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

Second workshop with relevant NSC projects

NanoDefine Technical Report D8.3

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The NanoDefine Consortium 2016

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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 providing 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 regularly 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.

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Table of Contents

1 Abbreviations and acronyms

AC	Analytical centrifugation
AF4	Asymmetrical flow field-flow fractionation
BET	Brunauer, Emmett and Teller
CEN	European Committee for Standardization
CLS	Centrifugal Liquid Sedimentation
CM	Characterisation Method
CPC	Condensation Particle Counter
DMA	Differential Mobility Analyser
DLS	Dynamic light scattering
EM	Electron Microscopy
ENM	Engineered NanoMaterials
ELS	Electrophoretic light scattering
ESI	Electrospray Ionization
EU	European Union
FDA	Federal Drug Administration
FFF	Field Flow Fractionation
FT-IR	Fourier Transform Infrared Spectroscopy
HRMS	High Resolution Mobility Spectrometry
H2020	Horizon 2020
ICP-MS	Inductively Coupled Plasma Mass Spectrometry
ICT	Information Communication Technology
ILC	Inter-laboratory Comparison
IPR	Intellectual Property Rights
ISO	International Organization for Standardization
LCA	Life Cycle Assessment
LS	Light Scattering
MALS	Multi-angle Light Scattering
MPI	Magnetic Particle Imaging
NDA	Non-Disclosure Agreement
Neth-ER	Netherlands house for Education and Research
NM	Nanomaterial
NMBP	Nanotechnologies, Advanced Materials, Biotechnology and Advanced Manufacturing and Processing
NP	Nanoparticle

NSC	NanoSafety Cluster
OECD	Organization for Economic Cooperation and Development
PC	Physical-Chemical
PSL	Polystyrene Latex
PTA	Particle Tracking Analysis
QCM	Quality Control Management
QSAR	Quantitative structure–activity relationship
TEM	Transmission Electron Microscopy
TG	Test Guideline
TRL	Technology Readiness Levels
REACH	Registration, Evaluation, Authorisation and restriction of Chemicals
SAXS	Small-Angle X-ray Scattering
SEM	Scanning Electron Microscopy
SME	Small and Medium Enterprises
SMPS	Scanning Mobility Particle Sizer
sp	Single Particle
SRA	Strategic Research Agenda
SOP	Standard Operating Procedure
UV-VIS	Ultraviolet–Visible spectroscopy
VSSA	Volume Specific Surface Area
WP	Work Package
WPL	Work Package Leader
WPMNM	Working Party on Manufactured NanoMaterials
XRD	X-Ray powder Diffraction

2 Summary

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

To this end 2 joint workshops with relevant NSC projects have been 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, such as the selection of 14 representative test materials, and planning joint inter-laboratory studies. The 1st NSC synergy workshop took place at the NRCWE in Copenhagen on 6 June 2014.

Organisation of a 2nd workshop began in summer 2015, and after discussions between RIKILT and NOMI it was decided to have the workshop at Neth-ER in Brussels on Tuesday 2nd February 2016. Key individuals from relevant NSC projects were identified and contacted to check their availability.

The workshop programme provided presentations from invited projects (NanoSolutions, NANoREG, SUN, GUIDEnano, NanoMILE, NanoMag, NanoDetector, eNanoMapper and NanoValid) and from NanoDefine on key topics, including test and reference materials (e.g. suppliers, formulations), protocols already tested (and/or validated) in various projects (e.g. equipment, numbers of iterations/round-robins and partners involved, outcomes, gaps in knowledge), planned activities and time schedules). One main focus was 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 areas and timeframes that best allow sharing of information/data or joint participation in experimental protocols.

All participants were advised to sign a non-disclosure statement and not to share confidential information presented with others outside 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 common interest and how they could be followed up.

