-Made Vitreous Fibres
MN	Manufactured Nanomaterials
MS	Mass Spectrometry
MWCNT	Multi-Walled Carbon Nanotube
NFT	Association Française de NORmalisation (ANFOR)
NIR	Near infrared
NIST	National Institute of Standards and Technology
nm	Nanometer, 10^{-9} m
NMR	Nuclear Magnetic Resonance spectroscopy
NTA	Nanoparticle Tracking Analysis
OECD	*OECD*, Organisation for Economic Co-operation and Development
OPC	Optical Particle Counter
OT	Optical Tweezer
PCS	Photon correlation Spectroscopy, see DLS
PCS	Particle Charge Sizer
PLS	Photoluminescence Spectroscopy
PTB	Physikalisch-Technische Bundesanstalt
Raman	Raman Spectroscopy
REACH	Registration, Evaluation, Authorisation of Chemicals
SEM/EDX	SEM with Energy Dispersive X-ray spectroscopy
ROS	Reactive Oxygen Species
SANS	Neutron Small Angle Scattering
SAXS	Small-Angle X-ray Scattering
SCCP	Scientific Committee on Consumer Products
SDFFF	Sedimentation separation
SDS	Safety Data Sheet
SEM	Scanning Electronbeam Microscopy
SIMS	Secondary Ion Mass Spectrometry
SLS	Static Light Scattering
SMPS	Scanning Mobility Particle Sizer, combination of DMA and CPC
SSP	Individual particle analysis methods (IPA) and spatially-separated particle analysis
STEM	Scanning TEM
SWCNT	Single-Walled Carbon Nanotubes
TED	Transmission Electron Diffraction
TEM	Transmission Electron Microscopy
TEM/EDX	STEM with Energy Dispersive X-ray spectroscopy
TEM/EELS	STEM with EELS
TEM/TED	TEM with TED
TEM/XRD	TEM with XRD
TG	Testing-Guidelines (*OECD*)
TGA	Thermo-Gravimetric Analysis
T_m	Melting temperature
TR	Technical rule

TWA	Technical Working Areas
USAXS	Ultra-Small Angle X-ray Scattering
USS	Ultrasound Spectroscopy
UV/Vis	UV/Vis Spectroscopy
VAMAS	Versailles Project on Advanced Materials and Standards
VSSA	Volume-specific surface area
WHO	World Health Organization
WPMN	Working Party on Manufactured Nanomaterials
XFA	X-ray Fluorescence Analysis
XPS	X-ray Photoelectron Spectroscopy
XRD	X-Ray Diffraction

Appendix 1 Important analytical methods and their statistical character

Appendix 1, Table 1 Important analytical methods and their statistical character.

Acronym	Method	Type	Information	Ref.
A4F	Asymmetric Flow Field-Flow Fractionation	EAA	Size separation	[173], [174]
AAS	Atom Absorption Spectroscopy	EAA	Elemental concentration	[175]
AES	Auger-Electron Spectroscopy	SSP	Chemical surface composition	[176], [177]
AFM	Atomic Force Microscopy	IPA	Size, Morphology, Topography	[178]
AFM/FDC	Atomic Force Microscopy with Force-Distance Curve measurement	IPA	Stiffness, surface functionality (for functionalised tips)	[179]
APS	Aerodynamic Particle Sizer	IPA	Aerodynamic radius	[180]
BET	Brunauer-Emmett-Teller method	EAA	Specific surface area	[131]
BJH	Barrett-Joyner-Halenda method	EAA	Pore volume and area	[181]
COM	Confocal Optical Microscopy	IPA	Microscopy with resolution below wavelength of light	
CPC	Charged Particle Counter	IPA	Particle number	
Cryo-TEM	TEM with cryogenic fixation	IPA	Particle morphology and agglomeration in (liquid) matrices	
DLS	Dynamic Light Scattering	EAA	Hydrodynamic radius	[182], [183]
DMA	Differential Mobility Analysis	EAA	Aerodynamic radius	[184]
DSC	Differential Scanning Calorimetry	EAA	Phase transition temperature	
DTA	Differential Thermo Analysis	EAA	Phase transition temperature	
DVS	Dynamic Vapour Sorption	EAA	Surface functionality and polarity	
EELS	Electron Energy Loss Spectroscopy	SSP	Elemental composition, chemical bonding	
EPR	Electron paramagnetic Resonance spectroscopy	EAA	Radical concentration	[185]
EPM	ElectroPhoretic Mobility	EAA	Hydrodynamic radius	[186], [187]
ESI-ToF-MS	Electron-Spray-Ionization Time-of-Flight Mass Spectrometry	IPA	Molecular mass	[188], [189]
Fluorescence	Fluorescence Spectroscopy	SSP	Detection of fluorescing immobilised markers	
FTIR	Fourier-Transformation-InfraRed spectroscopy	EAA	Chemical structure and chem. functionality	
GPC	Gel Permeation Chromatography	EAA	Apparent particle size	[190]
HR-TEM	High-resolution TEM	IPA	Morphology, Crystal lattice and atomic structure	
ICP-MS/OES	Inductively Coupled Plasma-Mass Spectrometry or optical ICP-Emissions spectrometry	EAA	Elemental concentrations	
IEP	Iso-Electric Point	EAA	Surface charge	[187]
IGC	Inverse Gas Chromatography	EAA	Surface energy and polarity	[191]

