NanoDefine

Development of an integrated approach based on validated and standardized methods to support the implementation of the EC recommendation for a definition of nanomaterial

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Author(s):	S. Weigel, H. Marvin, W. Beek, P. Müller, E. H. Larsen, D. Hodoroaba, M. Stintz, F. von der Kammer, R. Köber, H. Rauscher, L.Tobin, R. Reuther
Beneficiary(s):	RIKILT, BASF, DTU, BAM, TUD, UNIVIE, JRC, ION, NOMI

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PU	Public	X
PP	Restricted to other participants	
RE	Restricted to a group specified by the consortium	
CO	Confidential, only members of the consortium	

1 SUMMARY DESCRIPTION OF PROJECT CONTEXT AND OBJECTIVES

The European Commission has recommended a definition of nanomaterial (NM) as reference to determine whether an unknown material can be considered as 'nanomaterial' (2011/696/EU). One challenge in the implementation of the definition consists in the limited availability development of methods that reliably can identify, characterize and quantify nanomaterials both as substance and in various products and matrices. The NanoDefine project will explicitly address this question. Based on a comprehensive evaluation of existing methodologies and a rigorous intra-laboratory and inter-laboratory comparison, validated measurement methods and instruments will be developed that are robust, readily implementable, cost-effective and capable to reliably measure the size of particles in the range of 1–100 nm, with different shapes, coatings and for a wide range of materials, in various complex media and products. Case studies will assess their applicability for various sectors, including food/feed and cosmetics. One major outcome of NanoDefine the project will be the establishment of an integrated tiered approach including validated rapid screening methods (tier 1) and validated in depth methods (tier 2), with a user manual to guide end-users, such as manufacturers, regulatory bodies and contract laboratories, to implement the developed methodology. NanoDefine will be strongly linked to main standardization bodies, such as CEN, ISO and OECD, by actively participating in TCs and WGs, and by proposing specific ISO/CEN work items, to integrate the developed and validated methodology into the current standardization work.

2 DESCRIPTION OF WORK PERFORMED AND MAIN RESULTS

In the first year of the project, the focus was on laying the fundament for the elaboration of the main products to be delivered by NanoDefine, i.e. a tiered approach for the implementation of the definition in the form of an electronic decision support scheme and workflow, a methods manual with detailed advise on the use of different technologies as well as a bundle of related protocols, tailored instruments and software and finally methods (work items) for standardisation under CEN or ISO.

Real world test materials were sourced, pre-characterised and distributed to partners for method development, and dispersion protocols for these materials were developed. For the first time, a broad range of potentially suitable techniques was evaluated against the performance criteria that were identified to be relevant for the implementation of the definition. Instrument and software development has been started and already resulted in some prototypes. The fundamentals for the creation of the NanoDefiner e-tool have been gathered (material classification system, method performance requirements according to the different purposes of the definition) and the architecture of the required software has been drafted, yielding a pre-alpha version. Visibility to the outside world has been established via the project website, interaction with the NanoSafety cluster and a number of dissemination events.

In the second period of the project (the next 18 months) tailored methods will developed, optimised and validated, using the test materials produced within the project. Instrument and software development will continue as well as the iterative construction of the NanoDefiner e-tool.

A summary of the work conducted and achieved and expected results are presented in more detailed per work package in the sections below.

Work package 1: Test and reference materials

The main objective of WP1 in the 1st year was the selection, sourcing and distribution of test materials. The following activities have been conducted and results achieved:

- A review on currently available calibration standards meeting the EC proposal for a definition of nanomaterial was carried out and reported.
- Monomodal and trimodal calibration standards were sourced/fabricated and distributed to the participating laboratories for harmonised evaluation of techniques and calibration of instruments.
- 15 relevant and representative test materials for evaluation of techniques and validation of methods were selected (in consultation with stakeholders) and sourced. These include inorganic materials (e.g. BaSO4, CaO3, nano-steel, TiO2, kaolin, zeolite), carbon nanotubes, organic materials (pigments and polymers) as well as products (sun screen, toothpaste, plastics).
- All selected test materials (substances) and preselected test materials (products) were distributed to the project partners.

