The EU FP7 NanoDefine Project

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

First workshop with relevant NSC projects

NanoDefine Technical Report D8.2

Mark Morrison

The NanoDefine Consortium 2014

The research leading to these results has received funding from the European Community's Seventh Framework Programme (FP7/2007-2013) under Grant Agreement n° 604347

NanoDefine in a nutshell

The EU FP7 NanoDefine project was launched in November 2013 and will run until October 2017. The project is dedicated to support the implementation of the EU Recommendation on the Definition of Nanomaterial by the provision of the required analytical tools and respective guidance. Main goal is to develop a novel tiered approach consisting of (i) rapid and cost-efficient screening methods and (ii) confirmatory measurement methods. The "NanoDefiner" eTool will guide potential end-users, such as concerned industries and regulatory bodies as well as enforcement and contract laboratories, to reliably classify if a material is nano or not. To achieve this objective, a comprehensive inter-laboratory evaluation of the performance of current characterisation techniques, instruments and software is performed. Instruments, software and methods are further developed. Their capacity to reliably measure the size of particulates in the size range 1-100 nm and above (according to the EU definition) is validated. Technical reports on project results are published to reach out to relevant stakeholders, such as policy makers, regulators, industries and the wider scientific community, to present and discuss our goals and results, to ensure a continuous exchange of views, needs and experiences obtained from different fields of expertise and application, and to finally integrate the resulting feedback into our ongoing work on the size-related classification of nanomaterials.

Bibliographic data

NanoDefine Technical Report D8.2

Report title: First workshop with relevant NSC projects

Author(s): Morrison, M.

Affiliation(s): The Institute of Nanotechnology, 50 Richmond Street, G1 1XP Glasgow, Scotland.

Publication date: 04/07/2014

Publisher: The NanoDefine Consortium

Place of publication: Wageningen, The Netherlands

Citation: Morrison, M.: First workshop with relevant NSC projects, NanoDefine Technical Report D8.2, NanoDefine Consortium, Wageningen, 2014

URL: http://www.nanodefine.eu

Contact: coordinator@nanodefine.eu, www.nanodefine.eu

Table of Contents

1 Abbreviations and acronyms

BET	Brunauer, Emmett and Teller
CEN	European Committee for Standardization
CM	Characterisation Method
DoW	Description of Work
EC	European Commission
EM	Electron Microscopy
ENM	Engineered NanoMaterials
EU	European Union
FFF	Field Flow Fractionation
ISO	International Organization for Standardization
LCA	Life Cycle Assessment
MNM	Manufactured Nanomaterial
MS	Member States (EU)
NDA	Non-Disclosure Agreement
NM	Nanomaterial
NP	Nanoparticle
NSC	NanoSafety Cluster
OECD	Organization for Economic Cooperation and Development
PC	Physical-Chemical
RA	Risk Assessment
SOP	Standard Operating Procedure
SPR	Surface Plasmon Resonance
VSSA	Volume Specific Surface Area
WP	Work Package
WPL	Work Package Leader

2 Summary

NanoDefine includes a specific task for liaison with other projects in the EU NanoSafety Cluster (NSC). One of its purposes is to ensure complementarity of planning and delivery timing.

To this end joint workshops with relevant NSC projects will be organised for the purpose of defining the exchange of materials, alignment of tasks to avoid overlap and realise synergies, and to support activities in NanoDefine, namely the selection of 14 representative test materials, and planning joint interlaboratory studies.

Organisation of this workshop began in early 2014, and after discussion with the PMT and one of the members of the Scientific Advisory Board (Keld Alstrup Jensen), it was decided to hold the workshop at NRCWE in Copenhagen. Key individuals in relevant NSC projects were identified and contacted to check availability, and based on this Friday 6th June 2014 was selected.

The workshop contained presentations from NanoDefine WPLs and the invited projects (NANoREG, SUN, NanoValid, MARINA, NanoDetector) on key topics including test and reference materials (e.g. suppliers, formulations), protocols already tested (and/or validated) in other projects (e.g. equipment, numbers of iterations/round-robins and partners involved, outcomes, gaps in knowledge), planned activities (including timelines for these). It will focus on areas of overlap and complementarity to identify the best sources and formulations of test materials (i.e. sourcing materials from the same suppliers and using the same formulation methods will support cross-referencing with other projects' results) and identify areas and timeframes where there could be sharing of information/data or joint participation in experimental protocols.