MALDI-ToF-MS	Matrix-assisted Laser-Desorption/Ionization Time-of-Flight Mass Spectrometry	IPA	Molecular mass	[192]
NMR	Nuclear Magnetic Resonance spectroscopy	EAA	Chemical structure	
NTA	Nanoparticle Tracking Analysis	IPA	Hydrodynamic radius	[178]
OT	Optical Tweezers	IPA	Interaction strength	[193]
PCS	Photon correlation Spectroscopy, see DLS	EAA	Hydrodynamic radius	[182]
PCS	Particle Charge Sizer	EAA	Surface charge	
Raman	Raman Spectroscopy	SSP	Chemical bonding, defects, DW/SWCNT diameter	[194]
SEM	Scanning Electron Microscopy	IPA	Size, Morphology, Topography	[195]
SEM/EDX	SEM with Energy Dispersive X-ray spectroscopy	SSP	Chemical composition	
SANS	Neutron Small Angle Scattering	EAA	Agglomerate size	[196]
SAXS	Small-Angle X-ray Scattering	EAA	Particle-core/shell size and form factor	[173], [197]
SDFFF	Sedimentation separation	EAA	Hydrodynamic radius	[198]
SIMS	Secondary Ion Mass Spectrometry	EAA	Surface Composition Profile	
SLS	Static Light Scattering	EAA	Apparent particle size	[182]
SMPS	Scanning Mobility Particle Sizer, combination of DMA and CPC	IPA	Aerodynamic radius	[199], [200]
Solubility		EAA		
STEM	Scanning TEM	IPA		
TEM	Transmission Electron Microscopy	EAA	Size, Morphology	[201]
TEM/EDX	STEM with Energy Dispersive X-ray spectroscopy	IPA	Chemical composition	
TEM/ EELS	STEM with EELS	IPA	Chemical composition, chemical bonding	
TEM/XRD	STEM with XRD	IPA	Local crystal structure	
TGA	Thermo-Gravimetric Analysis	EAA	Oxidation stability, ash content, elemental/organic carbon	
Titration	Potentiometric acid-base titration	EAA	Surface acidity/basicity	[160]
USS	Ultrasound Spectroscopy	EAA	Concentration in suspension	
UV/Vis	UV/Vis Spectroscopy	EAA	Chemical structure	
XFA	X-ray Fluorescence Analysis	SSP	Elemental concentrations	
XPS	X-ray Photoelectron Spectroscopy	EAA	Chemical composition, chemical shifts	[201], [202]
XRD	X-Ray Diffraction	EAA	Crystallinity, domain size, lattice spacing	
Zeta potential	Zeta potential	EAA	Surface polarity, surface charge	[203]

EAA	: Ensemble-Averaging Analysis
IPA	: Individual Particle Analysis
SSP	: Spatially Separated individual particle Analysis – Individual particle analysis is possible for a spatial separated that exceeds the spatial resolution of the analysis technique used

Appendix 2 Standards and Test Guidelines

Appendix 2, Table 1 *ISO* standards explicitly developed for nanomaterials.

Standard	Description	Date of publication
ISO/TR 27628:2007	Workplace atmospheres -- Ultrafine, nanoparticle and nano-structured aerosols -- Inhalation exposure characterization and assessment	22.01.2007
ISO/TS 27687:2008	Nanotechnologies -- Terminology and definitions for nano-objects -- Nanoparticle, nanofibre and nanoplate	11.08.2008
ISO/TS 80004-3:2010	Nanotechnologies -- Vocabulary -- Part 3: Carbon nano-objects	19.04.2010
ISO/TR 11360:2010	Nanotechnologies -- Methodology for the classification and categorization of nanomaterials	12.07.2010

Appendix 2, Table 2 Standards in consultation for nanomaterials.

