NanoSafety Cluster

Compendium of Projects in the European NanoSafety Cluster

2014 Edition

June 2014

Editor:

Iseult Lynch
University of Birmingham, United Kingdom

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PREFACE

This is the fourth edition of the Nanosafety Cluster compendium. It documents the status of important EU-funded projects on nanomaterial toxicity and exposure monitoring, integrated risk management, research infrastructure and coordination and support activities as well as regulatory-focussed research on nanosafety.

The compendium is not intended to be a guidance document for human health and environmental safety management of nanotechnologies, as such guidance documents already exist and are widely available.

Neither is the compendium intended to be a medium for the publication of scientific data and research results, as this task is covered by scientific conferences and the peer reviewed press.

The compendium aims to showcase the exciting and important European-wide collaborative research being undertaken to ensure the safe implementation of nanotechnologies, and to act as a one-stop-shop for all stakeholders interested in acquiring an overview of current research activities. This years' compendium contains information on 30 running (or very recently finished) projects, including new entries describing the projects resulting from the last call of FP7, including eNanoMapper, NanoDefine and FutureNanoNeeds. It also includes presentations of several of the projects funded via the SIINN ERA-NET such as INSTANT, NANOHETER and NanOxiMet, as well as two projects funded via the LIFE topic (SIRENA and NanoRISK). In addition, updates from several of the Nanosafety Cluster Working Groups (WGs) are included, outlining their short, medium and long term goals, and progress to date.

The compendium also aims to bring the research community closer together and show them the potential for synergy in their work. It is a means to establish links and communication between them during the actual research phase and well before the publication of their results. It thus focuses on the communication of projects' strategic aims, extensively covers specific work objectives and the methods used in research, and documents human capacities and partnerships. As such, the compendium supports collaboration on common goals and the joint elaboration of future plans, whilst compromising neither the potential for scientific publication, nor intellectual property rights.

Of course this publication alone will not be able to achieve these targets. However, we hope that it will help the research community to make significant progress towards them. The compendium will continue to be a dynamic, frequently updated, web-based document available free of charge to all interested parties.

We hope that you find it useful, and please do feel free to cite it, and to contact the project coordinators and participants for more information or to collaborate on specific topics of interest. As ever, information sharing and fostering of collaborative activities are key goals of the Nanosafety Cluster. Feedback on the 2014 Compendium is most welcome, including ideas for additional information that could be included in future editions. Please email suggestions / ideas to: i.lynch@bham.ac.uk.

More information about the NanoSafety Cluster can be found at http://www.nanosafetycluster.eu

ACKNOWLEDGMENTS

I would like to thank the project coordinators / managers for their contributions in the creation of this publication. This compendium would not have been possible without their help. The compendium attests to the hard work, the outstanding ideas, the frustrations and successes, and the satisfaction of the researchers. Their commitment is the foundation for this publication.

Projects appearing in this compendium are supported financially by the European Union and the Governments of the Framework Programme Associated States. We gratefully acknowledge their continued support.

The editing of this year's Compendium was kindly supported by the QualityNano project (Grant Agreement SP4-Capacities-2010-262163 under the EC's 7th Framework Programme).

Iseult Lynch, Editor and Co-Chair of the Dissemination WG of the NanoSafety Cluster

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Foreword

Dear Readers of the Compendium, Dear Friends,

As FP7 hands over to Horizon2020, nanosafety research remains a centrally important topic to ensuring Europe's vision of the knowledge economy and the safe implementation of nanotechnologies for the benefit of society. Indeed, nanotechnologies are one of the Key Enabling Technologies for investment of research effort, with safety and public acceptance of nanotechnologies at the heart of these efforts. Indeed, as will become apparent from the project descriptions in this 2014 Edition of the NanoSafety Cluster Compendium, there is an increased emphasis on advanced materials and materials as they exist in products and following environmental transformation & ageing, as well as on research with a regulatory and/or market focus, which will continue and expand as the first Horizon2020 projects commence later this year. However, many of the projects reported here will continue to be active until 2017, so FP7 has lots more to deliver before its final completion, so "watch this space" for further updates!

The NanoSafety Cluster Compendium of projects is intended as to disseminate knowledge about European Commission funded research projects on various aspects of nanosafety to a wide variety of stakeholders, including international research communities, the regulatory authorities, parallel activities such as the OECD Sponsorship Programme, the EU DGs, industry and interested NGOs. It is intended to provide a concise snapshot of each project's aims, approaches and progress to date, thereby facilitating gap analysis, collaboration and provide a directory of European research and researchers. Given the scale of the 30 projects outlined here, resulting from the active funding efforts of the Commission, Europe has taken a position of global leadership of nanosafety research, including establishing the Nanosafety Cluster itself and leading on the iniation of the EU-US Communities of Research (CoRs). The Nanosafety Cluster was established as a mechanism for ongoing projects to benefit from one another, and from recently finished projects, through information sharing, as well as to collectively define strategic agendas for research (published 2013 – Strategic Research Roadmap 2020), regulation (in preparation) and industry (in preparation). Its 8 Working Groups address different aspects of the nanosafety challenge, from materials, through hazard, exposure, risk assessment, with cross-cutting themes including databases, dissemination and systems biology. For the first time, several of the Working Groups provide summaries of their roadmaps as part of the NanoSafety Cluster compendium, and these will also be updated periodically.