All participants were advised that they would sign a non-disclosure statement and not to share confidential material at the workshop. Any future collaboration requiring such information could be achieved through bilateral NDAs and discussion.

The workshop was structured to allow ample time for discussion on areas of complementarity and how these could be followed up.

3 Introduction

The overall objective of the workshop was to identify areas of mutual interest and the means by which to take these forward. The workshop set out to achieve this through:

1. A better understanding of each project including as far as possible:
    a. Its objectives.
    b. Materials and products investigated.
    c. Instruments and methodologies being used and developed.
    d. Scenarios being investigated.
    e. Responsible partners for delivering these activities.
    f. Status of these activities.
2. Identification of potential overlaps/synergies between projects and opportunities to collaborate in terms of:
    a. Materials – suppliers, known/studied PC-characteristics, formulations.
    b. Instruments – range in use by different projects for specific PC-characterisations, numbers of facilities involved, experimental parameters employed.
    c. Methodologies and SOPs – for free nanomaterials and those embedded within products.
    d. Case-studies/scenarios – e.g. work-place exposure, end of life disposal.
    e. Participation in inter-lab round-robins – to gain additional data, improves validity of results, complement each other.
    f. Joint training activities.
    g. Participation in project meetings/working groups – to share information between specific project partners/working groups.
3. Agreeing on an action plan
    a. What is of interest to participants?
    b. Who will follow up with whom?
    c. Dates by which information should be shared/further discussion scheduled?
    d. Expected outcomes?
    e. Date of review?

To achieve this each presenter was asked to prepare a short presentation based on the following structure (see Appendix 1 for presentations):

1. Overview of the project/NanoDefine WP objectives.
2. Organisations involved.
3. Materials studied.
4. Sample preparation methods (being) developed, and whether these are validated (or under validation) or not.
5. Dispersion methods (being) developed, and whether these are validated (or under validation) or not.
6. Analytical/measurement methods (being) developed, and whether these are validated (or under validation) or not.
7. Inter-laboratory round robins planned and the timelines for these.
8. Topics of interest – information or input needed and/or offers to other projects (e.g. identified materials or methodology needs, (future) developments potentially of interest to other projects),

The purpose of each presentation was to provide an overview of what is being done or planned in each WP/external project and the reasons why.

4 Workshop Agenda

The external participants were provided an opportunity to present an overview of their projects according to the structure above. Originally this was anticipated to last 90 minutes, with approximately 1 hour for each NanoDefine session, however much of the workshop's discussion happened during this period and was therefore not required during the NanoDefine sessions.

Time	Item
08.30-08.45	**Arrival**
08.45-08.50	**Welcome and purpose of workshop** - Mark Morrison (ION)
08.50-09.05	**Round table introductions**
09.05-09.15	**Overview of NanoDefine** - Hans Marvin (RIKILT)
09.15-12.30	**Invited project presentations** (NANoREG, NanoValid, MARINA, NanoDetector, SUN)
12.30-13.30	*Lunch*
13.30-15.30	**Session 1 - Test and reference materials: selection and methods for their preparation, dispersion and sampling -** Philipp Müller (BASF) & Katrin Löschner (DTU) 14 representative test materials have been chosen within NanoDefine, and representative consumer products will be selected. Standardised methods for preparing, dispersing, and sampling materials/products of industrial relevance for different analytical methods will be developed. **Discussion points**: methods, materials timelines, activities/experience in other projects, opportunities to collaborate across different projects (e.g. data input, choice of materials/methods, round-robins).
	Session 2 - Evaluation and selection of techniques and methodologies - Johannes Mielke (BAM) Evaluating different methods for their standardisation potential (particularly for real-world products). **Discussion points**: timelines, methods, activities/experience in other projects, opportunities to collaborate across different projects (e.g. data input, choice of materials/methods, round-robins).
	Session 3 - Two tiered approach to screen and assess products for presence/absence of nanomaterials - Michael Stintz (TUD) & Frank von der Kammer (UNIVIE) Cost effective, rapid and validated first screening methods for determining particle size ranges and numbers (nano/non-nano), followed by validated methods for particle characterisation in broad distributed, non-dispersible industrial samples, products (e.g. cosmetics, food), biota and environmental samples. **Discussion points**: timelines, methods, activities/experience in other projects, opportunities to collaborate across different projects (e.g. data input, choice of materials/methods, round-robins).
	Validation and standardisation - Robert Koeber (JRC IRMM) Ensuring harmonisation of the validation processes, for the purpose of proposing new work items to standardisation committees.
15.30-16.00	**Further discussion, agreement on follow-up activities and timelines**
16.00	**Close**