Primary particle size	
ISO/PRF TS 10797	Nanotechnologies -- Characterization of single-wall carbon nanotubes using transmission electron microscopy
ASTM WK1127	New Guide for Powder Particle Size Analysis
ASTM WK26321	New Guide for Measurement of particle size distribution of nanomaterials in suspension by nanoparticle tracking analysis (NTA)
Aggregation or agglomeration state	
ISO/CD 12025	Nanomaterials -- General framework for determining nanoparticle content in nanomaterials by generation of aerosols
ISO 1437:2007	Rubber compounding ingredients -- Carbon black -- Determination of sieve residue
EN ISO 787-18:1995	General methods of test for pigments and extenders. Determination of residue on sieve. Mechanical flushing procedure
Particle shape	
ISO/DTS 10797	Nanotechnologies -- Characterization of single-wall carbon nanotubes using transmission electron microscopy
ISO/DTS 10798	Nanotechnologies -- Charaterization of single-wall carbon nanotubes using scanning electron microscopy and energy dispersive X-ray spectrometry analysis
ISO/DTR 10929	Messmethoden zur Charakterisierung von MWCNT
ASTM WK28561	New Test Method for Airborne Carbon Nanotube Concentration in Ambient and Indoor Atmospheres as Determined by TEM Direct Transfer
Chemical composition	
ISO/DTR 10929	Nanotechnologies -- Characterization of multiwall carbon nanotube (MWCNT) samples
ISO/DTS 10797	Nanotechnologies -- Characterization of single-wall carbon nanotubes using transmission electron microscopy
ISO/PRF TS 10867	Nanotechnologies -- Characterization of single-wall carbon nanotubes using near infrared photoluminescence spectroscopy
ISO/DTS 10868	Nanotechnologies -- Characterization of single-wall carbon nanotubes using ultraviolet-visible-near infrared (UV-Vis-NIR) absorption spectroscopy
Surface chemistry	
ISO/PRF 10810	Surface chemical analysis -- X-ray photoelectron spectroscopy -- Guidelines for analysis
ASTM JAI13174	Characteristics of Beryllium Oxide and Beryllium Metal Powders for Use as Reference Materials
ISO/WD TR 14187	Surface chemical analysis -- Characterization of nanostructured materials
Zeta potential	
ISO/FDIS 13099	Colloidal systems -- Methods for zeta-potential determination
ASTM WK21915	New Guide for Zeta potential measurement by electrophoretic mobility

Appendix 2, Table 3 Standards applicable to nanomaterials.

Primary particle size, particle size distribution, hydro- or aerodynamic diameter	
OECD-TG 110	Particle Size Distribution/Fibre Length and Diameter Distributions
JIS H 7804:2005	SEM, TEM
ISO/TS 13762:2001	Particle size analysis -- Small angle X-ray scattering method
ISO/CD 15900:2009	Determination of particle size distribution -- Differential electrical mobility analysis for aerosol particles
ISO 9277:2010	Determination of the specific surface area of solids by gas adsorption using the BET method
ISO 9276	Representation of results of particle size analysis
ISO 22412:2008	Particle size analysis -- Dynamic light scattering (DLS)
ISO 21501	Determination of particle size distribution -- Single particle light interaction methods
ISO 20998-1:2006	Measurement and characterization of particles by acoustic methods -- Part 1: Concepts and procedures in ultrasonic attenuation spectroscopy
ISO 16700:2004	Microbeam analysis -- Scanning electron microscopy -- Guidelines for calibrating image magnification
ISO 15900:2009	Determination of particle size distribution -- Differential electrical mobility analysis for aerosol particles
ISO 14887:2000	Sample preparation -- Dispersing procedures for powders in liquids
ISO 14488:2007	Particulate materials -- Sampling and sample splitting for the determination of particulate properties
ISO 13322	Particle size analysis -- Image analysis methods
ISO 13321:1996	Particle size analysis -- Photon correlation spectroscopy
ISO 13320:2009	Particle size analysis -- Laser diffraction methods
ISO 13318:2004	Determination of particle size distribution by centrifugal liquid sedimentation methods
CEN: *EN* 725-5:2007	Advanced technical ceramics. Methods of test for ceramic powders. Determination of particle size distribution
ASTM B859-03	Standard Practice for De-Agglomeration of Refractory Metal Powders and Their Compounds Prior to Particle Size Analysis
ASTM E2490-09	Standard Guide for Measurement of Particle Size Distribution of Nanomaterials in Suspension by Photon Correlation Spectroscopy (PCS)
ASTM E2456-06	Standard Terminology Relating to Nanotechnology
ASTM E1919-07	Standard Guide for Worldwide Published Standards Relating to Particle and Spray Characterization
ASTM E112-96	Standard Test Methods for Determining Average Grain Size
Aggregation or agglomeration state	
ISO 16700:2004	Microbeam analysis -- Scanning electron microscopy -- Guidelines for calibrating image magnification
ISO 13322-1:2004	Particle size analysis -- Image analysis methods -- Part 1: Static image analysis methods
Partikel shape	
ISO 16700:2004	Microbeam analysis -- Scanning electron microscopy -- Guidelines for calibrating image magnification
ISO 13322-1:2004	Particle size analysis -- Image analysis methods -- Part 1: Static image analysis methods
OECD-TG110	Particle Size Distribution/Fibre Length and Diameter Distributions
ASTM D6281-06	Standard Test Method for Airborne Asbestos Concentration in Ambient and Indoor Atmospheres as Determined by Transmission Electron Microscopy Direct Transfer (TEM)