3 Introduction

The overall objective of this 2nd NSC synergy workshop was to identify areas of mutual interest with other relevant projects and the means to utilize them. To achieve this, the workshop was structured by the following specific objectives:

1. To get 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. To identify 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. To agree 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 these goals, each presenter was asked to prepare *a priori* a short presentation based around a specific aspect of their project and by using the following structure:

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 agenda of the workshop was set up to allow ample discussion after each presentation and a final discussion on how to follow up the results and agreements achieved:

2nd NanoDefine "NSC Projects Synergy Workshop"

Exchange of experience, expertise, materials and protocols, alignment of tasks to avoid overlaps and use synergies

2 February 2016, Netherlands house for Education and Research (Neth-ER) 22 rue d'Arlon, 1050 Brussels, Belgium

Chair/co-chair: Hans Marvin (RIKILT) and Rudolf Reuther (NOMI)

08:30-09:00 **Arrival and registration**

09:00-09:10 **Welcome and purpose of workshop**
Rudolf Reuther, NOMI

09:10-09:20 **Round table introductions**
All

09:20-09:30 **Overview of NanoDefine**
Hans Marvin, RIKILT

Session 1 – Invited project presentations:

09:30-09:45 **Development of a safety classification system for ENMs**
Kai Savolainen, NanoSolutions

09:45-10:00 **Characterisation methods considered and their "Technology Readiness Levels"**
Keld Alstrup Jensen, NANoREG

10:00-10:15 **Approach used for characterisation of pristine CuO and *safer by design* modified samples**
Magda Blosi, SUN

10:15-10:30	**Approach of GUIDEnano leading to a web-based tool for risk assessment of nano-enabled products** Socorro Vazquez, GUIDEnano
10:30-10:45	**Coffee break**
10:45-11:00	**Protocols for ENM synthesis, characterisation and safety assessment** Iseult Lynch, NanoMILE
11:00-11:15	**Standardisation of analysis methods for magnetic nanoparticles** Christer Johansson, NanoMag
11:15-11:30	**Detection and identification of nanoparticles using NanoDetector-technology** Vladimir Mirsky, NanoDetector
11:30-11:45	**Ontology for physicochemical measurement techniques and guidelines for experimental design** Barry Hardy, eNanoMapper
11:45-12:00	**Development of reference methods and materials for characterization and testing of nanomaterials** Rudolf Reuther, NanoValid
12:00-13:00	**Lunch**

Session 2 – Update of on-going work in NanoDefine:

13:00-13:25	**NanoDefine test materials and sample preparation** Katrin Loeschner, Technical University of Denmark and Robert Koeber, Joint Research Center-IRMM
13:25-13:50	**Method evaluation and selection** Dan Hodoroaba**,** Federal Institute for Materials Research and Testing, Germany
13:50-14:15	**2-tiered analytical approach to classify materials and products** Michael Stintz, Technical University Dresden

14:15-14:40 **Method validation and standardisation**

Robert Koeber, Joint Research Center-IRMM

14:40-15:05 **The NanoDefine eTool and Method Manual**

Hubert Rauscher, Joint Research Center-IHCP

Session 3 – Coffee break/discussion and follow-up activities:

15:05-16:00 **Discussion points:**

- identifying synergies and overlaps
- sharing methods and materials
- opportunities and ways to cooperate
- common dissemination including training activities

16:00 **Close of workshop**

Workshop participants (with initials of participants in brackets)**:** Keld Alstrup Jensen (KAJ), Magda Blosi (MB), David Carlander (DC), Manuel Correia (MC), Arno Gutleb (AG), Barry Hardy (BH), Dan Hodoroaba (DHod), Dick Hoeneveld (DHoe), Christer Johansson (CJ), Rune Karlsson (RKa), Robert Koeber (RKo), Katrin Loeschner (KL), Iseult Lynch (IL), Hans Marvin (HM), Vladimir Mirsky (VM), Hubert Rauscher (HR), Rudolf Reuther (RR), Alex Rinkus (AR), Kai Savolainen (KS), Nicolas Segebarth (NS), Michael Stintz (MS), Tom van Teunenbroek (TvT), Kristof Tirez (KT), Socorro Vasquez (SV), Eveline Verleysen (EV) and Sandra Verstraelen (SV).

5 Workshop Presentations

In the following a short description is given of some of the main topics discussed during the meeting and of the course of debates as reflected by the minutes taken by RK.

Welcome and purpose of workshop

RR gave a short introduction and outlined the purpose of the workshop.

Round table introductions

All participants gave a short introduction of their organization and their affiliation and role.

Overview of NanoDefine

HM continued with an overview of NanoDefine. He talks briefly about the project details and strategic objective. Provide affected industries and regulatory agencies with the tools to support the implementation of the definition in regulatory contexts.