Work package 2: Sample preparation, dispersion and sampling methods

The main objective of WP2 in the 1st year was the evaluation and development of dispersion protocols and sample preparation methods for electron microscopy. The following activities have been conducted and results achieved:

- Available dispersion protocols have been reviewed and tested on real world materials, a grouping concept was developed and a concept developed for harmonising energy input in ultrasonic dispersion. Tailoring of existing and new protocols towards the need of the definition has started.
- Sample preparation approaches for extraction of nanoparticles (NPs) from products were reviewed, suitable candidate methods identified and developed into a concept.
- Sample preparation for Electron Microscope (EM) was largely improved by (i) development of an ultracentrifuge sample deposition method; (ii) construction of an Electrospray Deposition System (prototype); (iii) development of a high vacuum baking station for EM sample carrier pre-treatment.

Work package 3: Evaluation and selection of techniques

The main objective of WP3 in the 1st year was the evaluation of techniques. The following activities have been conducted and results achieved:

- A comprehensive techniques evaluation based on literature review and expert evaluation has been carried out and a respective report completed; particularities (analytical strengths and weaknesses) of the characterisation techniques to be considered in the NanoDefiner e-tool and NanoDefine Method Manual have been highlighted.

- A ranking of techniques potentially applicable for the implementation of the EC Definition based on a quantitative evaluation of the performance criteria has been accomplished.
- The practical evaluation of the techniques performance characteristics has been started on the distributed real-world test material samples.
- The evaluation of conversion algorithms has started.
- Collaboration with the EU FP7 project "NanoReg" with respect to VSSA determination was established.

Work package 4: Screening methods

The main objective of WP4 in the 1st year was instrument and software development. The following analytical techniques have been assessed and improved:

- Scanning mobility particle sizer (SMPS): Concerning the electric mobility spectroscopy, the electrospray was modified in order to cover broader particle distributions
- High Resolution Mobility Spectrometer (HRMS) prototype for 1-5 nm range was designed and manufactured.
- Particle Tracking Analysis (PTA): Initial work identified a list of parameters which effect concentration measurement and categorised them. Software has been developed, which can provide repeatable concentration measurements by fixing and reporting all adjustment parameters.
- Single particle Inductively Coupled Plasma Mass Spectrometry (spICPMS): Data evaluation concept was developed and respective software development 50% completed.

Work package 5: Confirmatory methods

The main objective of WP5 in the 1st year was the development of an auto-EM toolbox and an interface for the coupling of separation with particle counting methods. The following activities have been conducted and results achieved:

Electron microscopy

- Microscope and programming language environment decided
- Basic functions needed for auto EM –toolbox written and tested

Particle separation coupled to conventional detection techniques

- Concept for Field Flow Fractionation (FFF) method development agreed on
- Test runs for conversions of particle mass into particle number available

Particle separation coupled to particle counting techniques

- Final technical design of dilution unit for FFF-spICPMS coupling available
- Technical modifications with the ESI source to allow coupling of FFF – ESI – EL or CPC/SMPS completed. Compatibility of FFF eluents and CPC ensured.

Work package 6: Validation and standardisation
The main objective of WP6 in the 1^{st} year was the establishing of links with standardisation organisations. The following activities have been conducted and results achieved:

- Contact was established to standardisation organisations and the liaison of the NanoDefine project with CEN/TC 352 was requested.
- A first draft of a guideline for the harmonised in-house validation of methods was prepared.