This compendium is a highly interesting piece of reading to all those who are interested in knowing how European nanosafety research projects tackle with the emerging safety and health challenges of novel engineered nanomaterials and nanotechnologies. The Compendium provides descriptions of the EU funded nanosafety projects in sufficient detail, and contact information of the coordinators of the projects. Please, make contacts, network, and increase collaboration further within Europe and globally. I wish that this compendium again proves be an extremely useful source of information of European nanosafety research.

Overview matrix: Research themes of the NanoSafety Cluster projects

Project Acronym	eNanoMapper	FutureNanoNeeds	GuideNano	INSTANT	MARINA	MembraneNanoPart	ModNanoTox	NanoDefine	NanoDetector	NanoFATE	NanoHeter	NanoMiCex	NanoMILE	NanoPolyTox	NanoPuzzles	NANoREG	NanoRISK	NanoSolutions	nanoSTAIR	NanoSustain	NanoTransKinetics	NanoValid	NanOxiMet	PreNanoTox	QualityNano	SanoWork	Scaffold	SINN	SIRENA	SUN
Start year	2014	2014	2013	2012	2011	2013	2011	2013	2012	2010	2013	2012	2013	2010	2013	2013	2013	2013	2012	2010	2011	2011	2013	2013	2011	2012	2012	2013	2013	2013
End year	2017	2017	2016	2015	2015	2015	2013	2017	2015	2014	2016	2014	2017	2013	2015	2016	2016	2017	2014	2013	2014	2014	2016	2016	2015	2015	2015	2016	2015	2016
measurement		X			X			X	X	X				X				X		X		X								
Physico-chemical properties		X	X	X	X	X		X	X	X	X	X	X	X		X	X	X		X		X	X	X	X	X		X		X
Analysis of "next generation" nanomaterials (2nd, 3rd or 4th generation)		X	X					X									X											X		X
Exposure assessment for humans and the environment		X	X		X					X	X	X		X			X			X		X								X
Develop & validate exposure measurement and modelling methods		X	X		X		X			X	X	X		X	X	X	X				X	X					X	X	X	X
Human Exposure: Application of measurement and		X	X		X					X		X		X		X				X		X		X	X	X	X			X
Environmental Exposure Assessment		X	X		X		X			X	X	X		X		X	X			X		X						X	X	X
Interaction of NM with biological systems		X			X					X				X				X		X		X								
Interaction with physiological mechanisms		X			X	X				X				X	X	X				X	X	X	X	X	X	X				
Toxicokinetics					X	X				X		X		X	X	X				X		X				X				
variability										X				X	X	X									X					
Predictive models					X	X	X			X		X		X	X	X		X		X	X	X	X				X			
Long term monitoring and assessment										X				X		X														
Human Health					X												X	X		X		X								
Develop & validate testing & assessment strategy					X									X	X	X	X	X		X	X	X			X					
Apply testing and assessment strategy					X							X		X	X	X	X	X		X		X				X				
Coexposures / Mixture		X																				X								
Ecotoxicology		X	X		X					X			X		X			X		X		X								
Develop testing and assessment strategy		X	X		X					X		X	X		X	X		X		X		X			X					X
Apply testing and assessment strategy			X		X		X			X			X		X	X		X		X		X								X
Control measures at workplace					X												X			X		X								X
Develop & validate methods to evaluate control measures at workplaces					X				X							X	X	X		X		X					X			X
Apply methods to evaluate control measures at workplaces					X				X			X				X	X			X		X				X	X			
Control banding approach					X							X					X									X	X			
Preliminary handling guidelines					X										X		X	X		X		X								X
Collect available and ongoing approaches					X										X	X	X	X		X		X					X		X	X
Evaluation and further development		X			X										X	X	X	X		X		X				X	X		X	X
Information transfer		X	X							X								X				X								
Database generation	X	X	X		X		X			X	X		X	X	X	X		X		X	X	X	X	X	X	X	X		X	X
Public dialogue			X		X					X	X				X		X					X				X	X			
Information to and training of workers, business and		X	X							X		X	X	X			X			X		X	X			X	X		X	X
National and international collaboration					X					X	X			X	X			X	X	X		X								
Development	X	X	X		X				X	X	X			X	X	X	X	X		X		X	X	X	X	X	X	X	X	
Testing	X	X	X		X	X			X	X	X	X		X	X	X	X	X		X		X	X	X	X	X	X	X	X	
Validation	X		X		X	X		X		X	X			X	X	X		X		X	X	X		X	X		X	X	X	
Standardisation	X				X			X		X	X			X	X	X			X		X	X	X	X	X		X		X	
Assessment activities			X		X					X	X	X		X	X	X		X		X	X	X		X	X	X	X			

eNanoMapper

A Database and Ontology Framework for Nanomaterials Design and Safety Assessment

Contract Agreement: 604134 Website: www.enanomapper.net
Coordinator: Dr. Barry Hardy, Douglas Connect GmbH, Switzerland

Table 1 Consortium List.

