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# PHYSICAL-CHEMICAL PROPERTIES OF NANOMATERIALS: EVALUATION OF METHODS APPLIED IN THE OECD-WPMN TESTING PROGRAMME

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# PHYSICAL-CHEMICAL PROPERTIES OF NANOMATERIALS: EVALUATION OF METHODS APPLIED IN THE OECD-WPMN TESTING PROGRAMME

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# FOREWORD

The OECD Joint Meeting of the Chemicals Committee and Working Party on Chemicals, Pesticides and Biotechnology (the Joint Meeting) held a Special Session on the Potential Implications of Manufactured Nanomaterials for Human Health and Environmental Safety (June 2005). This was the first opportunity for OECD member countries, together with observers and invited experts, to begin to identify human health and environmental safety related aspects of manufactured nanomaterials. The scope of this session was intended to address the chemicals sector.

As a follow-up, the Joint Meeting decided to hold a Workshop on the Safety of Manufactured Nanomaterials in December 2005, in Washington, D.C. The main objective was to determine the “state of the art” for the safety assessment of manufactured nanomaterials with a particular focus on identifying future needs for risk assessment within a regulatory context.

Based on the conclusions and recommendations of the Workshop [ENV/JM/MONO(2006)19] it was recognised as essential to ensure the efficient assessment of manufactured nanomaterials so as to avoid adverse effects from the use of these materials in the short, medium and longer term. With this in mind, the OECD Council established the OECD Working Party on Manufactured Nanomaterials (WPMN) as a subsidiary body of the OECD Chemicals Committee in September 2006. Since then, this programme has focused on human health and environmental safety implications of manufactured nanomaterials (limited mainly to the chemicals sector), and aimed to ensure that the approach to hazard, exposure and risk assessment is of a high, science-based, and internationally harmonised standard. This programme promotes international co-operation on the human health and environmental safety of manufactured nanomaterials, and involves the safety testing and risk assessment of manufactured nanomaterials.

This document is published under the responsibility of the Joint Meeting of the Chemicals Committee and Working Party on Chemicals, pesticides and Biotechnology of the OECD.

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# INTRODUCTION

1. The Sponsorship Programme for the Testing of Manufactured Nanomaterials (further referred to as "the Testing Programme") was concluded in March 2013, and the publication of the dossiers via the OECD website (www.oecd.org/science/nanosafety) started in June 2015. As indicated in the "Guidance manual for the testing of manufactured nanomaterials: OECD's sponsorship programme" (1), after conclusion of the Testing Programme a next step is to consider "the status, need for, and coordination of further test development" (1). Parallel to concluding the final stages of the Testing Programme, a series of workshops have taken place (2) (3) (4) (5), in which for different topics the applicability of existing OECD test guidelines for nanomaterials was discussed and the need for new ones analysed.

2. One workshop focussed on physico-chemical methods (4), addressing in detail the relevance of each physico-chemical endpoint proposed in the Testing Programme for the regulation of nanomaterials[1]. The methods were discussed in more general terms. However, as most of the proposed endpoints are new to the OECD Test Guidelines Programme, a much more detailed evaluation of the applied methods would be highly relevant.

3. To this extent, the Netherlands volunteered to lead an initial detailed evaluation of the applicability of the test methods applied to determine the physico-chemical properties of different types of nanomaterials in the Testing Programme. This initial focus on physico-chemical properties was prompted by the essential need for an adequate and complete characterisation of nanomaterials to enable a further evaluation of their (toxicological) properties.

4. A number of experts from several delegations volunteered to review and evaluate the methods applied to determine the physico-chemical properties of the nanomaterials in the Testing Programme (Annex I). The evaluation started by asking these experts to answer several questions on each method described using a web-based questionnaire (see Annex II). Thus, the different experts could provide their expert opinion on each of the methods applied in a uniform way. As a conclusion to their evaluation the experts were asked to rate the validity of the specific method with a score on a scale from 1 to 10. As experts may have interpreted the rating of the validity differently (e.g. some included the way the method was reported, while others did not), these scores are not reported in this document, but the motivation for the score is used in a qualitative way.

5. This evaluation started in the spring of 2014 and used the dossiers as they were available in May 2014. The dossier versions that were finally published (from June 2015 onwards) in some cases show considerable differences with the versions evaluated. Nevertheless, a quick check of the dossiers as available in May 2015 showed that all methods applied to determine the physico-chemical properties were evaluated for at least one of the dossiers (unless insufficient methodological details were provided).

6. The parameters assessed include chemical composition, aggregation/agglomeration, particle size distribution, crystalline phase, dustiness, specific surface area, water solubility/dispersibility, zeta potential, photocatalytic activity, porosity, redox potential, radical formation potential, crystallite size, and surface chemistry. Additional information on pour density and *n*-octanol-water partition coefficient ($K_{\mathrm{OW}}$) is available in the dossiers as well, but was not evaluated as such. The assessment only involved evaluation of whether a specific method applied to determine a certain physico-chemical property is suitable for the specific parameter, either for a specific nanomaterial or for a (broad) range of different nanomaterials. The

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[1] No.63, Physical-chemical parameters: measurements and methods relevant for the regulation of nanomaterials (2016)

usefulness of the different parameters for risk assessment was not assessed. Neither should information in this report be seen as preference for a certain method to be used in risk assessment, as the focus is on methods used in the Testing Programme and other methods may exist as well. Furthermore, suitability of a certain method may strongly depend on the material considered as well as the purpose of the use of a certain parameter in risk assessment.

7. In general the *n*-octanol-water partition coefficient is considered not relevant for nanomaterials, and only in two of the dossiers (fullerenes and dendrimers) this information was supplied. Many of the nanomaterials in the Testing Programme are inorganic, insoluble or only slightly soluble materials for which the parameter cannot be determined. In addition, for non-nanomaterials the parameter is often used to predict passive diffusion from a water phase to a lipid (organism) phase. For nanomaterials, however, uptake is generally not driven by passive diffusion. This limits the usefulness of the parameter for nanomaterials, thus also for the two dossiers that include the parameter. Likewise, pour density data is only available in a few dossiers, and thus was considered of less priority for this evaluation.

8. The evaluated dossiers[2] include information on multi-walled carbon nanotubes (MWCNT), single-walled carbon nanotubes (SWCNT), silver (Ag), silicon dioxide ($SiO_2$), cerium dioxide ($CeO_2$), zinc oxide (ZnO), titanium dioxide ($TiO_2$), fullerenes, dendrimers, gold (Au), and nanoclay.

9. In the current evaluation exercise, at least one combination of applied method(s) and nanomaterial(s) was evaluated for each dossier (Table 1). Where possible, conclusion(s) from these evaluations were extrapolated to other materials as well. However, the quality of the reported data in the evaluated dossiers was not always sufficient to allow its use. For instance for chemical composition, details on presence of any residual materials (for example residual catalyst material), by-products, impurities, etc. was not always provided. Moreover, details on the methods used to assess chemical composition were often lacking in the dossiers. Such information gaps may affect the evaluation of subsequent test results pertaining to toxicity, ecotoxicity and fate, where the absence of information on physico-chemical properties means that it may not be possible to fully establish the cause(s) of an observed effect, and the presence allows a deeper understanding of the outcomes.

10. In the following sections the methods used for each of the physico-chemical parameters will be discussed, based on the input to the questionnaire by the experts[3]. In addition, some of the discussion is based on the information provided for and discussions at the “Meeting on Nanomaterials Physical-Chemical Parameters: Measurements and Methods” as well[4], and further amended by reviewers of draft versions of this document (see Annex II).

11. In general, for many (if not all) methods, a well-standardised sample preparation protocol is needed as sample preparation can have large influence on the outcome of a certain test method. In some

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[2] The dossiers that were thoroughly evaluated were those that were available in May 2014. These dossiers may show differences with the ones that were published on the website in 2015.

[3] It should be noted that where dossiers are assessed, in most cases a particular parameter and its methods have been thoroughly evaluated by one expert only, although in the reviewing process other experts added additional views.

[4] The objectives of that meeting were to identify the appropriate test methods for physico-chemical parameters for manufactured nanomaterials, building on the experience from the testing programme as well as the OECD Expert Meeting on Physical-Chemical Properties of Manufactured Nanomaterials and Test Guidelines in collaboration with ISO/TC 229: Nanotechnologies. The expertise of the experimenters and other physico-chemical and metrology experts at the meeting were used to determine, if possible, which test methods are appropriate for both a particular parameter and particular type(s) of nanomaterials.

dossiers, the evaluation of the physico-chemical methods was hampered because insufficient information had been provided on the sample preparation methods.