Table 1: workshop agenda

5 Project- and NanoDefine WP presentations

NANoREG: The project has more than 63 partners (with continuing opportunity to join- in a number of ways). It is driven by European Union Member States (13 plus Norway and Switzerland), participation from outside Europe includes South Korea (participation on a national level requires contribution of >€500k to the funding pot). There is also strong interest from Australia, Canada, Turkey, China and Brazil. The total funding is >€50m (€10m from European Commission).

Various inter-linking activities, essentially want to know what ENMs (engineered nanomaterials) are on the market and provide regulators with decision-making tools, through new characterisation and testing strategies.

The main goal of the project is establishing close collaboration with authorities and industry to develop knowledge appropriate for strong risk management. The questions that need to be answered originate from the MS and other partner organisations (industry).

Process taken is to identify, develop, harmonise and apply reliable SOPs. Looking at value chains; so applying these along a product's life-cycle. One thing that was realised is that there are a number of projects developing such toolboxes, e.g. NANoREG, SUN, NanoDefine.

A lot of the data generated within the project will be made publicly available (via NANOhub). NANoREG has good links with international organisations, e.g. OECD, ISO, and CEN.

19 core nanomaterials (NM) have been identified and agreed with the whole consortium. There are a further 40 additional NMs. Project is working with materials that are already validated, not possible to develop new benchmark NMs for use within NANoREG. However, the project will develop other NMs for later use.

Have developed SOPs for manufactured NM (MNM) dispersion for PC characterisation and (eco)toxicity testing. These are probe sonication and ultrasound baths, and are based on NANOGENOTOX, ENRA and PROSPECT (for ectotox). Have a guidance document and relevant SOPs for minimum requirements (agreed a set of minimum characterisations required, e.g. hydrodynamic size distribution).

In terms of links with the 'topics of interest' identified by participants pre-workshop and included in briefing document, NANoREG sees NM preparation, dispersion protocols, and benchmark materials being relevant (however only a few genuine reference materials and this is a problem).

NanoDefine WP2 is concerned with synthesis, supply and characterisation of NMs. A critical mass of partners is needed for round-robin testing and validation. WP3 is concerned with assessing exposure through LCA. WP5 is about regulatory risk assessment and testing.

The following were identified as key areas for collaboration:

1. Number size distribution
2. VSSA (volume specific surface area)
3. Characterisation of new JRC benchmark materials

NanoValid: Main goal is to produce validated reference methods and materials for measuring biological effects of ENMs (hazard identification, risk assessment and LCA). PC characterisation is approx. 20% of NanoValid effort. WP2 is similar to WP1 in NanoDefine. Overview of materials tested and sources (see presentation). NanoValid is not focused on material development, rather methods for PC characterisation and dispersion methods. Ultrasonication dispersion method is being validated just now. There are a number of round-robins completed and more planned.

A number of topics of interest, for example:

1. Characterisation SOPs and validated protocols
2. Core-shell NPs – need these materials
3. Zeta-potential - sample preparation

MARINA: Developing validated and standardised methods and materials, and also risk assessment tools. There are 2 WPs related to materials and dispersion protocols. The project is working on materials that are of most concern (high volume and/or high value), most from JRC repository but some directly from industry.

Dispersion protocols being developed for materials in water and experimental media and for mixing in solid media. These are coordinated by JRC Ispra. No round-robins have yet been performed. There is real interest in *in situ* characterisation, so less to do with pristine materials. More characterisation in soil, water and biological matrices is on the way. There is a greater focus on the use phase of nanomaterials rather than production.