No.	Beneficiary name	Short name	Country
1	Douglas Connect GmbH	DC	SWITZERLAND
2	National Technical University of Athens	NTUA	GREECE
3	In Silico Toxicology GmbH	IST	SWITZERLAND
4	IdeaConsult Ltd	IDEA	BULGARIA
5	Karolinska Institutet	KI	SWEDEN
6	Technical Research Centre of Finland VTT	VTT	FINLAND
7	European Molecular Biology Laboratory - European Bioinformatics Institute	EMBL-EBI	GERMANY
8	Maastricht University	UM	THE NETHERLANDS

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1 Summary

Project Duration: 36 months (1 February 2014 – 31 January 2017)

Project Funding: € 4 998 227 (€ 3 996 870 EC contribution)

The eNanoMapper project proposes an ontology and computational infrastructure for toxicological data management of engineered nanomaterials (ENMs) based on open standards, ontologies and an interoperable design to enable a more effective, integrated approach to European research in nanotechnology. The eNanoMapper concept builds on common components and Application Programming Interfaces (APIs) to address data and models interoperability challenges.

The flexible computational infrastructure for this project will be implemented based on interoperable, standards-compliant and modular web services maximising cross-talk and interaction between different databases. This will include key services for ontologies, data storage, data analysis and modelling as well as supporting services (e.g. for authentication and authorisation) and prototype graphical user interfaces (GUIs) for data submission and analysis.

In order to achieve maximum impact and to avoid duplication, the system will be designed to be interoperable with resources already existing in this area; we will adhere to established standards and actively contribute to standards and new ontology development whenever necessary. The consortium has assembled partners with specific experience in predictive toxicology database and modelling resource development (DC, NTUA, IST, IDEA, UM), ontology creation and curation (EMBL-EBI, UM), and existing interactions with European nanotechnology and NanoSafety Cluster projects (IST, KI, VTT).

The eNanoMapper project is divided in seven Work Packages (WPs):

WP1 - Community Outreach: communication between the eNanoMapper project and the wider nanotechnology community. It establishes various mechanisms we use to ensure collaboration with and feedback on our project. The goals are to collaboratively develop interoperability documents together with definitions of personas and use cases that capture the community needs and working practices.

WP2 - Ontology Development: develop and disseminate a comprehensive ontology for the nanosafety domain, encompassing nanomaterials and all information relating to their characterisation, as well as information describing relevant experimental paradigms, biological interactions, safety indications and experimental paradigms.

WP3 - Database Development: design, implement and provide the data infrastructure and search capabilities, supporting all aspects of ENM characterization, namely synthesis and processing, physicochemical characterization, LCA, environmental and health hazards assessment, high throughput and high content datasets. Implementation of modules and a web service API for different types of queries, namely free text search for protocol description and relevant free text data, SPARQL endpoint for ISA-TAB metadata, and where relevant, a chemical structure and similarity search for ENMs and functionalized ENMs.

WP4 - Analysis & Modelling: to ensure the ontology and data warehouse developed in WP2 and WP3 meets the safety-by-design and community needs, WP4 will develop computational infrastructure capable to analyse and extract knowledge out of diverse types of ENM-related theoretical descriptors, experimental data and associated metadata, including provenance of experimental data and experimental conditions and protocols.

WP5 - User Application Development, Integration and Testing: to ensure the ontology and data warehouse developed in WP2 and WP3 meets the safety-by-design and community needs, WP4 will develop computational infrastructure capable to analyse and extract knowledge out of diverse types of ENM-related theoretical descriptors, experimental data and associated metadata, including provenance of experimental data and experimental conditions and protocols.

WP6 - Dissemination & Training: effective dissemination and development of news, findings, progress, lessons learned, practices, resources and services created, scientific discoveries and inventions from the eNanoMapper project.

WP7 - Management: effective planning and management of the project, coordination between partners, administration, and facilitating communications between partners within the project, and between the project consortium and the EC.

2 Scientific and technological challenges

eNanoMapper concept builds on common components and Application Programming Interfaces (APIs) to address data and models interoperability challenges. It will use semantic technologies to characterize ENMs, link databases and ensure a flexible data model. Minimum information standards and existing formats and tools such as ISA-TAB will be employed to capture experimental metadata and protocols. The proposed distributed web services technology will allow flexible data sharing and industry strength security solution to guarantee data protection.

The common API will allow building user friendly interfaces tailored to the needs of different user communities, as well as low level access to the data and computations for advanced computational science users.

- Ontologies
- Linking Existing databases
- Data management challenges and proposed solution
- Reporting of experimental information challenge and proposed solution
- Modelling and Analysis
- Computational infrastructure
- Optimal Experimental Design
- Inter-laboratory comparison
- Collaborations
- Associate Partner Program
- Sustainability

3 Objectives

eNanoMapper will support the collaborative safety assessment for ENMs by creating a modular, extensible infrastructure for transparent data sharing, data analysis, and the creation of computational toxicology models for ENMs. Building on recent developments of consortium partners in predictive toxicology, biology and nanotechnology research, we will develop resources, tools and standards for a scientifically sound risk assessment of ENMs that will support the design of new safe and environment-friendly ENMs as well as the assessment of existing materials.