**Table 1 – Overview of physico-chemical parameters that have been evaluated for nanomaterials in the dossiers.**

Numbers inside the cells indicate the number of forms of the respective nanomaterial for which the data and associated testing description(s) were evaluated, and the number of forms available in the specific dossier for each parameter (e.g. 0/6 in the upper left cell means that for 0 of the 6 available MWCNT forms the methods used for determining chemical composition has been evaluated). Empty cells indicate that the respective parameter was not measured for the specific nanomaterial. Grey cells indicate that the methods used for determining the specific parameter have been evaluated for at least part of the nanomaterial forms in the dossier, or that the method was evaluated for other material(s) and is considered generally applicable; white cells with numbers indicate that evaluation of methods was not performed (justification is given in notes below the table). Pour density and octanol-water partitioning coefficient ($K_{OW}$) were considered of less priority and methods for these parameters were not evaluated.

| | **Nanomaterial dossier** | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **Parameter** | **MWCNT** | **SWCNT** | **Ag** | **$SiO_2$** | **$CeO_2$** | **ZnO** | **$TiO_2$** | **Fullerenes** | **Dendrimers** | **Au** | **Nanoclay** |
| Chemical composition | 0/6$^{a}$ | 0/6$^{a}$ | 1/5 | 5/5 | 0/3 | 0/4 | 0/6 | 0/1$^{a}$ | 0/4$^{a}$ | 0/1$^{a}$ | 0/1$^{a}$ |
| Aggregation/agglomeration | 2/6 | 2/3 | 2/4 | 4/4 | 1/4 | 1/4 | 1/6 | 0/1 | 0/4 | 1/1 | 1/1 |
| Particle size distribution | 6/6 | 0/2 | 1/3 | 2/4 | 3/3 | 0/4 | 1/6 | 1/1 | 2/4 | | 1/1 |
| Crystalline phase | 2/3 | 1/5 | 0/1 | 1/4 | 0/4 | 4/4 | 2/5 | 0/1$^{a}$ | | 0/1 | 0/1$^{a}$ |
| Dustiness | 3/3 | 1/1 | | 4/4 | 3/4 | 4/4 | 5/6 | 1/1 | | | |
| Specific surface area | 3/5 | 0/5 | 0/1$^{a}$ | 4/5 | 2/4 | 2/4 | 5/7 | 0/1 | | 0/1 | 0/1 |
| Water solubility/dispersibility | 0/6$^{a}$ | 0/2$^{a}$ | 0/2$^{b}$ | 2/4 | 0/4 | 4/4 | 0/6 | 0/1$^{a}$ | | 0/1 | |
| Zeta potential | 3/3 | 2/3 | 2/4 | 4/4 | 3/4 | 4/4 | 1/6 | 0/1$^{a}$ | 4/4 | 1/1 | 0/1 |
| Photocatalytic activity | 1/1 | 2/2 | | | 3/3 | 4/4 | 1/3 | 1/1 | | | |
| Porosity | 2/2 | 2/2 | | 4/4 | 3/3 | 4/4 | | 0/1 | | | |
| Redox potential | | | | 4/4 | 3/3 | 3/3 | | | | | |
| Radical formation potential | | | | 4/4 | 3/3 | 3/4 | 3/3 | | | | |
| Crystallite size | 0/6$^{a}$ | 0/6$^{a}$ | 0/1$^{a}$ | 0/4$^{c}$ | 0/3 | 4/4 | 0/6 | 0/1$^{a}$ | | 0/1$^{c}$ | |
| Surface chemistry | | 1/2 | 1/1 | 0/5 | 0/4 | 0/4 | 0/3 | | 1/1 | | 1/1 |
| Pour density | 0/4 | 0/1 | | 0/1 | 0/3 | 0/4 | | 0/1 | | | |
| Octanol-water partitioning coefficient ($K_{OW}$) | | | | | | | | 0/1 | 0/2 | | |

[a] These dossiers did not include sufficient details on the methods used to enable evaluation of the methods.

[b] Water solubility of silver could not be evaluated, as the available studies focussed on the dispersion stability of silver nanoparticles instead.

[c] The data indicate that the material is not crystalline, so crystallite size was not determined.

## CHEMICAL COMPOSITION

12. To determine chemical composition the method(s) energy dispersive X-ray analysis (EDX) and inductively coupled plasma/optical emission spectrometry (ICP/OES) were used for silicon dioxide, silver, and titanium dioxide. Only for silicon dioxide and silver these methods were further analysed. The evaluated dossiers for MWCNT, SWCNT, fullerenes, dendrimers, gold and nanoclay did not include sufficient information to evaluate the method(s) applied, or did not indicate a method at all. An exhaustive list of applied methods could therefore not be compiled.

13. Energy dispersive X-ray analysis (EDX) is a generally recognised analytical tool, which provides elemental analysis for chemical composition characterisation. It is typically suitable for any elements above carbon (although limited for elements below sodium), e.g. titanium dioxide, silver, zinc oxide, iron, nanoclay and cerium oxide, and especially useful to retrieve information on atom distribution. However, experts indicated that the method is unsuitable for nanoparticles of complex composition, in complex matrices, and for large aggregates. Sample preparation should be documented in detail, because it influences the final physical form (e.g. density) of the sample, which affects the elemental composition. For different forms of nanomaterials, different calibration curves may be needed. Overall the method appears suitable, provided that sample preparation is standardised and reported in sufficient detail.

14. Inductively coupled plasma/optical emission spectrometry (ICP/OES) is a generally accepted method for the detection of trace metals, which has been evaluated for silicon dioxide. The ICP part of the method provides excited atoms and ions that emit electromagnetic radiation at wavelengths characteristic of a particular element. The intensity of this emission is indicative of the concentration of the element within the sample. The evaluating expert judged the method to be suitable for other nanomaterials as well. Not all elements, however, can be detected by this method[5] and emitted radiations of different elements may show interference.

15. For cerium oxide and zinc oxide x-ray photoelectron spectroscopy (XPS) was used, next to the EDX discussed above. This method was not evaluated for the parameter ‘chemical composition’, nor was it evaluated for these compounds for the parameter ‘surface chemistry’ where it was also used. Nevertheless, the evaluation of this method for the parameter ‘surface chemistry’ of SWCNTs, nanoclays and dendrimers below (paragraph 78) suggests that it is a suitable method for determining chemical composition of the core, provided that the surface layer (0–10 nm) is representative for the core material (i.e. for uncoated materials). As it is a method for surface chemistry, for coated materials XPS will only provide information on the coating.

16. Inductively coupled plasma/mass spectrometry (ICP-MS), electron microscopy (EM) and scanning tunnelling microscopy (STM) were suggested as additional methods to determine chemical composition, but these were not indicated as being used in the evaluated dossiers. Evaluation of these methods is therefore beyond the scope of the current document.

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[5] In the silica dossier that was evaluated for this method (6), samples were screened for 68 elements: Ag, Al, As, Au, B, Ba, Be, Bi, Ca, Cd, Ce, Co, Cr, Cu, Dy, Er, Eu, Fe, Ga, Gd, Ge, Hf, Hg, Ho, In, Ir, K, La, Li, Lu, Mg, Mn, Mo, Na, Nb, Nd, Ni, P, Pb, Pd, Pr, Pt, Rb, Re, Rh, Ru, S, Sb, Sc, Se, Si, Sm, Sn, Sr, Ta, Tb, Te, Th, Ti, Tl, Tm, U, V, W, Y, Yb, Zn and Zr.

## AGGLOMERATION / AGGREGATION

17. Methods to determine aggregation/agglomeration were analysed for MWCNT, SWCNT, silver, silicon dioxide, cerium oxide, zinc oxide, and nanoclay. Although titanium dioxide, fullerenes and dendrimers were not fully analysed, some of the same methods as described below were also applied to these materials. In general, it is difficult to distinguish between an aggregate and agglomerate directly, unless advanced sample preparation methods or a combination of techniques (e.g. (7)) are used. Many of the methods used to determine agglomeration/aggregation are also used for the particle size distribution (e.g., DLS, TEM, SEM, cf. paragraphs 24–35). The issues identified for the different techniques used for particle size distribution clearly have an impact on the current section as well and vice versa. Suitability of methods to some extent depends on purpose of the data. If agglomeration/aggregation data are being used for fate and transport modelling, aerosol or liquid based methods are better suited for determining agglomeration/aggregation than microscopy methods, as these are better suited for inline measurements.

18. Transmission electron microscopy (TEM) is suitable for most nanoparticles, provided that they are not affected by the vacuum or electron beam used by this technique. In addition, attention should be paid to the dispersion conditions to avoid (additional) agglomeration on the grid. Furthermore, the pH of the analysis medium could influence the results, as electrode errors are high in media without ionic strength. A disadvantage of TEM is that it is resource intensive and creates two dimensional projections of three dimensional particles. Also large agglomerates are frequently lost in the sample preparation or not readily imaged. In addition, the issues described for particle size distribution may be valid here as well (cf. paragraph 29).

19. Similar to TEM, scanning electron microscopy (SEM) could be suitable for most nanoparticles (especially electron-dense materials), provided that they are not affected by the test conditions. One expert (evaluating nanoclays) stated that the use of vacuum is an important drawback and drying of the sample may induce agglomeration of primary particles[6], thereby making the technique unsuitable for measuring agglomeration state. Environmental scanning electron microscopy (ESEM)[7] would be more appropriate in this case (similar argumentation may be applicable to TEM as well, but TEM was not evaluated by this specific expert). Another expert commented that the limited resolution of the ESEM technique may sometimes render its use less suitable. SEM is not suitable for very small nanoparticles (< 5–10 nm). In comparison to TEM, SEM is less accurate for small nanoparticles, but the sample preparation is easier and in many cases it is more accurate in the vicinity of 100 nm[8]. Like TEM, SEM is also a resource intensive technique, and also the vacuum used in both techniques may interfere with agglomeration state. In addition, the issues described for particle size distribution may be valid here as well (cf. paragraph 29).