Foreseen solution: easy to implement, cost efficient: a tiered approach from tier 1 (screening methods) to tier

2 (confirmatory methods), flexible: adapted to changing regulatory requirements, sustainable: developed approach implemented in structures.

HM showed slide over work packages (WP1-WP10) and interdependencies and discuss this briefly.

WP6 is crucial and will deliver the NanoDefiner e-tool and the NanoDefine Method Manual. Other main products are SOPs for analysis of materials and products, CEN/ISO Work Items of key methods, calibration standards and reference materials, instruments prototypes and technology transfer.

Development of a safety classification system for ENMs (NanoSolutions)

KS gave an introduction to NanoSolutions. NS will develop a safety classification system based on the biological identity and will be a hazard tool. Nine ENMs are used with different functional groups. EU wants testing material-by-material but is not feasible. There has been no break-through really. Hierarchic approach considered. Refined in vitro assays are developed that will replace animal testing. KS discussed the WP interdependencies. NS has a bioinformatics approach, systems biology analysis and mathematical algorithms. Observe NS is about hazard assessment, not risk assessment.

Some comments on the Strategic Research Agenda (SRA) update. This is a strategy document, does not include details. New parts are bioinformatics, close-to-the-market, regulatory research.

DHod: A need to reduce uncertainty of the physico- chemical characteristics that determine toxic potential.

HM: How do you use the data in eNanoMapper?

BH: Use original data from many projects such as on bioinformatics.

NS: What data in the prediction model is associated with physico- chemical characteristics? How about data from all biological testing, proteomics, transcription? This is very important. Crucial with a more accurate model and validate with materials from industry.

NS: The next NMBP work-program may be published in 2016 already.

KS: Need to find time to update SRA soon.

Characterisation methods considered and their "Technology Readiness Levels" (NANoREG)

KAJ presented WP2 of NANoREG. WP2 has about 20 participants. Two main objectives: Provide ENMs and develop characterisation protocols based on OECD guidelines as well as new methods based on existing "state-of-the-art" procedures or completely new methods. Among, the new procedures/methods are protocols for identification and quantification of coatings; solubility and reactivity testing; as well as harmonized test item preparation and a probe-sonicator calibration. Protocols for solubility, reactivity are also important. KAJ explains TRL, a method of estimating technology maturity of critical technology elements, has 9 levels in H2020. Challenge – require tested/validated SOP for different methods that are laboratory and software dependent, i.e. extraction procedure for inter-laboratory calibration procedure.

Primary characteristics are harmonised and tested based on OECD SOPs. Comparison to OECD test guidelines (TGs) and WPMNM list of end-points: Some are applicable to ENMs, i.e. dispersion/dissolution but uncertain in some cases. TG 109/110 and 115 used and modified in NANoREG.

SOPs for size-distribution of primary nano-objects (phase 1, 2, 3). Size distribution of primary nano-objects: works well for spherical particles and work is currently in progress to test the reliability in sizing aggregates and "non-spherical" particles. Volume specific surface area: important to discriminate between internal and external surface area (t-plot). Lesson from NANoREG Deliverable 2.3 – need to know relative density. SOP development – cooperation with NanoDefine.

Regarding purity of MN and chemicals registration, a material containing up to 20 wt% impurities is still one substance in REACH (ECHA). This is a lot! A screening system is needed to identify and quantify inorganic surface-chemical modifications. Coatings/impurities need to be known through a combination of well-known / well-established methods.

Technical guidelines will be at a lower TRL3 (proof of concept). Regarding the TRL of the methods, most NANoREG methods will be at lower TRLs after the NANoREG evaluations and modifications. Most methods are high TRL, but SOPs are lower TRL. The next phase will be to harmonize, validate and implement methods for general use, depending on the end-point in question.

NS: So the OECD guidelines need to be revised?

KAJ: Yes, a lot of work capability to standardise methods that can distinguish between nano/non-nano materials, is needed. 20% impurities are not acceptable!

Approach used for characterisation of pristine CuO and safer by design modified samples (SUN)

MB gave overview of SUN. Current risk knowledge is enough to make use of LCA, and guide sustainable nano-innovation (safe-by-design approaches). These will lead to greener nanotechnologies. SUN supports NANoREG and other projects. The project includes long term exposure and effects. SUN includes 8 case studies, with 8 different materials, to follow life cycle of materials.