The main objectives of the eNanoMapper project are:

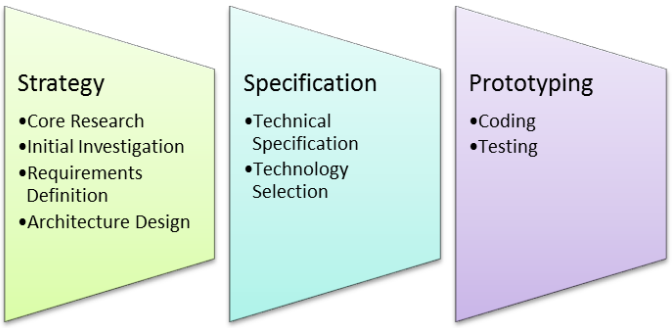
- Improving the utilisation of data through the implementation of a modular infrastructure for data storage, searching and sharing, based on open standards and semantic web technologies, minimum information standards and established security solutions;
- Accelerating knowledge exchange and reuse through the development of ontologies for the categorisation and characterisation of ENMs (pristine and in situ) in collaboration with other projects, including those launched following the NMP.2013-1.3-3 call (Development of a systematic framework for naming and assessing safety of the next generations of nanomaterials being developed for industrial applications);
- Enabling the creation of new computational models in nanomaterials safety through the implementation of interfaces for toxicity modelling and prediction algorithms which may process all data made available through eNanoMapper (e.g. using algorithms available from the OpenTox FP7 project or statistical/data mining software);
- Enabling the meta analysis of nano-bio interactions supporting "safe-by-design" ENMs development by pursuing a Linked Data approach which integrates data and metadata originating from diverse sources within nanoscience, chemistry, biology and toxicology;
- Creation of tools for the exchange, quality assurance and reporting of research protocols and data for regulatory purposes;
- Creation of a community framework to accelerate interdisciplinary collaboration between experimental and computational scientists in the establishment and use of data management and analysis infrastructure and to mutually develop quality-driven guidelines for experimental design and optimal production and sustainable maintenance of new datasets;

- Closely aligning the eNanoMapper infrastructure development with user needs to enhance the research cohesion, integration and advancement of the EU NanoSafety Cluster agenda.

4 Organisation

The eNanoMapper project implementation is arranged in 7 Work Packages (WPs). The user-driven requirements analysis, design and application testing activities of WP1 will provide extremely valuable guidance for all eNanoMapper RTD. WPs 2-4 provide the core component development parts of eNanoMapper in the areas of ontology, data management and computational processing and analysis infrastructure, with WP5 providing a regular integrated testing and release of components including GUIs for use application testing and deployment. The use cases developed in WP1 will guide all component (WP2-4) and integrated application development focus (WP5) and validation by providing software applications to users, who will use the applications on an ongoing basis throughout the project, providing valuable feedback in an iterative manner, and scaling up the number of users in later stages of the project. WP6 on Dissemination and Training and WP7 on Project Management and Reporting are pervasive throughout the duration of the project to ensure quality outcomes and results, and rely on experienced EC project coordination and WP management skills of partners.

eNanoMapper will follow an iterative cycle of contextual design and inquiry with users, strategy and specification, followed by software development, testing and real world application. The user-centric design, agile SW development methodology and periodic integration and testing will reduce inherent project risk by breaking the WP tasks into smaller sub-tasks providing more ease-of-change across several iterations. Across all project development activities, the strategy, concepts, requirements analysis, and design of architecture will be developed, tested and refined by an iterative prototyping approach.



5 Expected Impact

The eNanoMapper project has the potential to have significant positive impact on the nanotechnology industry, regulators and citizens in the European Union and markedly strengthen the cohesive growth, influence and outreach of European nanosafety and research-driven industrial nanotech-based innovation within a global perspective. Our eNanoMapper data warehouse and ontology platform can be used to capture data and knowledge along the full lifecycle of ENMs, from research to product development to manufacturing procedures, human and environmental exposures, (eco)toxicological effects and degradation processes. Its flexible design will support the safety, environmental, regulation, and standardisation aspects of ENMs, which are all important for safety-by-design and risk assessment. Our linked data approach will support real, achievable and operational interoperability with external ontologies and databases and enable the application of statistical and data mining procedures for data analysis, as well as to support the information transfer activities of the NanoSafety cluster. Thus, these deliverables of eNanoMapper will be directly applicable to exploit and couple together the many diverse data types of both past and current nanosafety research projects.

In particular the eNanoMapper platform will consist of:

- an ontology addressing all standardisation and regulatory requirements;
- a data warehouse to share data and accelerate knowledge flow along the full nanomaterial research and production chain in a secure way (supported by authorization and authentication on all resources);
- human and machine interfaces for the data warehouse;
- data analysis platforms supporting toxicological and environmental risk assessment, safety-by-design principles and experimental design;
- guidelines for experimental design capturing statistical evidence and community-selected standards;
- ISA-Tab templates to capture experimental data using spreadsheets.

These results will have the following impact on the European nanosafety community:

- the open, community-agreed language formalized in an ontology will enable comparing and combining private and public data and knowledge;
- there will be an open platform for integrating ENM data sources to provides uniform access to Open and confidential data;
- the platform will support the exploration and highlighting of patterns in ENM structure-activity relations at a US-EU community scale;
- the platform will simplify entering data, reducing the cost of safety studies and comparisons;
- the European publishing industry will be provided with clear reporting requirements and usable and well-supported tools, allowing their authors to make their data available to the eNanoMapper platform.