20. Dynamic Light Scattering (DLS) is an ensemble light scattering technique that measures the hydrodynamic diameter. It is a suitable technique to assess dispersion stability, but may give misleading results on agglomeration/aggregation, because it cannot distinguish between individual particles and aggregates/agglomerates. Another expert indicated that DLS may still be a useful method for agglomeration/aggregation, provided that besides the maximum, the polydispersity index is given.

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[6] This implies that it is likely that already agglomerated particles will also further agglomerate.

[7] The environmental scanning electron microscope (ESEM) is a SEM that allows for the option of collecting electron micrographs of specimens that are “wet”, uncoated, or both by allowing for a gaseous environment in the specimen chamber.

[8] Most techniques do not have a size cut off that is not tied to other physical parameters, so size cut-offs are generally a range.

21. Atomic Force Microscopy (AFM) has been evaluated for SWCNTs. If performed in solution it can provide reliable data on agglomeration. However, the technique is much more difficult to use under these conditions. Drying of samples may facilitate the use of the technique, but as this has influence on the agglomeration state, this should be avoided. From a round robin study (8) with silicon dioxide nanoparticles it was concluded that a cleaved mica substrate is the preferred reference surface for this technique, because it has the least influence on measurement uncertainty.

22. Finally, measuring turbidity was evaluated for nanoclays. It was indicated as a useful technique for providing a qualitative indication of aggregation. However, this technique is not precise enough to determine the agglomeration state of nanoparticles. In practice, this is usually a first screening method that is followed by more quantitative particle sizing measurements.

23. Other potential techniques for agglomeration/aggregation have been suggested by OECD (1), but these were not used in the Testing Programme to determine aggregation/agglomeration. These methods include Brunauer-Emmett-Teller (BET) surface area[9] and small-angle X-ray scattering (SAXS)[10] to determine mean particle size for solids, and Differential Mobility Analysis (DMA, indicated in the dossier as Scanning Mobility Particle Sizer – SMPS) to determine mean particle size for aerosols. One expert, however, noted that centrifugal liquid sedimentation and analytical centrifugation are the commonly applied and perhaps the most quantitative methods in this area, and ultrasonic spectroscopy was suggested as a fairly good method for the characterisation of agglomeration and aggregation states as well. Evaluation of these methods, however, is beyond the scope of the current document.

## PARTICLE SIZE DISTRIBUTION

24. Particle size distribution was evaluated for silicon dioxide, cerium oxide, silver, titanium dioxide, MWCNTs, dendrimers, and nanoclay. SWCNTs and zinc oxide were not individually evaluated, but the methods used were the same as for other materials. Many of the methods used to determine the particle size distribution are also used for agglomeration/aggregation (e.g., DLS, TEM, SEM). The issues identified for the different techniques used for agglomeration/aggregation (cf. paragraphs 17–23) clearly have an impact on this section as well and vice versa.

25. A general remark regarding the determination of particle size distribution is that there is a potential problem with mass-based methods versus number-based methods to establish size, and the way to interpret their results. A mass-based distribution is generally dominated by a relatively small number of larger and heavier particles, while the number-based size distribution is dominated by the smaller (nano)particles. For high resolution weight based methods this can be mathematically corrected, but it is then often assumed that the particles are more or less spherical. For non-spherical particles such mathematical correction is more difficult and usually not automated. Number-based methods, in particular microscopy methods, often have the disadvantage that only a relatively small number of particles is counted that may not be fully representative for the full size distribution.

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[9] Surface area calculated based on the Brunauer-Emmett-Teller theory (9).

[10] This method was used in the dossier for titanium dioxide, but details on the methodology were not provided in time (i.e. after May 2015) for the current evaluation.

26. The most commonly used method to determine particle size distribution was Dynamic Light Scattering (DLS). DLS is a generally accepted method for particle size distribution for which an ISO standard exists (ISO 22412:2008). This ISO standard lists DLS as a method for the estimation of an average particle size and the measurement of the broadness of the size distribution. The applicability to nanomaterials depends on several factors, both related to the material and to the test setup. DLS can give good information in a narrow size range, and provides three-dimensional information instead of the two-dimensional information from microscopy techniques. As one expert remarked, DLS is generally a reliable method, but is often inappropriately used. The applicability is limited to stable particle suspensions of monomodal and relatively narrowly dispersed size distributions, and shape of the particles plays a role in the interpretation of the results[11]. DLS measures the hydrodynamic diameter which has the advantage of providing three-dimensional and hydrated data (although shape issues still play a role).

27. On the other hand, some drawbacks for DLS were identified as well. Coatings may affect the outcome by giving raise to corona formation, and for organic particles a high concentration is needed, although at very high concentrations, the extent of aggregation exceeds the working range for DLS. Results may further be influenced by the type of solvent (e.g. water vs. alcohol), the dispersion medium (e.g., presence of organic matter, pH), and the model applied for calculating the results (mass/intensity-based vs number-based via mathematical transformation). DLS may give misleading results, because it cannot distinguish between individual particles and aggregates/agglomerates. Furthermore, DLS does not measure particle size directly, but based on several approximations light intensity is converted to particle radius[11]. This may result in missing smaller particles, because their lower light intensity is “overshadowed” by the higher intensity of larger particles.

28. For some materials, Diffusion-Ordered Spectroscopy with Nuclear Magnetic Resonance (DOSY-NMR) can provide atomic level size distributions for any nanomaterial that scatters light (for NMR, C- and H-based molecules are most suited). This technique was evaluated for dendrimers. However, this technique suffers from high detection limits, which makes high particle concentrations (in the range of g/L) necessary. Furthermore, the outcome is influenced by the composition of the particles and depends on field strengths and other factors. Although it is easily done for small dendrimers, it is much harder for larger materials, and also not suitable for a wide range of solid particle types (e.g., paramagnetic materials and those that would result in complex field modifications). Due to the specialised setup, this technique is more useful for research and development purposes than for routine industrial use.

29. Transmission electron microscopy (TEM) and scanning electron microscopy (SEM) were also evaluated as number-based methods for particle size distribution and found generally applicable to nanomaterials as a first investigation of particle size distribution provided by the Feret diameter. However, the sample preparation method may induce agglomeration, and the vacuum applied can alter the dimensions of some materials (e.g., hydrated particles). Their use is therefore, best limited to estimating primary particle size distributions. An additional drawback of TEM is that a relatively high particle concentration is needed, so this technique may not work well at environmentally relevant concentrations[12]. For materials with low atomic mass, contrast can be problematic. As also indicated in the evaluation of Aggregation/Agglomeration, the preparation of the sample is important and dispersion protocols should be described. One of the experts recommends to reduce artefacts by preparing TEM samples in hydrophilic resins, a technique that is currently rarely performed. From a round robin study (8) with silicon dioxide nanoparticles it was concluded that the application of a conductive sample coating is not recommended, as the thickness of the coating significantly influences the measured particle size distribution. For the use of

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[11] Mathematical models to interpret the results generally assume spherical particles, which may lead to (high) uncertainties and/or mistakes if this is not the case (e.g. for rod shaped particles).

[12] This statement may be true for other techniques as well, but for the use of TEM for particle size distribution it was specifically indicated by the evaluating expert.

TEM, the same study concluded that nanoparticles should preferably be collected by direct deposition on a TEM grid, rather than transferring them from a nucleoporous membrane onto a TEM grid.

30. Differential Mobility Analysis (indicated in the dossier as Scanning Mobility Particle Sizer – SMPS) was evaluated for cerium oxide and titanium dioxide. This number-based technique can be used for measuring aerosols and suspensions. However, analysis for non-spherical nanoparticles (and to some extent agglomerated nanoparticles) is difficult[13] and may require tandem differential mobility analysers. Another expert indicated that differential mobility analysis has fewer difficulties with agglomerated nanoparticles than most other methods.

31. Laser Diffraction is a light scattering method that was evaluated for MWCNTs and considered not particularly useful for small nanomaterials, but more suitable for larger materials, i.e. primarily above 50 nm[14]. Suitability depends on relative refractive index and instrument employed.

32. Centrifugal Liquid Sedimentation Disc Centrifuge (CLS) was evaluated for cerium oxide. One expert indicated that this mass based technique may be usable, although the forces used could introduce clustering of particles (depending on the type of CLS used). Because the readout is mass-based, it could be prone to calibration errors and errors in shape and size estimates. Another expert stated that centrifugation/sedimentation methods are generally considered gold standards and first principle measurements. Sometimes knowledge on the movement of a particle has more meaning than physical dimension approximated via microscopy. This technique was also used for studying the effects of different sonication methods on the particle size distribution of nanoclay. These sonication pre-treatments hampered proper evaluation of the CLS technique for particle size distribution of nanoclay. ISO has published several standards for this technique (ISO 13318-1:2001; ISO 13318-2:2007; ISO 13318-3:2004).