Work package 7: Implementation: NanoDefiner e-tool, manual and case studies
The main objective of WP7 in the 1^{st} year was the definition of performance requirements and the design of the e-tool. The following activities have been conducted and results achieved:

- Catalogue of technical performance criteria established according to the measurement requirements resulting from the EC definition of nanomaterial.
- Characterisation methods templates developed which specify the technical performance characteristic per method as needed for the implementation of the EC definition.
- Materials classification system developed as the major entry pathway into the decision flow scheme.
- NanoDefiner structure set up and translated into pre-alpha version of the e-tool

Work package 8: Dissemination
The main objective of WP8 in the 1^{st} year was to establish the visibility of NanoDefine to stakeholders and the public. The following activities have been conducted and results achieved:

- Project website launched: www.NanoDefine.eu
- Project flyer prepared
- NanoDefine contributions to 2 NanoSafety Cluster newsletters
- Workshop with other NSC projects organised for the establishment of collaborations

3 EXPECTED FINAL RESULTS AND POTENTIAL IMPACTS

WP1: A set of representative test materials will be available for method validation and interlaboratory studies. Some of them will be developed into reference materials by the involved metrology institutes and made commercially available to the public. The selection of real industrial products maximizes the relevance of the project for regulation, industry and science. The chemically labelled nanomaterials synthesised in the project will largely enhance the reliable quantification of nanoparticles from complex matrices and thus directly contribute to the implementation of the definition in a statutory context.

WP2: Standardised dispersion protocols will be available, which rely on a calibrated ultrasound energy input for the dispersion process, and therefore are applicable across laboratories with similar results. The efficiency of the calibrated dispersion protocol will have been established by way of common bench mark material(s) with known metrics. The risk of formation of nanoparticulate artefacts from the sonication process of the pristine nanoparticles will have been evaluated and recommendations for minimisation given. As the dispersion efficiency is a bottleneck in the reproducibility of NM characterisation this will be a breakthrough in the reliability and comparability of results and will thus largely facilitate the implementation of the definition. Sample preparation methods involving dissolution, hydrolysis or removal of the complex matrix of products will be available as Standard Operation Procedures (SOPs), yielding a high recovery rate of the embedded nanoparticles and optimised for quantification by FFF and detection by multiple detection techniques including light scattering and ICP-MS. In this way, these complex techniques can be adopted by a broader range of (statutory) laboratories for the monitoring of products (e.g. cosmetics, food) according to the definition. Harmonised protocols for the representative sampling of particulate bulk materials will be publically available and contribute to the reduction of the measurement uncertainty.

WP3: Available characterisation techniques will have been compared for the first time against the same performance requirements (resulting from the implementation of the definition) and on the same test materials (with high practical relevance). Strengths and limitations of individual techniques for specific purposes will be documents and serve to select the most appropriate method for a given task. This includes instrument manufacturers' mathematical conversion algorithms for non-counting methods to the number-based particle size distribution. Where these are inappropriate new algorithms will are provided by NanoDefine. The relationship of the volume specific surface area and number based particle size distribution using the real-world materials is established and possibly integrated into the NanoDefiner e-tool as a pre-screening step, thus largely reducing the costs for testing.

WP4: SMPS: A fully developed HRMS prototype instrument covering the size range of 1-5 nm and related analytical procedures (SOPs) will enable especially product development and optimization in this range, which is accessible until now only by ultracentrifuge and TEM. The main application advantage is the high number of analysed particles in comparison to TEM and the high size resolution in comparison to ultracentrifuge. CLS: Protocols for lowering noise level and increasing baseline stability in the region of small particles in disc centrifugation methods will be delivered due to producing better quality data suitable for conversion to number size distributions. Dedicated light scattering and light absorption models for known particle morphologies result in the improvement of data quality for internal mathematical conversion to number distributions. A tailored SOP for cuvette centrifugation will enable higher sensitivity and proof of small and large particle size tails of the distribution (to validate baseline drift of disc centrifuge). A PTA method for number concentration measurement in liquids will be available. But optimized specific calibration procedure for the concentration measurement limits the application range. ICP-MS: platform independent evaluation software that allows the implementation of spICPMS for particles counting and size determination in a broad range of laboratories that are equipped with conventional ICP-MS instruments. This will ad hoc multiply the number of laboratories being capable classifying nanomaterials according to the definition.