6 Directory

Table 1 Directory of people involved in this project.

First Name	Last Name	Affiliation	e-mail
Barry	Hardy	Douglas Connect GmbH	barry.hardy@douglasconnect.com
Haralambos	Sarimveis	National Technical University of Athens	hsarimv@central.ntua.gr
Pantelis	Sopasakis	National Technical University of Athens	
Christoph	Helma	In Silico Toxicology GmbH	helma@in-silico.ch
Nina	Jeliazkova	IdeaConsult Ltd	jeliazkova.nina@gmail.com
Bengt	Fadeel	Karolinska Institutet	bengt.fadeel@ki.se
Roland	Grafström	Technical Research Centre of Finland VTT	grafstromrc@gmail.com
Vidal	Fey	Technical Research Centre of Finland VTT	vidal.fey@vtt.fi
Christoph	Steinbeck	EMBL - European Bioinformatics Institute	steinbeck@ebi.ac.uk
Janna	Hastings	EMBL - European Bioinformatics Institute	hastings@ebi.ac.uk
Egon	Willighagen	Maastricht University	egon.willighagen@maastrichtuniversity.nl
Chris	Evelo	Maastricht University	chris.evelo@maastrichtuniversity.nl

7 Copyright

FutureNanoNeeds

A framework to respond to regulatory needs of future nanomaterials and markets.

Contract Agreement: NMP.2013.1.3-3 Website: http://www.futurenanoneeds.eu/
Coordinator: Kenneth Dawson, Centre for BioNano Interactions, University College Dublin, Belfield, Dublin 4, Ireland

Table 1 Consortium List.

No.	Beneficiary name	Short name	Country
1	University College Dublin, National University Of Ireland	NUID UCD	Ireland
2	Fundacio Privada Institut Catala De Nanotechnologia	ICN	Spain
3	Philipps Universität Marburg	PUM	Germany
4	Ecole Polytechnique Federale De Lausanne	EPFL	Switzerland
5	Institut Für Energie Und Umwelttechnik Ev	IUTA	Germany
6	Heriot-Watt University	HWU	United Kingdom
7	Centre De Recherche Public - Gabriel Lippmann	CRP-GL	Luxembourg
8	The University Of Birmingham	UoB	United Kingdom
9	Trinity College Dublin	TCD	Ireland
10	Nederlandse Organisatie Voor Toegepast Natuurwetenschappelijk Onderzoek	TNO	Netherlands
11	Istituto Di Ricerche Farmacologiche Mario Negri	MNI	Italy
12	Rijksinstituut Voor Volksgezondheiden Milieu	RIVM	Netherlands
13	Vlaamse Instelling Voor Technologisch Onderzoek N.V.	VITO	Belgium
14	Commissariat A L Energie Atomique Et Aux Energies Alternatives	CEA	France
15	Technicka Univerzita V Liberci	TUL	Czech Republic
16	Comitee Des Donnees Scientifiques Et Technologiques	CODATA	France
17	Nanofutures Asbl	NfA	Belgium
18	Filarete Servizi Srl	FILARETE	Italy
19	Nanogap Sub-Nm-Powder Sa	NANOGAP	Spain
20	Solarprint Limited	Solarprint	Ireland
21	Nanonica Europe Sl	Nanonica	Spain
22	Centro Ricerche Fiat Scpa	CRF	Italy
23	Universidade De Santiago De Compostela	USC	Spain

Contents

1 Summary

Project Duration: Starting in January 2014 for 48 months

Project Funding: FP7-NMP-2013-LARGE-7

Rapidly developing markets such as green construction, energy harvesting and storage, advanced materials for aerospace, electronics, medical implants and environmental remediation are potential key application targets for nanomaterials. There, nanotechnology has the potential to make qualitative improvements or indeed even to enable the technology. Impacts range from increased efficiency of energy harvesting or storage batteries, to radical improvements in mechanical properties for construction materials. In addition, concerns of these markets such as scarcity of materials, cost, security of supply, and negative environmental impact of older products can also be addressed by new nano-enabled materials (e.g. lighter aircraft use less fuel).

FutureNanoNeeds will develop a novel framework to enable naming, classification, hazard and environmental impact assessment of the next generation nanomaterials prior to their widespread industrial use. It will uniquely achieve this by integrating concepts and approaches from several well established contiguous domains, such as phylontology and crystallography to develop a robust, versatile and adaptable naming approach, coupled with a full assessment of all known biological protective responses as the basis for a decision tree for screening potential impacts of nanomaterials at all stages of their lifecycle. Together, these tools will form the basis of a "value chain" regulatory process which allows each nanomaterial to be assessed for different applications on the basis of available data and the specific exposure and life cycle concerns for that application. Exemplar materials from emerging nano-industry sectors, such as energy, construction and agriculture will be evaluated via this process as demonstrators. The FutureNanoNeeds consortium is uniquely placed to achieve this, on the basis of expertise, positioning, open mindedness and a belief that new approaches are required.