33. Other techniques to measure particle size distribution are available (10), but these have not been applied for particle size distribution in the Testing Programme and evaluation of these methods is beyond the scope of this document. For some of them standardised methods are available. These methods include optical microscopic examination, sieving, sedimentation (gravitational settling), electrical sensing zone (e.g., Coulter) method, phase Doppler anemometry SEM, ultrasonic spectroscopy (ISO/20998-1:2006), small angle X-ray scattering (SAXS) (ISO/TS 13762:2001)[15], and X-ray diffraction (XRD) (BS EN 13925-1, BS EN 13925-2 and BS EN 13925-3). Some of these techniques have been applied in the Testing Programme for other parameters (e.g. XRD for crystallinity, SAXS for specific surface area). In addition, experts suggested field-flow fractionation (combined with a detection method), AFM (cf. paragraph 21), particle tracking analysis, and single particle ICP-MS[16] as potentially useful.

34. According to experts' opinions, characterisation of particle size distribution of unknown materials should preferably be performed by microscopy (TEM/SEM), while DLS can be used for characterising particles of known shape (taking its limitations on certain shapes into account, and probably some size fractionation prior to DLS is needed as well). For aerosols, DMA can be used and it is more and more commonly used for sizing particles from suspensions.

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[13] This statement may be true for other techniques as well, but for the use of DMA for particle size distribution it was specifically indicated by the evaluating expert.

[14] Most techniques do not have a size cut off that is not tied to other physical parameters, so size cut-offs are generally a range.

[15] This method was used in the dossier for titanium dioxide, but details on the methodology were not provided in time (i.e. after May 2015) for the current evaluation.

[16] For this method a draft standard was developed by ISO (ISO/DTS 19590).

35. One of the experts indicated the existence of a round robin study by the Versailles Project on Advanced Materials and Standards (VAMAS). In this study airborne silicon dioxide nanoparticles were generated and the particle size distribution of the aerosols was assessed by different methods (8). Detailed protocols are described for the generation of the aerosols, online measurement of the particle size distribution by DMA, and offline measurement of the particle size distribution by TEM, SEM, and AFM. Measured particle size distributions showed good inter-laboratory agreement, as well as good agreement between results measured by the four different techniques. To improve reproducibility some specific recommendations are provided on sample preparation, transport and measurement conditions, and computational analysis.

## CRYSTALLINE PHASE

36. Crystalline phase was evaluated for silicon dioxide, titanium dioxide, and zinc oxide. For all these materials XRD was used. XRD was also used for silver and gold. Crystallite size is often determined as well with the same method (see paragraphs 74–76).

37. X-ray diffraction (XRD) is a generally accepted method to determine crystallinity of a substance. The technique is suitable for powder forms of nanomaterials. For zinc oxide, the method was evaluated for coated (triethoxycaprylsilane) as well as uncoated forms. The presence or absence of a coating had no impact on the capacity of XRD to determine crystalline phase. However, as the method is not standardised, a detailed description of the methodology should be included in the study report.

38. For two of the SWCNT tested, Raman spectral analysis was used. In principle, Raman can be used for CNTs and graphene to determine the extent of order/disorder (presence of defects). The technique has been used for determination of crystallinity of different carbon based nanomaterials. However, the specific information in the evaluated dossier was insufficient to allow evaluation of the method.

39. Similarly, TEM and SEM were used for the endpoint “crystalline phase” for two of the MWCNTs. However, evaluation of these methods for measuring crystalline phase was not possible because the information given in the evaluated dossier was too limited.

40. For one form of titanium dioxide, Scanning Transmission Electron Microscopy (STEM) coupled with Energy Dispersive X-ray (EDX) was used for physico-chemical characterisation. The results gave no information for “crystalline phase”, however, and the method was considered not suitable for this parameter.

## DUSTINESS

41. Methods for dustiness were evaluated for all available data (MWCNT, SWCNT, silicon dioxide, cerium oxide, zinc oxide, titanium dioxide, and fullerenes).

42. The rotating drum technique is a standardised method to evaluate dustiness[17] under EN-15051:2006. It is suitable for nanomaterials that are in powder form. The method provides results on health-relevant size dustiness, based on mass. The small rotating drum is a down-scaled version of the rotating drum (12). In the applied set-up respirable dust is collected by a cyclone and particle size distributions are measured both in the submicron and the micron size area (6) (13). The advantage of the down-scaled version is that smaller amounts of nanomaterial powder are needed to perform the test. The small rotating drum has similar properties to the rotating drum under EN-15051:2006 and the results have shown good agreement with the ones obtained by the EN-15051:2006 version. The small rotating drum should be applicable to all powder forms of nanomaterials. However, the small rotating drum is not standardised yet. In one of the dossiers (titanium dioxide), recommendations were given on harmonisation of operation procedures, sampling and measurement methods to enable round-robin tests and comparison between test methods.

43. The continuous drop method is also part of the standardised methods under EN-15051:2006 and a continuous drop dustiness tester is available. Like the rotating drum method, it is suitable for powder forms of nanomaterials and presents a health-relevant mass-based dustiness index. However, this method is less suitable for powders that are sensitive to caking and for fluffy powders, because these may be affected by the feeder system, resulting in discontinuous drop of the powder in the tube.

44. The vortex shaker method represents the evaluation of dustiness under relatively high drag forces. ISO/TS 12025 describes the advantages of this method as “it is easy, simple, and compact, is constructed from generic lab items, and can continuously generate particles (several tens of minutes or more) with a small amount (approximately 1 $cm^3$ or less) of sample powder.” However, the main disadvantage of this method is that it is not clear whether the resulting dustiness index is representative for health-relevant dustiness. Furthermore, the method is not standardised. For these reasons this method was considered a less suitable technique. In one of the dossiers (titanium dioxide), recommendations were given on harmonisation of operation procedures, sampling and measurement methods to enable round-robin tests and comparison between test methods.

45. Currently, it is under discussion whether additional metrics (other than mass) should be added to the dustiness index, e.g. particle size distribution or total particle number concentrations.

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[17] Dustiness is defined as the propensity of a material to emit dust during agitation; it is important to note that dustiness is not an intrinsic physical or chemical defined property of a powder and its level depends on e.g. characteristic properties of the powders and the activation energy in the simulated handling, and thus different values may be obtained by different test methods. A European standard (EN15051) has been established containing two methods (the rotating drum and continuous drop methods); however, EN15051 is not fully suitable for nanomaterials, as also stated in EN 15051. Other procedures are therefore currently under investigation. Specifically for nanomaterials ISO/TS 12025:2012 describes several dustiness methods, including vortex shaker, rotating drum and continuous drop methods. Furthermore, the OECD Guidance on Sample Preparation and Dosimetry for the Safety Testing of Manufactured Nanomaterials (11) also contains some noteworthy descriptions regarding dustiness testing.

## SPECIFIC SURFACE AREA

46. Methods to determine specific surface area were evaluated for silicon dioxide, zinc oxide, cerium oxide, titanium dioxide, and MWCNTs. BET was used in these evaluated dossiers, and in addition was also used for SWCNTs, fullerenes, gold, and nanoclay.

47. For all of the indicated nanomaterials, Gas adsorption by BET technique[18] was used. This method is standardised under ISO (ISO 9277:2010). The method is applicable to all nanomaterials that do not absorb the gas used and are either disperse, nonporous, mesoporous with pore diameter between 2–50 nm, or macroporous solids. The technique is not suitable for substances which are microporous solids, although a specific annex (Annex C) is included in ISO 9277:2010 which contains a strategy for measurement of surface area for these substances as well. For some of the evaluated dossiers the level of reporting could be improved.

48. In two dossiers (titanium dioxide and silicon dioxide) the use of Small Angle X-ray Scattering (SAXS) was reported and results obtained by SAXS were compared with those obtained by BET. The specific surface area measured by SAXS did not correlate with that measured by BET and therefore SAXS is not recommended as a primary method to measure specific surface area.

49. ISO 18757:2005, which is listed by ECHA (10), describes a technique for determination of the total specific external and internal surface area of disperse or porous (pore diameter > 2 nm) fine ceramic materials. This method was not used in the testing programme and its evaluation is therefore beyond the scope of the current document.

## WATER SOLUBILITY / DISPERSIBILITY

50. Information on water solubility / dispersibility[19] was available in the dossiers for silicon dioxide, zinc oxide, titanium dioxide, cerium oxide, gold and SWCNTs. The information available in the dossier of SWCNTs was insufficient to enable evaluation of the methods used.

51. For silicon dioxide the shake flask method was evaluated. This method is standardised in OECD TG105 (14). The same method was used for gold. The method was found suitable, but needs further validation for nanomaterials. In the OECD-WPMN Expert Meeting on Ecotoxicology and Environmental Fate of Manufactured Nanomaterials experts identified several limitations of OECD TG105 (3). As a result, a project is currently running in which new OECD TGs and an accompanying GD on agglomeration and dissolution behaviour are being developed.

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[18] Surface area calculated from gas adsorption, based on the Brunauer-Emmett-Teller theory (9).