NanoDetector: Project started June 2012 and finishes November 2015. It focusses on supporting SMEs (6 involved), using surface plasmon resonance (SPR) to detect individual NPs (in the work place and in different liquid and gas media). System contains a gold layer which can be functionalised in different ways (charged groups, antibodies) to bind different NPs (this gives some information about the surface chemistry of the NP). This is an integrated system with sample delivery, SPR and image analysis. Both aerosols and liquids can be applied, and have so far used Ag, Au, polystyrene, and a protein NP (drug complex from one of the partners) of different sizes to test the system. Intensity of SPR signal is proportional to the size of particle (mass) and its refractive index, however correlation needs to be developed further to be able to extract size data from image data.

System can image the binding of individual NPs to the Au surface. Detector is ~1mm^2 and capable of detecting a single NP, up to several thousand NPs in the sample.

The system could be potentially implemented on commercial imaging systems, and used to detect a number of different materials. NanoDetector is interested in testing core-shell NPs as signal is strongly influenced by core. However, need a high refractive index; so TiO2 can be detected, but not silica.

It should be possible to measure NP concentration - work to correlate image analysis with particle concentration is ongoing. However, discriminating between different shapes is not possible.

Minimum detected size is ~20-40nm, depending on the material. Agglomerates would be detectable by the large mass, however would not be able to discriminate these from larger sized primary particles (of the same material – should be able to distinguish between similar sized particles of different materials, in unknown samples).

One issue is that NPs bind strongly to the detector surface, and after a period of time this needs to be regenerated (requiring harsh chemical treatment). Only three groups in world working on such a system, and this is the only one in Europe.

SUN: Project started October 2013 and lasts 42 months. Its aim is to support the safer design of new nanotechnology innovations through providing answers to regulatory question on EHS risks. Looking at a number of case studies which represent supply chains of real products from SUN's industrial partners. Analysing and comparing pristine NMs and the same materials within product matrices. To date have performed primary characterisation of a number of pristine NMs. Have used protocols from NanoValid and NanoGenoTox (SUN will not develop its own validated sample preparation and characterisation methods, and no round-robins are planned in the DoW.

The project is looking at a number of matrices including food, soil, sediment and different animal species. Main interest is in material characterisation protocols for environmental and biological media and in situ characterisation in tissues and cells.

SUN is looking at how NM properties are affected and changed by different environments through their life-cycle. It is this interaction that defines the biological impacts of the ENM, not the PC characterisation of the pristine ENM. SUN is focused on developing tools, rather than new data on different materials. Nevertheless a lot of characterisation work (in various media) is planned in the project that is aimed to capture the transformation of NMs in the different life-cycle stages of nano-enabled products.

NanoValid and NANoREG are also looking at how different media affect NP dispersion and their interaction with biological systems.

SUN is not planning to look at chronic exposure, but is looking at long-term effects using an adapted short-term inhalation and ingestion protocol (RIVM responsible for this).

SUN looks at 4 broad LCA stages: NM synthesis, product manufacture, use and end-of-life. There is more data for some materials at different LCA stages than others.

Industry will be the end-user of the tools being developed: mainly for workplace assessment, but also considering consumer exposure including dermal and inadvertent aerosol exposure. Project partners are developing data for consumer products (e.g. dustiness).

SUN will further develop a decision support system for practical use by industry and regulators that is aimed to estimate risks and environmental impacts and support decisions on technological alternatives and risk management measures to reduce the risks/impacts, including indication of their costs.

NanoDefine WP1 and WP2: The purpose of these WPs is to supply representative materials (commercial and consumer relevance) for testing within the project and develop some of these into reference materials. Pre-characterising these materials and developing sampling protocols. The WPs are looking at three different types of material: calibrants (SiO2 and polystyrene), substances (relevant to industry products) and products (food, cosmetics and other relevant matrices). Three products decided upon (SiO2 in tomato soup, TiO2 in sunscreen (with and without other inorganic materials) and a fourth still to be chosen). Dispersion methods are mainly probe sonication (some bath sonication may be used). Sub-sampling methods not yet decided. Chemical and enzymatic methods will be used for sample preparation. Round-robins planned for final year of project.