2 Background

Without doubt one of the most difficult challenges faced in the exploitation of nanotechnology for the benefit of European society (and beyond) has been the uncertainty surrounding the potential associated risks. The impact of this intrinsic uncertainty (often associated with many new technologies) might have been exacerbated by the fact that 'legacy' nanomaterials were already on the market when the discussion began. In reality there is no way of knowing whether the intrinsic safety issues could have been handled in more measured manner in early discussions, had this 'legacy' issue not been present. The facts are that such early materials emerged more by an evolutionary process of colloidal product optimization. Nanotechnology in general (nanoparticulates) was never afforded the opportunity to evolve into the market via a considered process, with balanced scientific consensus underpinning its arrival. No one can tell at this point, if this alternative scenario could have been workable, and what would have been the differences in the outcome. Still, the opportunity to reset the discussion is arising again, with the advent of qualitatively new materials.

Furthermore, even if a well-developed policy-level strategy for technology transfer into the market had been available, one has to face the real facts on the ground, during that period. Scientists were not prepared to provide the information, let alone deeper insights, into the new issues involved. Indeed, when called upon to respond, the challenge of new science requiring a highly integrated interdisciplinary approach lead to a fragmented scientific response from a community that was itself just assembling. Some of the information published was simply factually incorrect, some over-interpreted (or, sometimes worse, not at all) and the discussion turned into a debate with 'sides' taken by different stakeholders. In the end, the scientific response admixed with a broader societal debate, with significant mutual misunderstanding of what science could, and could not, do on the time-scales available.

It must be considered a remarkable achievement of policy makers, European (US, and other) institutions, and a scientific community gaining increasing confidence, that the situation has now sufficiently stabilized to allow for a rethink of the overall approach. There is still uncertainty (some significant) about some aspects of the legacy materials, but certainly the most severe concerns about acute hazard that were widely publicized several years ago are (for most common materials) no longer considered justified. A more systematic and thorough scientific investigation of the legacy materials is now underway, as is also a systematic effort to address the outstanding issues.

Within this program an opportunity has arisen (possibly for the first time world-wide) to look to the future. The implications and impact of this go far beyond science, though that is important, and a point to which we return below. Perhaps the most dramatic impact could be to modify the terms on which the present discussion has taken place, and to unblock the overall process of nanotechnology (safely) transiting to the market.

3 Scientific and technological challenges

Much has been made of the general revolution associated with Nanoscience and Nanotechnology, and all of the associated potential. Likely future perspective will shed a new light on these developments, confirming the potential, but perhaps stressing new aspects not immediately visible to us. For example, for the first time in human history vast numbers of distinct (in composition, geometry, morphology, topology and surface structure) novel engineered structures, with different properties (and different functions) are being created. Essentially nothing is known about the interactions of these new objects with living organisms and the rest of the environment. Only a small fraction has been characterized in detail. The process has just begun. Future technological innovation and products will employ the benefits connected to these unique material properties.

It is safe to assume that within decades more distinct engineered (non-natural) structures will be produced than those from the beginning of humanity. We and our environment will for the first time in human history be exposed to large amounts of surface area with a topography and morphology not previously seen by either. This surface will be covered by molecules derived from the surroundings in which particles are prepared, formulated, or from

the environment (including our own bodily fluids) to which they are exposed along their life cycle, for specific value chains (Figure 1). While it is true that many of these new features will not lead to novel impacts, certainly some will.

Figure 1. The emerging concept of 'biological identity' has been mainly applied to legacy particles. It involves size and, crucially, the nature of the surface, the latter usually significantly modified due to biomolecules that have adsorbed to it. These concepts to date are laid out in a review by partners of FutureNanoNeeds written in Nature Nanotechnology 7, 779–786 (2012). However, for new materials these ideas will have to be significantly evolved (right panel). There the size, shape, and adsorbed biomolecules will all combine in a manner yet to be determined. Note (right panel) that biomolecules on novel particles may now be associated with unique areas of the particle. Many of these ideas are also increasingly seen to be relevant in environmental exposure scenarios where, again, new materials will require new thinking.

The scientific program of FutureNanoNeeds will transform our understanding of how engineered materials interact with our world into a new, encompassing framework. Biology is designed to process information on the scale of tens of nanometres. Though often not stressed, this is the length-scale on which much biological processing functions. Organisms throughout the environment possess endogenous mechanisms, based on specific biological recognition (largely absent for small molecules), to deal with complex structures on these length scales, largely in a regulated and systematic manner.

Partners in this program have pioneered many synthetic routes for functional nanomaterials and link it to biological mechanisms, their biological identity, including impact on living beings. The project will enlarge the paradigm and progress this far beyond current thinking, and methodologies.