[19] Strictly speaking water solubility and dispersibility are different parameters, but in practice it is difficult to distinguish between the two in an experimental set-up. In particular where nanoparticles are small, available methods can often not distinguish between solute and dispersed particles. Also in the evaluated dossiers, water solubility and dispersibility (and hydrophilicity) are often reported under the same heading, where a distinction between the two is not always clear.

52. Another method used for silicon dioxide is based on spectrometry and labelling silicate with a colouring agent (malachite green) (15). The method was found suitable for silicon dioxide, but it needs to be determined if the indicator can be used for other nanomaterials.

53. For zinc oxide, results from the PROSPEcT project[20] were evaluated (16). Here dissolution of the nanomaterial in colloidal suspension was determined (expressed as a proportion of the total mass). Different fractions were obtained by filtration and centrifugation and were analysed by ICP-MS at several time intervals. A similar methodology was used for titanium dioxide and cerium oxide. The method should be applicable to soluble forms that are available to be dissolved, as well as for other dispersible and soluble nanomaterials where there is an analytical method to determine the soluble species. The suitability should be determined for nanomaterials that are embedded in a matrix (e.g. cream).

54. Other methods were also available in the dossier for zinc oxide (e.g. turbidity meter based on light scattering, colourimetry), but insufficient details on the method were given for a proper evaluation.

55. For silver, studies were focussed on the dispersion stability of silver nanoparticles using ELS[21] spectrophotometer and TEM instead of determining water solubility. These methods were not further evaluated for their applicability to measuring the water solubility parameter.

## ZETA POTENTIAL (SURFACE CHARGE)

56. Laser-Doppler electrophoresis was evaluated for a wide range of nanomaterials (cerium oxide, zinc oxide, silicon dioxide, titanium dioxide, silver, gold, SWCNTs and MWCNTs). The method was found to be suitable for stable and disperse suspensions of nanomaterials. This means that it would be unsuitable for hydrophobic nanomaterials in aqueous media, or application in high conductivity media (e.g. seawater). Furthermore, in the evaluation of this method in the gold dossier it was mentioned that citrate coating may impact the zeta potential measurement when diluting in water, and thus diluting in equilibrium supernatant was recommended.

57. Electrophoretic light scattering (ELS) was evaluated to be very appropriate for silver nanoparticles (NM-300). In general, it is a standard method to measure zeta potential (ISO 13099-2:2012) and it is suitable for all nanoparticles that can be dispersed in a liquid. A similar methodology was used for nanoclay, but not specifically evaluated.

58. In addition, DLS (also indicated as photon correlation spectroscopy – PCS) was used to determine zeta potential of titanium dioxide, but insufficient information was provided in the dossier to enable evaluation of the method used.

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[20] Further information on this project is available at www.nanotechia.org/activities/prospect-ecotoxicology-test-protocols-representative-nanomaterials-support-oecd.

[21] Electrophoretic light scattering is a technique that is based on dynamic light scattering. In the case of dynamic light scattering, Brownian motion causes particle motion. In the case of electrophoretic light scattering, oscillating electric field performs the same function.

## PHOTOCATALYTIC ACTIVITY

59. Photocatalytic activity is difficult to assess, and many methods can only determine an “apparent photocatalytic activity”. It is sometimes difficult to translate the outcome from probes to different particles with different affinities to the probe molecules, due to the interplay between surface affinity/adsorption and photocatalytic activity.

60. Rhodamine-B dye degradation in the presence of nanomaterials under simulated sunlight (150 W Xenon lamp) using UV-Vis spectroscopy was evaluated for zinc oxide and cerium oxide and was found to be suitable and sufficient for measuring photocatalytic activity of nanomaterials in light-coloured suspensions. The difference in activity of the nanomaterials can be discriminated. However, this technique is unsuitable for nanomaterials that form coloured suspensions, as this interferes with the UV-Vis spectroscopy. In such cases centrifugal ultrafiltration is typically used to remove nanoparticles and allow for analysis of the dye degradation.

61. Similar to Rhodamine-B dye degradation, DPPH degradation measurement under UV light is suitable for nanomaterials in lightly coloured suspensions, but not for nanomaterials in coloured suspensions.

62. Hydroxyl radical generation under UV light, measured with electron paramagnetic resonance (EPR) and Orange II degradation under UV exposure, detected by UV-Vis spectroscopy both gave good results for titanium dioxide nanoparticles, but there was not sufficient information available in the dossier to evaluate its suitability to other nanoforms or nanoparticles.

63. A standardised method (ISO 22197-2) was evaluated for SWCNTs, MWCNTs, and fullerenes. This method determines degradation of acetaldehyde in the presence of nanomaterials, measured under UV light and $CO_2$, using gas chromatography. Apart from carbon nanomaterials the expert indicated the method to be suitable for oxide forms of nanomaterials. However, the method was found to be insufficient for quantitative measurements, which may be improved by optimisation of the sample preparation.

## POROSITY

64. Two widely accepted methods for measuring porosity, both standardised under ISO 15901-2 (mesopore analysis by gas adsorption), have been evaluated. Gas adsorption, modelled by BJH method (17) was evaluated for zinc oxide and cerium oxide and Gas adsorption, modelled by BET method (9) was evaluated for silicon dioxide. Both methods were found suitable for non-microporous nanomaterials, but not for microporous nanomaterials, because in the latter situation the true surface may be underestimated.

65. It is important to realise that the values obtained from these gas adsorption modelling methods depends on the pressure and temperature during the test, as well as on the model used to calculate the results. These parameters should be accurately described in the report.

66. Mercury porosimetry was evaluated for SWCNTs, and MWCNTs. In addition it was used for fullerenes, but an expert indicated that for fullerenes porosity is hardly relevant, unless a sample contains

(amorphous) impurities. This method is standardised under ISO 15901-1 (mercury porosimetry). The method is valid for studying porosity of nanomaterials, except for metal-containing nanomaterials, because mercury can react (form amalgams) with the metals contained in the nanoparticles[22]. Another limitation of mercury porosimetry is the physical limits to the pore size that can be analysed, which may result in measuring inter-particle porosity only, and not intra-particle porosity.

67. OECD (1) suggested additional techniques for measuring porosity, including micropore analysis by gas adsorption (standardised under ISO 15901-3) and dye absorption techniques, but these were not applied in the testing programme and their evaluation is therefore beyond the scope of the current document.

## REDOX POTENTIAL

68. The two methods for measuring redox potential that were evaluated for zinc oxide, cerium oxide and silicon dioxide are both unsuitable for measuring redox potential of nanomaterials. The first method, potentiometry, is more sensitive to ions and other solubilised substances in the test medium than to the nanomaterials themselves. The second method, OxoDish® $O_2$-detection, comprises the monitoring of oxygen levels inside a 24-well plate during 24-hour incubation using a fluorescence method. It was found unsuitable for measuring redox potential in nanomaterials, because the test endpoint is oxygen level and not redox potential. Although dissolved oxygen may correlate with redox potential, this is not always the case.

69. Some possible alternate methods for (indirect) measurement of redox potential were suggested by the expert in the evaluation of the potentiometry method. These include X-ray photoelectron spectroscopy, electron paramagnetic resonance, electron energy loss spectroscopy, monitoring of ion solubility upon applied voltage, monitoring of surface transformation, and cyclic voltammetry (for metallic nanomaterials).

## RADICAL FORMATION POTENTIAL

70. The use of Benzoic acid PBS was evaluated for different forms of silicon dioxide. This technique comprises the formation of 4-hydroxy-benzoic acid in a phosphate buffered hydrous solution (PBS), as detected via HPLC-UV. This test assumes that hydroxyl radicals are the principle free radicals initiated from the particle surface, which may not always be a valid assumption. The test was found unsuitable for the evaluated nanomaterials. A comprehensive motivation of the limitations in the suitability of this test was given by the evaluating expert: "*For the detection of free radicals, a suitable interaction with the surface has to occur with the analyte. Phosphates have the potential to block, passivate or turn over*

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[22] In most cases, CNTs contain metal catalyst from the synthesis, which may impact the usefulness of this method for these types of nanomaterials as well.

*surface silanol sites. Adsorption/interaction of benzoic acid with silica under these conditions is also unknown. Hence, the results may be an artefact of the sample preparation and analysis conditions. Free radical generation in a pure state as well as in a confounded or exposure related state should both be measured. The dossier provides little information regarding the test other than that it was negative. Other results in the literature for SAS* [synthetic amorphous silica] *via ESR show detectable hydroxyl radical response levels (e.g., see Zhang et al. 2012* (18)*). Hydroxyl radical activities of similar SAS materials have been shown to depend on synthesis route and hydroxyl ring structure by Zhang et al. 2012. Those measurements were not performed in PBS, but highlight the need for identifying a meaningful conditioning method when determining surface reactivity endpoints. The impact of PBS and the use of benzoic acid as the indicator for hydroxyl radical generation for silica are still not clear. Direct comparisons with ESR prior to broad implementation would be useful*”.

71. For zinc oxide and cerium oxide a method based on measuring the oxidative activity by reactive oxygen species (ROS) using potassium iodide and measuring the optical absorbance was identified. This method was found suitable for nanomaterials that generate hydroxyl radicals ($OH^{\bullet}$).