Primary characterisation methods are TEM, XRD, DLS, ELS, BET, FT-IR and ICP-MS.

Case study on nano-CuO for anti-bacterial use in wood-paint: size XRD ≅ 15nm; TEM ≅ 3-35 nm; BET ≅ 20nm. MB presented step-by-step, safe-by-design strategy for CuO. CuO is going through a process from synthetic to biological identity pathway: a/ dispersion in PO_4^{3-} buffer, b/ addition of surface modifiers, c/dispersion in salts, d/interaction with proteins, dispersion in complete TOX media.

Conclusion: Enforce “Safer by design” approach a/ wet state characterization in environmental or biological relevant media, b/ modelling in support of safe design, c/ categorisation, identification of key risk relevant factors.

Approach of GUIDEnano leading to a web-based tool for risk assessment of nano-enabled products

SV talked about GUIDEnano. The project develops innovative methodologies to evaluate and manage human health and environmental risks, considering the whole product life cycle. Experimental data are produced to fill data gaps and for validation purposes. Exposure levels and ENMs in relevant environmental /human compartments are determined. Validation is done through case studies, also from the industrial sector. ENMs in case studies/products: ZnO, Al_2O_3, TiO_2, nanocellulose, Ag, Fe^o.

GUIDEnano version 1.3 is now available. This is a web-based risk assessment tool covering the hotspots of exposure and hazard important for human and environmental health during the entire nano-enabled products life cycle, including risk management. One of the main parts of the tool includes the description of the NM form in all its life cycle stages.

V1.3 focuses on the material flow, identifying relevant exposure/release and hazard hotspots. Future versions will incorporate hazard and risk assessment as well as a decision module to support the user in selection of appropriate risk refinement and risk mitigation activities.

TvT: Do you generate your own data?

SV: 1st part is to gather data from other projects, 2nd is to generate our own data.

NS: How do you assess the quality of the data? We need to make "expert" decisions. I assume that sources of data and data quality are clearly stated in the project reports. In some areas there are a lot of data gaps.

KS: How do you determine probability of risk?

SV: Risk assessment strategy is incorporated in the GUIDEnano tool. This strategy is evaluated by real case studies within the project.

Protocols for ENM synthesis, characterisation and safety assessment (NanoMILE)

IL presented the NanoMile project. NanoMile has 16 EU academic, 7 EU SME, 3 large EU industry, and 2 US academic partners. Main focus is on interaction of ENMs with organisms. It is important to understand function of the corona. QSAR (Quantitative structure–activity relationship) models are central.

Key features a/ Focus on high through-put methods, b/ Iterative process towards safer by design ENMs, c/ Building on efforts from NanoReTox and other FP7 projects, d/Development of systematic libraries of MNMs varying by one property, e/ Investigate fresh and "aged" ENMs.

Functionalisation of JRC's SiO_2 and Au NPs. Materials studied CeO_2, Ag, TiO_2, ZnO, SiO_2, CNTs and Au. Effects of coatings, surface charge, and functionalisation are studied. Method developments are taking place. For example, Attana is performing QCM measurements for ENM-protein and ENM-cell interactions. A number (4) of dispersion control methods are developed. Some data presented on corona evolution by following uptake in cells (HCA NM corona evolution).

NS: How about validation of methods?

IL: No ILC studies are planned.

DHod: Why is XPS missing under "methods developed"?

IL: Overlooked some common surface characterisation methods. XPS is available at JRC.

BH: What is the NanoMile – NanoSolutions difference?

IL: NM has a broader range of nanomaterials, test species and test conditions and takes a broader screening approach, with NS going deeper into specific systems / organisms. The differences/similarities will also be discussed at joint workshop in April and there are good possibilities in terms of sharing of computational tools developed in each project to be validated in the sister project.

KS: Classification system in NS based on mathematical models. There are many similarities, e.g. QSAR and practical outcomes.

Standardisation of analysis methods for magnetic nanoparticles (NanoMag)

CJ gave an introduction to NanoMag. The project wants to standardise, improve, harmonise and redefine measurement/analysis methods of magnetic nanoparticles. The coordination organisation is ACREO with about 140 people and provides cutting edge resources and technologies within sensors and actuators, power electronics, digital communication and Life Science. Magnetic ENMs has mainly biomedical applications. Today two ENMs approved by FDA: magnetite and maghemite. Ferrocite can be used as magnetic fluid.