ASTM D5755-03	Standard Test Method for Microvacuum Sampling and Indirect Analysis of Dust by Transmission Electron Microscopy for Asbestos Structure Number Surface Loading
Specific surface area	
ISO 9277:2010	Determination of the specific surface area of solids by gas adsorption -- BET method
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 15901	Pore size distribution and porosity of solid materials by mercury porosimetry and gas adsorption
ISO 13322-1:2004	Particle size analysis -- Image analysis methods -- Part 1: Static image analysis methods
ASTM: B922-02	Standard Test Method for Metal Powder Specific Surface Area by Physical Adsorption
Chemical composition	
ISO 22309:2011	Microbeam analysis -- Quantitative analysis using energy-dispersive spectrometry (EDS) for elements with an atomic number of 11 (Na) or above
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
Surface chemistry	
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/TR 18394:2006	Surface chemical analysis -- Auger electron spectroscopy -- Derivation of chemical information
ISO/TR 18392:2005	Surface chemical analysis -- X-ray photoelectron spectroscopy -- Procedures for determining backgrounds
ISO 24237:2005	Surface chemical analysis -- X-ray photoelectron spectroscopy -- Repeatability and constancy of intensity scale
ISO 24236:2005	Surface chemical analysis -- Auger electron spectroscopy -- Repeatability and constancy of intensity scale
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 23812:2009	Surface chemical analysis -- Secondary-ion mass spectrometry -- Method for depth calibration for silicon using multiple delta-layer reference materials
ISO 21270:2004	Surface chemical analysis -- X-ray photoelectron and Auger electron spectrometers -- Linearity of intensity scale
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 20341:2003	Surface chemical analysis -- Secondary-ion mass spectrometry -- Method for estimating depth resolution parameters with multiple delta-layer reference materials
ISO 19318:2004	Surface chemical analysis -- X-ray photoelectron spectroscopy -- Reporting of methods used for charge control and charge correction
ISO 18516:2006	Surface chemical analysis -- Auger electron spectroscopy and X-ray photoelectron spectroscopy -- Determination of lateral resolution
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
ISO 18117:2009	Surface chemical analysis -- Handling of specimens prior to analysis
ISO 18115	Surface chemical analysis – Vocabulary
ISO 18114:2003	Surface chemical analysis -- Secondary-ion mass spectrometry -- Determination of relative sensitivity factors from ion-implanted reference materials
ISO 17974:2002	Surface chemical analysis -- High-resolution Auger electron spectrometers -- Calibration of energy scales for elemental and chemical-state analysis
ISO 17973:2002	Surface chemical analysis -- Medium-resolution Auger electron spectrometers -- Calibration of energy scales for elemental analysis

ISO 17560:2002	Surface chemical analysis -- Secondary-ion mass spectrometry -- Method for depth profiling of boron in silicon
ISO 15472:2010	Surface chemical analysis -- X-ray photoelectron spectrometers -- Calibration of energy scales
ISO 15471:2004	Surface chemical analysis -- Auger electron spectroscopy -- Description of selected instrumental performance parameters
ISO 15470:2004	Surface chemical analysis -- X-ray photoelectron spectroscopy -- Description of selected instrumental performance parameters
Zeta potential	
JIS R 1638:2000	Availlable in Japanese
Crystallinity and crystalline phase	
JIS R 7651:2007	Availlable in Japanese
Water solubility	
CEN : *EN* 12457-1:2002	Characterisation of waste - Leaching - Compliance test for leaching of granular waste materials and sludges
Fatty acid solubility / Oleophilic properties	
OECD-TG116	Fat Solubility of Solid and Liquid Substances
Octanol-water partition coefficient	
OECD-TG 107	Shake-flask method [151]
OECD-TG 117	HPLC Method [152]
OECD-TG 123	Slow-stirring method [153]
Dustiness	
ASTM: Paper ID: STP25370S	Evaluation of Airborne Dust Measurement Methods for Agricultural Chemical Carriers
EN 15051:2006	Workplace atmospheres. Measurement of the dustiness of bulk materials. Requirements and reference test methods