72. In addition, it was indicated that electron paramagnetic resonance (EPR) / electron spin resonance (ESR) is broadly applied and is regarded as one of the better methods for free radical determination. It is suitable for a broad range of nanomaterials including zero valent iron, various forms of iron oxide, silicon dioxide, titanium dioxide, zinc oxide, cerium oxide, SWCNTs, MWCNTs and fullerenes. However, the method is not standardised, and to reflect exposure scenarios the sample conditions need careful consideration.

73. A general question was raised about which specific radicals should be identified to determine the radical formation potential of a nanomaterial. Furthermore, evaluation was sometimes hampered by a lack of details in the reporting.

## CRYSTALLITE SIZE

74. Crystallite size is often related to crystalline phase (paragraphs 36–40). For some of the materials crystallite phase and crystallite size were determined by the same method, i.e. XRD.

75. For cerium oxide, titanium dioxide and zinc oxide, X-ray diffraction (XRD) was used to determine crystallite size. This method, although not standardised, is regarded as generally suitable to measure both crystalline phase and crystallite size. Nevertheless some caution is warranted. For most materials only crystallites sizes under 100 nm can be detected and assumptions must be made in the analysis (e.g. mathematical analysis of the XRD signal).

76. For some materials (silicon dioxide, gold) the crystalline phase determination indicated that the materials are amorphous and thus it was not relevant to determine crystallite size. For other materials (silver, fullerenes) the evaluating experts indicated that the details in the dossiers were insufficient to evaluate the method.

## SURFACE CHEMISTRY

77. Surface chemistry was evaluated for SWCNTs, nanoclays and dendrimers. Different techniques were used, including x-ray photoelectron spectroscopy (XPS), energy-dispersive x-ray spectroscopy (EDX), and liquid chromatography interfaced to a hybrid quadrupole/time-of-flight mass analyser. For all of these techniques the evaluating expert indicated that it may be difficult to separate surface chemistry from that of the core material (high concentrations/masses may be required), which strongly limits the use of these techniques for surface chemistry[23]. Another expert acknowledges the short-comings, but indicates that XPS is a well-defined reliable method and "at the moment this seems to be the only useable method for this endpoint". Where the core material is known a mixed signal can be corrected for the signal of the core. Furthermore, a third expert indicate that for true surface sensitive methods XPS (<10 nm), the related Auger electron-spectroscopy method (<10 nm AES) and Time-of-Flight-Secondary-Ion-Mass-Spectrometry (<2 nm) (ToF-SIMS) are the most commonly available methods which could be considered as giving information which is closer to being truly "surface" relevant. XPS was also used for silicon dioxide, cerium oxide, zinc oxide, and titanium dioxide, but for these materials the method was not evaluated.

## CONCLUSIONS

78. Many of the methods used to determine the physico-chemical parameters were thoroughly evaluated by only one expert, although in the reviewing process other experts added additional views. Furthermore, methods were not always evaluated for all materials. Nevertheless, some conclusions can be drawn based on the information provided, taking note that these conclusions may not always represent a consensus of the experts. Furthermore, it should be emphasised that the assessment only involved evaluation on whether a specific method applied to determine a certain physico-chemical property is suitable for the specific parameter, either for a specific nanomaterial or for a (broad) range of different nanomaterials. The usefulness of the different parameters for risk assessment was not assessed. Neither should information in this report be seen as preference for a certain method to be used in risk assessment, as the focus is on methods used in the Testing Programme and other suitable methods may exist as well. Furthermore, suitability of a certain method may strongly depend on the material considered as well as the purpose of the use of a certain parameter in risk assessment.

79. For most parameters, one or more suitable methods appear to be available that can be used for nanomaterials (summarised in Table 2), although some of the methods may be applicable only to a subset of nanomaterials or under certain conditions. Suitable and widely accepted methods are available for a broad range of nanomaterials to measure the chemical composition, aggregation and agglomeration, particle size distribution, crystalline phase, dustiness, water solubility, zeta potential, porosity, radical formation potential and crystallite size. However, most of these methods (including sample preparation)

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[23] For example, XPS measures the top surface layer (0–10 nm), which may comprise both surface chemistry and chemistry of the core material. Where a surface modification is applied, the technique may give a mixed signal, but such mixed signals can be recalculated, if the core material is known.

are not standardised (yet) or not standardised for nanomaterials, which generally hampers their use in risk assessment of nanomaterials.

80. Standardised methods for measuring specific surface area, zeta potential and porosity are available, as well as non-standardised methods for measuring photocatalytic activity. These methods have been found to be applicable only to some of the different types of nanomaterials. For redox potential, experts indicated that all evaluated methods were unsuitable for nanomaterials, but suggestions by the experts could be further explored. Similarly additional methods suggested by the evaluating experts for other parameters could be further explored[24].

81. It must be noted that in a number of the dossiers, important information was missing regarding sample preparation and test conditions. This severely hampered the evaluation of the specific methods. In addition, in some cases, adaptions to the methods used have been described in the dossier or have been recommended by the expert's evaluation, often related to sample preparation. This highlights the importance of a detailed and complete description of the methodology used when reporting physico-chemical results.

## RECOMMENDATIONS

82. While recognising the limitations of the current evaluations of methods (e.g. a limited number of experts provided input), some general recommendations can be made. First of all the evaluations showed that many of the methods have limitations, which to some extent can be addressed by developing (sufficiently) standardised methods.

83. Based on the overall conclusions in the previous section it is recommended to prioritise some of the methods for further work towards standard test methods. As sample preparation may have an influence on the outcomes (11) a well-standardised sample preparation protocol (including dispersion) is needed and this should be taken into account when developing these methods[25]. Furthermore, detailed reporting of methods and data is needed, which should be addressed in standardised method protocols.

84. It is recognised that ISO and CEN standards are available for some of the methods and that others (e.g. ISO) may already have started activities on (some of) them. This includes the following methods[26]:

- Dynamic Light Scattering (DLS) for measuring particle size distribution[27], with special focus on whether it is suitable for number distributions.

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[24] Although highly recommended, such exploration goes beyond the scope of the current evaluation.

[25] It should be acknowledged that each specific method for determining a physico-chemical parameter may need its own separate sample preparation protocol.

[26] This list of methods is based on the current evaluation and not intended as being exhaustive, since other methods, not being used in the Testing Programme, may be available as well.

[27] Specifically for this method, unreliable results are often due to flaws in sample preparation and other methodological aspects. Tackling these aspects in a proper method description appears feasible. An ISO standard is available (ISO 22412:2008).

- Centrifugal Liquid Sedimentation (CLS) methods for particle size distribution[28].
- Differential Mobility Analysis (DMA) methods for particle size distribution.
- Energy dispersing X-ray analysis (EDX) for measuring chemical composition.
- Inductively coupled plasma/optical emission spectrometry (ICP/OES) for measuring chemical composition. Instead of OES mass spectrometry (MS) may also be useful[29].
- Transmission electron microscopy (TEM) for measuring aggregation and agglomeration and particle size distribution[30].
- Scanning electron microscopy (SEM) for measuring aggregation and agglomeration and particle size distribution[31].
- Atomic Force Microscopy (AFM) for measuring aggregation and agglomeration.
- X-ray diffraction (XRD) for measuring crystalline phase and crystallite size.
- Small rotating drum for measuring dustiness, as a supplement to standardised rotating drum and continuous drop tester (EN 15051-1:2013, EN 15051-2:2013 and EN 15051-3:2013).
- Electron paramagnetic resonance (EPR)[32] / electron spin resonance (ESR) for measuring radical formation potential.

85. For the development and validation of standard test methods, development of reference materials is also strongly recommended. In addition, certain parameters are non-intrinsic, i.e. parameters are influenced by the surrounding medium. For such properties, efforts are needed to define standardised media as well, including standardised “biologically or experimentally relevant” media. The tests in “standard” media are used to calibrate the material or handling of the material with other studies. The tests in “experimentally relevant” media provide information of the material behaviour as it pertains to the specific study.

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[28] ISO has published several standards for this technique (ISO 13318-1:2001; ISO 13318-2:2007; ISO 13318-3:2004).

[29] Both CEN/TC 352 and ISO/TC 229 are developing a document for single particle ICP-MS.

[30] Both CEN/TC 352 and ISO/TC 229 are developing a document for TEM methods.

[31] CEN/TC 352 is developing a document for SEM methods. ISO/TC229 considers developing a document for SEM methods.

[32] ISO/TC 229 WG3 is developing a document for the electron paramagnetic resonance method.