Magnetic particles can be single-core or multi-core, e.g. magnetite/maghemite. Harmonise measurement/analysis methods for magnetic particles. Promote standardisation of methods.

Magnetic Particle Imaging (MPI); this is quite a new method (Phillips, 2006). There are no validated protocols for measure and analyse particles using this method. NanoMag has 17 partners and a 15 member stakeholder committee.

Planning a publication (get this out soon) on matrices of analysis methods, sizing techniques based on different measurement principles. Particles are synthesised in the project. Analysis methods include TEM and DLS. Monte Carlo simulation employed for multi-core particles.

Detection and identification of nanoparticles using NanoDetector-technology

VM on NanoDetector: development of technologies for detection and analysis of single nanoparticles in complex environments. New measurement principle: single sub-wavelength objects give rise to optical signals in surface plasmon resonance microscopy. The instrument detects particles by scattering of surface plasmons. Size determined from scattering intensity and allows direct visualisation of adsorption of ENMs to surfaces. Instrument based on real-time detection principle, high sensitivity (in- sensitivity to very small NPs), high dynamic range and high stability. Method works in difficult matrices (e.g. orange juice). Different examples are shown during the presentation, e.g. 40 nm Au particles.

Ontology for physicochemical measurement techniques and guidelines for experimental design (eNanoMapper)

BH introduced the project. Ontology – formal naming and definition of the types, properties, and interrelationships of the entities that really or fundamentally exist for a particular domain of discourse. Example with the cat on what distance it feels comfortable from home. Not only a number (m), needs also to describe the context. It's about how e.g. scientists communicate with each other.

E.g. eNM is developing ontologies for the categorisation and characterisation of ENMs in collaboration with other projects. Ontology assembled from multiple sources - 6,690 classes identified in eNM.

eNM based on open standards and common data management. A prototype database is developed. All protocols should be linked together with data. Dose-response investigated. Evidence made from drug development. eNM active in the NSC. Please attend the eNM workshop in Basel on 10th February, organised together with SUN and NanoFase.

NS: Is there ontology for pc characterisation in place?

BH: Check with JRC. Practical test when incorporating SOPs from NANoREG.

KAJ: ICT-data base? This has not been use before and is important for quality control of data.

DC: You mention about 6600 classes. Can these be ranked? How do you check quality and IPR/security check?

BH: Depends what you are looking for. At the moment, we're working on ontology for hazard assessment.

NS: Important to have ontology in place when start to bring in a lot of data from other projects.

Development of reference methods and materials for characterisation and testing of nanomaterials (NanoValid)

RR on premises for NanoValid: 1/ how applicable are current methods to ENMs? 2/ develop validated methods (pc characterisation, biological testing etc.) for ENMs. A project overview is described with main tasks and objectives. NV had a bottom-up approach. RR shows high-lights from different WPs.

DHod: Was the pc characterisation methods validated using all ENMs?

RR: A selected number of ENMs was used to test and validate the performance of a method for a particular measurand and/or end-point.

RR: We are planning to publish all SOPs developed in NanoValid in a book. After publication all the raw data should be made available.

TvT: I think there should be open access to all data since NanoValid funded by the EU.

HM: There is an issue with IPR, 100% funding is not provided by EU. This is specified in Grand Agreement.

NS: Data should be provided after publication after a certain time, maybe 2 years. We need a plan for future dissemination.

BH: Current practice needs to be changed.

DHod: A lot of data from NanoDefine is already in the public domain.

NanoDefine test materials and sample preparation

RKo presented material systems on NanoDefine. RKo gave an overview of material systems in NanoDefine, products and substances.

Toothpaste and sunscreen were prepared; since the "real" products are have all kind of sizes and structures.

Two types of materials used: calibrants/test materials and reference materials.

Test materials are mono-modal SiO_2, Au, Ag, PS; tri-modal SiO_2 and labelled Al_2O_3/TiO_2.

Possible ref materials tested with CLS, DLS and BET. DLS is not so good for proving "real" world materials. Some adaptation of sample preparation is needed. No significant inhomogeneity or instability. No indication these materials are unsuitable as ref materials.