4 Objectives

FutureNanoNeeds focusses on the following objectives:

- Rapidly engage with new generations of materials, as well as the upstream researchers and innovators from which they are emerging.
- Identify ways in which safety and innovation can partner for overall success.
- Identify potential (generic) hazards early, and help provide an early framework for their resolution; and provide a scientific and technical basis to identify 'safe pathways' or platforms which might be exploitable faster, and at lower cost.
- Provide the basis for changing the nanosafety dialogue, reducing 'generic' criticisms of 'uncertainty' by proactively road-mapping the issues ahead of any realized risk to society.
- Change the way in which nanosafety research is conceived and applied, qualifying the concept of 'toxicity' or hazard along specific value chains.
- Reframe the role of nanosafety research by studying materials along value chains and reporting the outcomes in a manner immediately relevant to that value chain.
- Sustain and position Europe as a scientific and technical leader in the underlying issues in nanosafety of next generation materials.
- Characterise the 'in situ' behaviour of nanoparticle interactions throughout their whole life cycle, hence advancing the scientific state of the art.
- To develop an understanding of the relationships between nanoparticle (pristine) structure, its properties (including in situ), and its biological and environmental activity (that is, structure and 'identity' broadly defined) thereby giving early support to the science of 'new nanomaterials, safe by design'.
- Support and influence developments within the standards and ontologies communities (including relevant EU programs), thereby supporting their relevance to the safety agenda.
- To connect nanomaterial properties (in given exposure scenarios) to elementary biological responses (known to be associated with pathological response) and use this relationship to signpost potential for hazard.
- Inform stakeholders and policy makers so that planning for future research priorities can be partly based on preliminary knowledge.

5 Progress and Outcomes to date

FutureNanoNeeds started its activity in 2014. The projects' kick-off meeting was held 9th-10th January 2014 in Dublin. A public session was realized in the morning followed by subgroup meetings of the individual Work Packages. All Work Packages closely interact between each other and the different disciplines supported by regular meetings on a monthly basis. To promote exploration of the opportunities and issues of new nanomaterials, FutureNanoNeeds helds an Open Global Workshop on New Perspectives on Nanomaterials Interactions and Classification in early 2015. The Workshop will bring together key thought leaders and actors from all sectors of the international nanomaterials community to discuss the emerging research results and identify new approaches to coordination and cooperation to facilitate achieving the potential value made possible by these results. Date and locality will be confirmed soon and published in the NanoSafety Cluster Newsletter.

6 Expected Impact

The materials FutureNanoNeeds will investigate are not yet on the market, and in many cases there is still sufficient flexibility of choice and time for different technological options to be explored for commercialization. Thus, rather than being caught up in a debate that seems to require black and white answers, or 'choosing of sides' in a policy debate, science can play its correct role; giving measured, careful and accurate answers and insights into any novel hazards or risks foreseen, and allowing policy makers to measure the whole complex of issues, and make the decisions, for which they hold final responsibility.

It is stressed throughout the program the value of planning the nanosafety research approach from the perspective of value chains, as well as from the scientific imperatives. This allows FutureNanoNeeds to prioritise the choice of general materials types, and then create homologous series of material variants and shapes around that basic choice. The project provides so the opportunity to analyse exposure scenarios, in-situ biological properties and impacts on living systems of a wide range of representative but highly differentiated materials, involving widely differing geometries, topologies, surfaces, compositions, and other parameters, and try to both rapidly screen them, as well as understand any new scientific paradigms associated with them. FutureNanoNeeds, while committed to cautious high quality studies, cross-checked, and communicated in a cautious manner, would enter this arena with the scientific preparation, experience of previous mistakes, and overall understanding to allow for a well-judged study to take place. The project emphasizes the potential positive impact of this approach which may unblock the overall process of nanotechnology (safely) transiting to the market, early and highly cost effective.

FutureNanoNeeds considers that public dialogues on nanosaftey were sometimes characterized by confusion and lost trust in technological innovation. The project looks to the future, and provides unbiased foresight of the issues, not just those of tomorrow, but for decades to come. Instead of simply, 'what are the risks', one could also add, 'which directions of innovation might be preferential and more easily proven to have lowered risks'. This could have the impact of lowering market entry, by identifying where regulatory and other associated costs might be limited. Cooperation on this topic provided by the project will best serve the overall interest of Europe, and globally, in ensuring that nanotechnologies can be safely applied.

While the issues around the topic of nanosafety and regulation go far beyond science, and (as we have argued) much larger impacts, the impact within science itself should not be understated nor forgotten. It must be considered remarkable that almost no studies of materials with anomalous shapes, geometries and topologies have appeared in the literature, especially given the large growth of information on legacy materials, and on simple shapes. There have been a few discussions of novel clusters, and of course, the rod paradigm, albeit in the limited and potentially misleading guise of carbon nanotubes, has been discussed. In itself this could be argued as fortunate, for it affords time for those studies to be considered well scientifically, and executed within a strong framework.

Still, the facts remain that neither within the Nanosafety community, nor nanomedicine, nor indeed any of the other contiguous communities, have we really begun to develop an understanding of the connection between the properties of radically new materials, and their biological impacts, let alone provide a deep basis for safety.

Strengthening the role and Prestige of the EU's Nanosafety Cluster, The European Nanosafety Cluster is composed of those scientists working in EU and many others nationally funded. It has contributed significantly to the stabilization of research quality in nanosafety, the defragmentation of operations and opinions. It is now visibly moving to another level in which it begins to see itself as a leader Cluster of scientific excellence, worldwide.