**Table 2 – Overview of suitability per parameter of methods used in the Testing Programme according to the current expert evaluations[33]. This table should be seen as a summary. Details are provided in the text.**

| Parameter | Suitable method(s) for a broad range of nanomaterials in general | Suitable method(s) for certain types of nanomaterials only | Method(s) found not suitable | Remarks (an overview of abbreviations used is given in Annex III) |
|---|---|---|---|---|
| Chemical composition | • XPS | • ICP/OES<br>• EDX |  | • ICP/OES is a generally accepted method for the detection of trace metals, but not all elements can be detected by this method.<br>• EDX is typically suitable for elements above carbon, but is unsuitable for nanoparticles of complex composition, in complex matrices, and for large aggregates.<br>• Evaluation of XPS for measuring surface chemistry suggested that it is a suitable method for determining chemical composition, provided that the surface and the core show the same chemistry (i.e. the nanomaterial is not coated). |

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[33] Many of the methods used to determine the physico-chemical parameters were thoroughly evaluated by only one expert, although in the reviewing process other experts added additional views. Consequently, conclusions may not always represent a consensus of the experts. Furthermore, it should be emphasised that the assessment only involved evaluation on whether a specific method applied to determine a certain physico-chemical property is suitable for the specific parameter, either for a specific nanomaterial or for a (broad) range of different nanomaterials. The usefulness of the different parameters for risk assessment was not assessed, nor should the information in this table be seen as a preference for a certain method to be used in risk assessment, as the focus is on methods used in the Testing Programme and other methods may exist as well. Furthermore, suitability of a certain method may strongly depend on the material considered as well as the purpose of the use of a certain parameter in risk assessment.

| Parameter | Suitable method(s) for a broad range of nanomaterials in general | Suitable method(s) for certain types of nanomaterials only | Method(s) found not suitable | Remarks<br>(an overview of abbreviations used is given in Annex III) |
|---|---|---|---|---|
| Aggregation and agglomeration | • AFM | • TEM<br>• SEM<br>• DLS | • Turbidity | • AFM can provide reliable data if performed in solution.<br>• TEM is suitable for most nanoparticles, provided that they are not affected by the vacuum or electron beam used, because there is a risk of agglomeration on the grid. A disadvantage of TEM is that it creates two dimensional projections of three dimensional particles.<br>• SEM has similar restrictions as TEM In comparison to TEM, SEM is less accurate for small nanoparticles, but the sample preparation is easier and in many cases it is more accurate in the vicinity of 100 nm[a]. As an alternative for SEM ESEM was suggested.<br>• DLS cannot distinguish between individual particles and aggregates or agglomerates, but may still be useful, provided that besides the maximum, the polydispersity index is given.<br>• Turbidity method is not precise enough (can only give qualitative information). It may be used as a first screening method that is followed by more quantitative particle sizing measurements.<br>• In general, when used for fate and transport modelling, aerosol or liquid based methods are better suited for determining agglomeration/aggregation than microscopy methods. |

| Parameter | Suitable method(s) for a broad range of nanomaterials in general | Suitable method(s) for certain types of nanomaterials only | Method(s) found not suitable | Remarks<br>(an overview of abbreviations used is given in Annex III) |
|---|---|---|---|---|
| Particle size distribution | • CLS<br>• DLS<br>• DMA<br>• TEM<br>• SEM | • DOSY-NMR | • Laser Diffraction | • CLS is mass-based and may be prone to errors in calibration and in size and shape estimates. Nevertheless, it is generally considered gold standard and first principle measurement.<br>• DLS was the most commonly used method and an ISO standard exists (ISO 22412:2008). The applicability is limited to stable particle suspensions of monomodal and relatively narrowly dispersed size distributions, and shape of the particles plays a role in the interpretation of the results. Also other methodological issues were identified (see paragraphs 26–27).<br>• DOSY-NMR requires particle concentrations in g/L range. Its applicability is limited to a few types of nanomaterials only (e.g. small dendrimers), and not suitable for a wide range of solid particle types.<br>• TEM is best limited to estimating primary particle size distributions. It may not work well at environmentally relevant concentrations[b]. A disadvantage of TEM is that it creates two dimensional projections of three dimensional particles.<br>• SEM has similar limitations as TEM. In comparison to TEM, SEM is less accurate for small nanoparticles, but the sample preparation is easier and in many cases it is more accurate in the vicinity of 100 nm[a].<br>• DMA can be used for aerosols and suspensions, but analysis for non-spherical nanoparticles is difficult[b]. It may have fewer difficulties with agglomerated nanoparticles than most other methods.<br>• Laser diffraction is not suitable for nanomaterials, but more appropriate for larger materials, i.e. primarily above 50 nm[a].<br>• In general, reproducibility of particle size distribution measurements can be improved by standardising of sample preparation, transport and measurement conditions, and computational analysis. |

| Parameter | Suitable method(s) for a broad range of nanomaterials in general | Suitable method(s) for certain types of nanomaterials only | Method(s) found not suitable | Remarks (an overview of abbreviations used is given in Annex III) |
|---|---|---|---|---|
| Crystalline phase | • XRD | | • Raman spectral analysis<br>• TEM<br>• SEM | • XRD is a generally accepted and suitable method.<br>• Raman spectral analysis, TEM, and SEM may in principal be useful for this parameter, but available information was insufficient for evaluation. |
| Dustiness | • Rotating drum<br>• Small rotating drum | • Continuous drop tester | • Vortex shaker | • The rotating drum is a standardised method[c] and provides results on health-relevant dustiness, based on mass.<br>• The small rotating drum is not standardised (yet), but appears to show similar results as the rotating drum.<br>• The continuous drop tester[c] is less suitable for powders that are sensitive to caking and for fluffy powders.<br>• The vortex shaker is considered easy, simple, and compact, but the resulting dustiness index is not representative for health-relevant dustiness. |
| Specific surface area | • BET | | • SAXS | • BET[d] is suitable for nanomaterials that do not absorb the gas used. For nanomaterials that are microporous solids specific adaptations are needed.<br>• SAXS is not recommended as a primary method to determine specific surface area. |
| Water solubility / Dispersibility[e] | • Shake flask method | • Spectrometry<br>• Filtration & centrifugation | | • The Shake Flask method needs further evaluation for nanomaterials[f].<br>• Spectrometry was found suitable for $SiO_2$, but needs to be evaluated for other nanomaterials.<br>• Filtration and centrifugation[g] is applicable to soluble nanomaterials. Suitability for nanomaterials embedded in a matrix should be determined. |

| Parameter | Suitable method(s) for a broad range of nanomaterials in general | Suitable method(s) for certain types of nanomaterials only | Method(s) found not suitable | Remarks (an overview of abbreviations used is given in Annex III) |
|---|---|---|---|---|
| Zeta potential | - ELS | - Laser-Doppler electrophoresis | | - ELS[h] is suitable for all nanomaterials that can be dispersed in a liquid.<br>- Laser-Doppler electrophoresis is not suitable for hydrophobic nanomaterials in aqueous media, or application in high conductivity media. |
| Photocatalytic activity | | - Rhodamine-B<br>- DPPH<br>- Hydroxyl generation under UV-light + EPR<br>- Orange II degradation + UV-Vis | - Degradation of acetaldehyde | - Rhodamine-B and DPPH methods are unsuitable for nanomaterials that form coloured suspensions. In such cases centrifugal ultrafiltration is typically used to remove nanoparticles and allow for analysis of the dye degradation.<br>- For hydroxyl generation and orange II degradation good results were reported for titanium dioxide, but insufficient information was available to evaluate these methods for other nanomaterials.<br>- Degradation of acetaldehyde was suitable for oxide forms of nanomaterials, but yields insufficiently quantitative results.<br>- In general photocatalytic activity is difficult to assess, and many methods can only determine an “apparent photocatalytic activity”. |
| Porosity | - BET/BJH | - Mercury porosimetry | | - BET/BJH[i] methods are not suitable for microporous nanomaterials.<br>- Mercury porosimetry[j] is not suitable for metal-containing nanomaterials and has similar limitations with small pores as BET/BJH.<br>- In general, interpretation of results for all indicated methods strongly depends on pressure and temperature during the test, and on the model used. |
| Redox potential | | | - Potentiometry<br>- Oxo-Dish $O_2$-detection | - The potentiometry method is more sensitive to ions in the test medium than to the added nanomaterials.<br>- Oxo-Dish measures oxygen levels, which may not correlate with redox potential. |

| Parameter | Suitable method(s) for a broad range of nanomaterials in general | Suitable method(s) for certain types of nanomaterials only | Method(s) found not suitable | Remarks<br>(an overview of abbreviations used is given in Annex III) |
|---|---|---|---|---|
| Radical formation potential | • EPR / ESR | • Potassium iodide and optical absorbance | • Benzoic acid PBS | • EPR / ESR is regarded as a suitable method for a broad range of nanomaterials.<br>• Potassium iodide and optical absorbance was found suitable for nanomaterials that generate hydroxyl radicals.<br>• Results from benzoic acid PBS may be an artefact of the sample preparation and analysis conditions.<br>• A general question is which specific radicals should be measured to identify the radical formation potential or a nanomaterial. |
| Crystallite size | • XRD | | | • XRD is a generally accepted and suitable method, but for most materials only crystallites sizes under 100 nm can be detected. |
| Surface chemistry | | • XPS | • EDX<br>• Liquid chromatography | • All of these methods have difficulties in distinguishing between properties of the core and of the specific surface.<br>• Nevertheless, at the moment XPS (which measures the top surface layer 0–10 nm) seems to be the only useable method for this endpoint and correction for a known core material may be possible. |

[a] Most techniques do not have a size cut off that is not tied to other physical parameters, so size cut-offs are generally a range.