KL presented development of dispersion protocols. Requirements for dispersion: reasonable sonication time, suitable volumes, minimum stability, compatible media, suitable concentration, transfer method to other labs. SOPs developed: 10-30 min, 2-10 mL, stable (except sedimentation), aqueous and 0.1-10 mg/mL. Quality: after sonication single primary particles and 2-10 primary particles per agglomerate. Use of dispersants/surfactants needed in some cases. Hydrophobic particles are difficult to handle such as MWCNT, pigments, methacrylate copolymer.

Sonication energy is critical. Check dispersion with DLS, CLS, LP and TEM with respective SOP. Need to have an idea of what size to expect.

To keep in mind: Probe may contaminate, use of vial tweeter for selected material. Sample preparation methods have been developed.

EV: Pre-treatment of TEM grids (SOPs), sample deposition TEM grids (SOPs). Electrospray system tested with calibration standards.

TvT: Drying by cryo-deposition. Why is this not used?

EV: It's a very difficult procedure.

KL: Could be used as confirmatory method; it's not standardised.

TvT: Method may be more realistic.

Method evaluation and selection (NanoDefine)

DHod introduced method evaluation and selection. Methods in NanoDefine encompassed screening methods and confirmatory methods such as EM and FFF. Quality Control Materials used as reference for sizing techniques, Representative Test Materials are the "real world" materials.

DHod showed overview of measurement techniques (counting techniques – CT) able to probe the size of ENPs.CTs are evaluated against material, performance, technical and economic criteria.

Both theoretical and experimental evaluation is going on. Evaluation is based on comprehensive measurements of QCMs and RTMs. Measurements according to SOPs. Uniform reporting templates for data reduction. Same dispersion protocols are used.

Experimental CT evaluation carried out, using TEM, SEM, DMA (2x), dAC-turb, cAC-turb, cAC-RI, AF4-LS, DLS (2x) and SAXS.

SiO_2-mono: easy to measure. PSL trimodal: optical methods unclear, estimate of NP fractions. BaSO4-ultrafine: Good agreement with TEM and SEM. Kaolin – problem.

Size by SEM analysis: usually within a factor of 2.

NanoDefine decision scheme for powders - VSSA correlation to e-Microscopy (EM): VSSA ($d_{minVSSA}$) vs EM ($d_{minFerret}$). Screening method: BET to determine VSSA + quick SEM.

Conclusions: reliable classification for "simple" materials, reliable classification for "real-world" materials by suited CTs (preparation, measurement and data evaluation), VSSA: simple solution to most powders, SEM: (simple?) solution to almost all materials, problems if agglomerates/aggregates, polydispersity, porosity.

KAJ: How do you know if you have aggregates or primary particles?

DHod: Need to apply manual approach to do this.

CJ: Tricky part to model size if not spherical.

2-tiered analytical approach to classify materials and products (NanoDefine)

MS described the 2-tiered analytical approach. NanoDefine has a 2-tiered analytical approach towards classify materials and products: screening and confirmatory methods.

Tier 1: set of validated, cost-efficient, robust, and easy implementable methods for rapid distinction between nano/non-nano.

Sample preparation method developed in WP2: validated. Dispersion methods: electrospray to aerosol – to be validated, ultrasonic dispersion in liquids – to be optimised.

Analytical method development: Centrifugal Liquid Analysis Disk Type and Cuvette Type, High Resolution Mobility Spectrometer, Particle Tracking Analysis, Single particle ICP-MS. Methods to be validated in NanoDefine.

CMs: Evaluation of limiting factors and possible improvements for disc and cuvette centrifugation method with optical detection.

HRMS: Extend range to 1 – 5nm. Need ref material of 5nm in suspension, latex or gold would be good. Need to be able to measure in suspension.

PTA: Improvement to both repeatability and reproducibility of concentration measurements.

sp ICP-MS: Platform independent software, automated determination of key evaluation parameter, selection and modification of threshold values for nanoparticle fractions.

Inter-lab calibration: validation of key methods from M36.

Topics of interest: applicability range and methods performance description – materials dependency, particle number concentration in suspensions - no liquid reference material available, particle number concentration in aerosols - no reference, only comparison method (ISO).

Tier 2: Establish methods for implementation of the recommendation for the EC definition (in "difficult" samples).