NanoDefine WP3: This starts with an evaluation of different characterisation methods (CM), comparing these in a semi-quantitative manner (in terms of a number of aspects including: size ranges, chemical selectivity, cost, turnaround). From this assessment will make recommendations for further development in NanoDefine (or not). Then will perform real-world testing of the CMs (particularly on multimodal NM preparations). Developing algorithms for converting non-counting methods to NP number based size distributions – in particular VSSA.

NanoDefine WP4: WP4's main objective is the development of a set of validated, cost-efficient, robust, and easy implementable methods for rapid distinction between nano/non-nano according to the definition. Many partners involved. Need to validate/optimise dispersion methods (electrospray for aerosols and ultrasonication for liquids) and sample preparation for real world materials (will use some from NanoLyse). The WP is interested in learning from others' experience on the applicable range of different methods and their performance (with different materials), particle number concentration in suspension and aerosols (if no reference material), and optimum ultrasonic dispersion methods (considering different surface chemistries and presence of contaminants, e.g. in real-world products). Want to also understand material behaviour between 100 and 1000nm (hence methods are included that don't have single digit nm resolution).

NanoDefine WP5: Tier 2 will be able to investigate nano and non-nano. Looking to develop number based size distribution from non-counting methods (e.g. mass or intensity measurements). Developing field flow fractionation (FFF) methods coupled to particle counting. The WP is also developing methods to remove liquid from samples and then aerosolise these (however this is difficult because of the other components, formation of salts, etc.) and to develop low-cost FFF for use by regulatory labs.

Sample preparation methods are being shared with WP2 and will be needed for both FFF and EM. Analytical methods will be validated if they perform satisfactorily during development. Round-robins will begin in M36.

NanoDefine WP6: Objective is to develop harmonised methods and submit these to standards committees. Target number of interlab methods to be validated is 4 (in M41-46). The interlab comparisons will be open to external participation including organisations in VAMAS and other NSC projects.

6 Conclusions

A number of key areas of collaboration and sharing of materials/results were identified by project partners (described in the minutes above). At this point these mainly involved access to SOPs for: sample preparation, sampling of materials, and material characterisation, and in addition to validation protocols. From NanoDefine's perspective, the most immediate collaborations affect WP 1 and WP2.

Appendix 1 - Workshop participants

Project	Organisation	Name	Responsibility for …	Other NSC Projects
NanoDefine	ION	Mark Morrison	NanoDefine WP8 Dissemina-tion, training & technology transfer	NanoValid
NanoDefine	RIKILT	Hans Marvin	NanoDefine WP10 Scientific coordination	Quality Nano
NanoDefine	RIKILT	Stefan Weigel	NanoDefine WP10 Scientific coordination	Quality Nano
NanoDefine	BASF	Philipp Mueller	NanoDefine WP1 Test and reference materials	SUN, NanoMile
NanoDefine	JRC IRMM	Robert Koeber	NanoDefine WP6 Validation and standardisation, Deputy - WP1	
NanoDefine	BAM	Johannes Mielke	NanoDefine WP3 Evaluation & selection of techniques & methodologies	NanoValid
NanoDefine	DTU	Katrin Löschner	NanoDefine WP2 Sample preparation, dispersion & sampling methods	SUN
NanoDefine	UNIVIE	Frank vd Kammer	NanoDefine WP5 Confirma-tory methods (tier 2)	SUN
NanoDefine	TUD	Michael Stintz	NanoDefine WP4 Screening methods (tier 1)	NanoSTAIR
NanoDefine	NIA	David Carlander	NanoDefine WP8 deputy	NANoREG WP5 leader, MARINA
NanoDefine/ NanoValid	NOMI	Rudolf Reuther	NanoValid coordinator & NanoDefine WP9 Project management	NanoValid
NanoDefine/ NanoValid	NOMI	Rune Karlsson	NanoValid coordinator & NanoDefine WP9 Project management	NanoValid
NANoREG	NRCWE	Keld Alstrup Jensen	SUN WP5 leader, NANoREG WP2 leader, NanoDefine, EAB	SUN, NanoMile
MARINA/ SUN	Aarhus	Janeck Scott-Fordsmand	MARINA WP13 leader, envi-ronmental risk assessment	WP4 leader SUN, MODERN
Nano-Detector	Brandenburg TU	Shavkat Nizamov	NanoDetector coordinator representative	
SUN	UNIVE	Danail Hristozov	SUN coordinator	