[b] This statement may be true for other techniques as well, but for this technique it was specifically indicated by the evaluating expert.

[c] Standardised under EN-15051-1:2013, EN 15051-2:2013 and EN 15051-3:2013.

[d] Surface area calculated from gas adsorption, based on the Brunauer-Emmett-Teller theory, standardised under ISO 9277:2010.

[e] Strictly speaking water solubility and dispersibility are different parameters, but in practice it is difficult to distinguish between the two in an experimental set-up.

[f] According to OECD TG105. It should be noted that the OECD-WPMN Expert Meeting on Ecotoxicology and Environmental Fate of Manufactured Nanomaterials identified several limitations of OECD TG105 (3). As a result, a project on these issues is part of the work plan of the OECD Working Group of the National Coordinators for the Test Guidelines Programme.

[g] According to the method described in the PROSPEcT study (16).

[h] Standardised under ISO 13099-2:2012.

[i] Surface area calculated from gas adsorption, based on either the Brunauer-Emmett-Teller (BET) theory, or the method of Barrett-Joyner-Halenda (BJH), Standardised under ISO 15901-2.

[j] Standardised under ISO 15901-1.

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## ANNEX I: VOLUNTEERING EXPERTS FROM THE DIFFERENT DELEGATIONS

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[34] The first names in each delegation coordinated activities in the different delegations. The actual evaluation of dossiers was done by experts in the bullet lists.

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## ANNEX II: QUESTIONNAIRE FOR ASSESSMENT OF OECD DATA ON NANOMATERIALS

**Objective of the assessment:**

Assess the methods applied for testing the physicochemical endpoints in the OECD-WPMN testing programme with the aim to assess the applicability of the methods used for the specific nanomaterials as well as their general applicability, and provide recommendations for potential modifications of OECD Test Guidelines as well as the need to develop new OECD Test Guidelines.

- Assess the applicability of the OECD Test Guidelines for determining physicochemical parameters of manufactured nanomaterials.
- Assess the need to develop new OECD Test Guidelines for determining the physicochemical parameters of manufactured nanomaterials.
- Assess the influence of different forms of nanomaterials on the physicochemical properties (in case more than one nanomaterial is included in the dossier).
- Assess which test methods are (in)appropriate for a particular parameter and particular types of nanomaterials.
- Identify data gaps (i.e. absence of data for a specific nanomaterial) and the reason for the gap (no available test methods, parameter not applicable). If applicable, identify what was done to fill the gap.
- Assess the quality of the method (based on expert judgement)

Select the parameter you will look at from the list: * --Select parameter--

Select the dossier you will look at from the list: * --Select dossier--

Select the specific nanomaterial you will look at from the list: * --Select nanomaterial--

Answer each of the questions below

Many of the questions ask for an expert opinion on the test method. Therefore, instead of answering with a simple yes or no, please provide clear explanations and justifications for your answers.

For several parameters (specifically those related to particle size, but potentially others as well), it may be important that the specific method distinguishes between primary particles and aggregates/agglomerates. Please consider this in answering the questions.

You can store your answers temporarily by using the button “Store entries, finish later” below. When you are finished with all questions, use the button “Send”. In case you press “Send” before answering all questions, you will get a warning indicating which answers are missing.

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## 1. Which test method was used?

Indicate the method/methods that was/were used. If available (e.g. where standardised methods were used), provide references (e.g. number of ISO Technical Standard or OECD Test Guideline). Where non-standardized methods were used, please give a short description of the method (this may simply be copied from the dossier). If the method was specifically developed for the endpoint and material, please indicate whether you think it is described in sufficient detail.

Title: *

For standard test guidelines please provide the title in a standardized way: e.g. OECD TG XXX, ISO TS XXXXX:XXXX).
For a non-standardized test method, please provide a short (3-word) description. In addition, please provide a full reference in the designated field below.

Description: *

Reference: *

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## 2. Were adaptions necessary to perform the test? ● Yes ○ No

Adaptations may include a different way of administration of the test material, or a different measurement method.

a. If yes, which adaptations were made? *

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## 3. Which dispersion method was used?

If the testing was not done on dispersed material, please indicate this. For dispersed materials, please provide a short description of the method used (if available, please provide the reference). If the method was specifically developed for the endpoint and material, please indicate whether you think it is described in sufficient detail.

In case different dispersion methods were used for testing this parameter with the method indicated at Question 1, please use a separate form for each individual dispersion method

Title: *

For standard test guidelines please provide the title in a standardized way: e.g. OECD TG XXX, ISO TS XXXXX:XXXX).
For a non-standardized test method, please provide a short (3-word) description. In addition, please provide a full reference in the designated field below.

Description: *

Reference: *

**4. Is the test method considered suitable and sufficient for this specific parameter and nanomaterial? *** ● Yes ○ No

This question refers to the method(s) used, including adaptations. If applicable, indicate what additional adaptations may be necessary to further improve the method. Also, indicate your opinion on the necessity of the adaptations used.

a. Provide the reason(s) for your answer *

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**5. Is the test method also considered suitable and sufficient for other forms of this nanomaterial? *** ● Yes ○ No

Many of the dossiers include data on different forms apart from the principal material(s). Differences between the forms may include differences in coatings, size, manufacturer or other. Measurements on the different forms of the nanomaterial would obviously help in answering this question. If such measurements are not available, your expert judgement is asked for (with justification).

a. Provide the reason(s) for your answer, and if yes for which forms? *

b. Alternatively, should the application of this method be limited to specific (group of) forms of the nanomaterial?
Provide the reason(s) for your answer, and if yes for which forms? *

---

**6. Is the test method also considered suitable and sufficient for other nanomaterials? *** ● Yes ○ No

This particular questions aims at a comparison of the information in the different dossiers, keeping the previous questions in mind. Nevertheless, also your opinion on suitability of the method for nanomaterials that were not included in the sponsorship programme are welcomed (in this case a reference for a measurement of such a nanomaterial is appreciated).

a. Provide the reason(s) for your answer, and if yes for which (group of) nanomaterials? *

b. Alternatively, should the application of this method be limited to specific (group of) nanomaterials?
Provide the reason(s) for your answer, and if yes for which (group of) nanomaterials? *

---

**7. Are recommendations for adaptations of the test method included in the dossier? *** ● Yes ○ No

If available, please provide a reference (other than the dossier) for the recommendations as well.

a. Provide the reason(s) for your answer, and if yes provide these recommendations *

---

**8. As an expert, how do you consider the validity of this method (including adaptations) for this parameter and nanomaterial? Please indicate your opinion as a score on a scale from 1 – 10 (1: not valid – 10: very valid). ***

○ 1 ○ 2 ○ 3 ○ 4 ○ 5 ○ 6 ○ 7 ○ 8 ○ 9 ● 10

If available, please provide a reference (other than the dossier) for the recommendations as well.

a. Please provide a justification for this score as well (keeping in mind your answers to the previous questions). *

**9. Any other comments** In case you have any additional remarks or comments, please provide these here.

## ANNEX III: ABBREVIATIONS OF ANALYTICAL TECHNIQUES

| | |
|---|---|
| AES | Auger electron spectroscopy |
| AFM | Atomic force microscopy |
| BET | Brunauer-Emmett-Teller calculation method for gas adsorption |
| BJH | Barret-Joyner-Halenda calculation method for gas adsorption |
| CLS | Centrifugal Liquid Sedimentation disc centrifuge |
| DLS | Dynamic light scattering; some authors used “photon correlation spectroscopy (PCS)” to indicate this technique. |
| DMA | Differential Mobility Analysis; some authors used SMPS as abbreviation for this technique. |
| DOSY-NMR | Diffusion-ordered spectroscopy with nuclear magnetic resonance |
| DPPH | 2,2-diphenyl-1-picrylhydrazyl; the degradation under UV light may be used as a measure for photocatalytic activity |
| EDX | Energy-dispersive X-ray spectroscopy / Energy dispersive X-ray analysis; some authors used EDS as abbreviation for this technique. |
| ELS | Electrophoretic light scattering |
| EM | Electron microscopy |
| EPR | Electron paramagnetic resonance |
| ESEM | Environmental scanning electron microscopy |
| ESR | Electron spin resonance |
| HPLC-UV | High-performance liquid chromatography (combined with) UV detection |
| ICP/OES | Inductively coupled plasma / Optical emission spectrometry |
| ICP-MS | Inductively coupled plasma – Mass spectrometry |
| PBS | Phosphate buffered solution; used in methods for radical formation potential |
| PCS | Photon correlation spectroscopy; DLS appears to me a more commonly used term for this technique. |
| SAXS | Small-angle X-ray scattering |
| SEM | Scanning electron microscopy |
| SMPS | Scanning mobility particle sizer |
| STEM | Scanning transmission electron microscopy |
| STM | Scanning tunnelling microscopy |
| TEM | Transmission electron microscopy |
| ToF-SIMS | Time-of-flight secondary ion mass spectroscopy |
| UV-Vis | Ultraviolet–visible spectroscopy or ultraviolet-visible spectrophotometry |
| XPS | X-ray photoelectron spectroscopy |
| XRD | X-ray diffraction |