Tier 2 methods are FFF, EM and sp ICP-MS. Materials/products are coated TiO_2, food containing SiO_2, TiO_2, and Fe_2O_3 in polymer, Al_2O_3 in toothpaste. Sample preparation methods validated for FFF and EM analysis.

Analytical method development: Automated EM image analysis, automated EM operation with NP analysis (image + elemental composition) and conventional FFF methodology are validated. FFF-single particle ICP-MS: under validation. FFF-single particle ESI-SMPS and ESI-CPC: not validated, incompatible with FFF carrier.

Automated EM developed at EAWAG: rapid particle sizing and automated image analysis. FFF + multi-detection techniques (MALS, DLS, UV-VIS, ICP-MS, etc.): need experienced operator.

Intra-laboratory validation for selected reference methods using selected materials/products from M36.

Topics of interest: Applicability range and methods performance description EM methods – material dependency, robustness towards difficult materials, Particle number concentration from non– counting techniques – accuracy of conversion techniques, requirements for the analytical technique, particle number concentration from counting detectors after FFF – accuracy, dynamic range interfacing, applicability ranges.

Method validation and standardisation (NanoDefine)

RKo spoke on validation and standardisation. WP6 is responsible for in-house validation in support of WP4/WP5. ND co-operates with standardisation organisations and submission of work item proposals. Evaluate inter-laboratory validation studies of key methods.

Definition of a method: Based on a sample preparation protocol, the measurement technique (e.g. TEM), and a specific type of sample/nanomaterial. The measurand needs to be clearly defined (in the SOP). Measurement equipment: proper calibration and performance control resulting in traceable property values.

RKo shows overview of in-house validation workflow. Inter-laboratory validation studies planned of 4 selected methods. Time-line: M41-M46 (Mar 2017 – Aug 2017). Topics of interest: validation concept; similar validation concepts in other projects with focus on solid ENMs.

DHod: I assume measurand for EM is size. How about measurand for other techniques?

RKo: Should be stated in the SOP what measurand is used, conversion algorithms etc.

NS: How many partners in the inter-laboratory studies?

RKo: Aiming for at least 8. The labs need to have competence.

HM: Is any training needed?

RKo: This should be provided

CJ: What materials do you intend to use.

RKo: Some of our ND materials. Use them as is.

The NanoDefine eTool and Method Manual (NanoDefine)

HR described eTool and Method Manual**.** The "NanoDefiner" eTool is a decision making framework over cost-efficient methods. This eTool will be accompanied of the NanoDefine Methods Manual for hands-on use of the methods.

The Decision-support flow scheme uses input from material classification, requirements and purpose, available methods and existing data. This scheme guides the user to do the measurements in the right way. Revision may be needed based on method development, quality control and regulatory aspects. The scheme takes sample preparation options into account. Unknown material can be tested (plausibility check) if it's nano/non-nano according the recommended definition.

Method development (selected techniques): substance-related criteria and method-related criteria depending on circumstances. All methods are evaluated against uniform criteria. Need to take into account stability (particle suspension, aerosol etc.), theoretical evaluation, experience from project.

First edition of Methods Manual published, but not yet public available due to IPR-issues. The first eTool with methods database should be available in 2 months.

BH: Both the eTool and the Methods Manual should be available through a cloud service.

Discussion

TvT: In this meeting the projects have presented themselves and just suggested how cooperation could start. But there is no time for continuation and discuss in detail how cooperation should start/continue. Therefore I propose a follow-up meeting as soon as within a month. NANoREG could even sponsor this meeting.

HM: For now we should collect all the presentations and upload to ND intranet.

NS: Next meeting should start with concrete steps to cooperate from each project.

BH: There is a need of systematic method compilation within the nanosafety community that reflect different levels of SOPs and templates.

RR: Yes, and we should find a proper structure within the NSC on how to coordinate and organise the implementation of the cooperation between the various projects.

6 Conclusions

A number of key areas have been identified and possible practical steps discussed to cooperate and share materials/results and SOPs by project partners (as described in the minutes above). One main focus was access to established new (and/or validated) SOPs for: sample preparation, sampling of materials, and material characterisation. It was agreed to arrange a follow-up workshop within the next 6-month period. NanoDefine will come up with a list of actions to be taken before and will organise the next follow-up meeting.

Appendix 1 - Workshop participants

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