WP5: Methods for the implementation of the EC definition in difficult samples, non-dispersible powders, complex matrices, products and the environment will be available. An automated EM toolbox for automated particle recognition, measurement, counting and analysis according to the EC definition will largely reduce the time and costs of EM measurements. Validated reference methods for classifications of materials with characteristics outside the applicability of rapid screening methods will be available. Procedures to generate number based distributions from raw data based on detection principles other than particle counting are established and validated. FFF coupled to particle counting detectors (liquid and aerosol based) to obtain true number based distributions after high-resolution separation will be established.

WP6: A common guideline for the validation of methods to be used in the characterisation of nanomaterials will largely contribute to the harmonisation of data quality and reproducibility between laboratories. The approach of harmonised validation (i.e. standardisation of data quality) is complementary to standardisation of methods and allows a higher degree of flexibility (rapid integration of emerging technologies and analytes). Interlaboratory validation of key methods has demonstrated their transferability and widespread applicability. NanoDefine methods will be established as international standards via the cooperation with international standardization bodies after submission of new work item proposals for the standardization technical committees.

WP7: General performance requirements are established which any method should meet in order to be suitable to be included in the decision framework. These general performance requirements will also support the EU Commission in developing formal decision criteria when implementing the Definition. Recommendations on the revision of the Definition based on analytical possibilities will be a significant input for the EC to adopt the definition according to technical limitations. The NanoDefine Methods Manual will provide guidance to all laboratories that analyse materials with respect to the classification into nano / non-nano according to the definition. The included SOPs can directly be used to implement the respective methods in a broad range of laboratories which is a pre-requisite for the widespread implementation of the definition. The manual will be complemented by the NanoDefiner e-tool which will guide the user to the most reliable and cost-efficient measurement method and support the classification of any substance or mixture according to the definition. The e-tool will have been validated with a number of case studies on real-world material to increase the end-user acceptance.

WP8: Ultimately, the wider community of stakeholders will be made aware of the resources being developed within the project and this will be achieved by executing strategies outlined in the exploitation plan and through targeted delivery to different stakeholder groups via a combination of electronic activities (website, mailshots, online meetings, presence in professional networks), physical engagement (membership of standardization committees, disseminations events, presentations to stakeholders) and publications (in peer-reviewed journals, trade and popular press). Additionally, stakeholders will be invited to comment on project results and direction through regular polls, surveys and feedback (primarily through the beneficiaries that are industrial associations).

Engagement with the stakeholder panel add value to NanoDefine through discussing and fine-tuning the project focus and the exploitation of results including reference materials, standardization protocols and instrumentation for commercial use by e.g. industry, testing laboratories and for monitoring purposes.

Internal training workshop for PhD students and post-docs within the consortium will take place during the evaluation and selection of methodologies for sampling and testing, and prior to commencing the inter-laboratory round-robins to promote competency in the methodologies used, prior to their use. Training for both internal and external researchers will take place following the elaboration of tier 1 and tier 2 methods, and in the final year of the project as a practical delivery of the NanoDefine Manual.

At least two technology transfer activities designed to engage with end-users will be organized in the final year of the project.

4 CONTACT INFORMATION

More detailed information is available via the project's public website and the contacts below:

Website URL	**Coordinator**
www.NanoDefine.eu	Dr. Hans Marvin / Dr. Stefan Weigel RIKILT - Institute of Food Safety PO Box 230 6700 AE Wageningen The Netherlands Tel: 0031 317 480342 / 485518 Fax: 0031 317 417717 email: hans.marvin@wur.nl / stefan.weigel@wur.nl