The research carried out by the partners of FutureNanoNeeds (most are highly involved members of the Cluster) will be significant within the broader Nanosafety Cluster, giving strong hints as to those arenas of interest needing additional early efforts, and improving efficiency of funding deployment within National activities within EU. Besides this, successful science in this key strategic area will lend the Cluster, and its activities, international prestige and recognition, further building its morale, and reinforcing its obligation to fulfil its new mission of 'excellence'. It is clear that this program will have a unique impact on the Nanosafety Cluster.

FutureNanoNeeds actively promotes the alliance between Nanosafty Cluster and other relevant NMP programs, regulatory agencies and programs like NanoReg and industry by ongoing communication, but especially by a workshop (see 5 Progress and Outcomes to date) on the key outcomes of the program.

7 Directory

Table 1 Directory of people involved in this project.

First Name	Last Name	Affiliation	Address	e-mail
Derk	Brouwer	TNO	Utrechtseweg, Zeist 3704 HE, Netherlands	derk.brouwer@tno.nl
Miroslav	Černík	TUL	STUDENTSKA, LIBEREC 1, 46117, Czech Republic	miroslav.cernik@tul.cz

First Name	Last Name	Affiliation	Address	e-mail
Simon	Clavaguera	CEA	Rue des Martyrs, Grenoble Cedex 9 38054 France	simon.clavaguera@cea.fr
Aurora	Conill	Nanonica	Corcega, Barcelona 08025, Spain	aconill@nanotargeting.eu
Kenneth	Dawson	NUID UCD	School of Chemistry and Chemical Biology, Centre for BioNano Interactions, Belfield, Dublin 4	kenneth.a.dawson@cbni.ucd.ie
Teresa	Fernandes	HWU	Riccarton, EDINBURGH EH14 4AS, UK	t.fernandes@hw.ac.uk
Daniel	Fernandez Mosquera	NANOGAP	RUA DA XESTA NAVE, AMES A CORUNA 15895, Spain	d.fernandez@nanogap.es
Bertrand	Fillon	CEA	Rue des Martyrs, Grenoble Cedex 9 38054 France	bertrand.fillon@cea.fr
Michael	Graetzel	EPFL	Lausanne, CH-1015, Switzerland	michael.graetzel@epfl.ch
Karen	Griffin	NUID UCD	School of Chemistry and Chemical Biology, Centre for BioNano Interactions, Belfield, Dublin 4	karen.griffin@cbni.ucd.ie
Yuri	Gunko	TCD	College Green, Dublin 2, Ireland	igounko@tcd.ie
Arno	GUTLEB	CRP-GL	Rue du Brill, Belvaux 4422, Luxembourg	gutleb@lippmann.lu
David	Jeng	Solarprint	Ground Floor Silverstone House,Ballymoss Road, Sandyford, Dublin 18, Ireland	djeng@solarprint.ie
Zeljka	Krpectic	NUID UCD	School of Chemistry and Chemical Biology, Centre for BioNano Interactions, Belfield, Dublin 4	zeljka.krpetic@cbni.ucd.ie
Thomas	Kuhlbusch	IUTA	Bliersheimer Strasse, Duisburg D-47229, Germany	tky@iuta.de
Manuel Arturo	Lopez Quintela	USC	Faculty of Chemistry, Avda. Das Ciencias, Santiago de Compostela 15782, Spain	malopez.quintela@usc.es
Iseult	Lynch	UoB	Edgbaston,BIRMINGHAM B15 2TT, UK	i.lynch@bham.ac.uk
Fabian	Meder	NUID UCD	School of Chemistry and Chemical Biology, Centre for BioNano Interactions, Belfield, Dublin 4	fabian.meder@cbni.ucd.ie
Paolo	Milani	FILARETE	Viale Ortles, MILANO 20139, Italy	paolo.milani@fondazionefilarete.com
Marco	Monopoli	NUID UCD	School of Chemistry and Chemical Biology, Centre for BioNano Interactions, Belfield, Dublin 4	marco.monopoli@cbni.ucd.ie
Inge	Nelissen	VITO	Boeretang, MOL 2400, Belgium	inge.nelissen@vito.be
Wolfgang	Parak	PUM	Renthof, Marburg 35037, Germany	wolfgang.parak@physik.unimarburg.de
Peter	Pechy	EPFL	Lausanne, CH-1015, Switzerland	peter.pechy@epfl.ch

First Name	Last Name	Affiliation	Address	e-mail
Willie	Peijnenburg	RIVM	Antonie Van Leeuwenhoeklaan, BILTHOVEN 3721 MA, Netherlands	willie.peijnenburg@rivm.nl
Victor	Puntes	ICN	Campus UAB, Building CIN2 Office 2040, Bellaterra 08193, Spain	victor.puntes.ing@icn.cat
Louise	Rocks	NUID UCD	School of Chemistry and Chemical Biology, Centre for BioNano Interactions, Belfield, Dublin 4	louise.rocks@cbni.ucd.ie
John	Rumble	CODATA	RUE AUGUSTE VACQUERIE, PARIS 75016, FRANCE	jumbleusa@earthlink.net
Mario	Salmona	MNI	Via Giuseppe La Masa, MILANO 20156, Italy	mario.salmona@marionegri.it
Mauro	Sgroi	CRF	Strada Torino, ORBASSANO (TO) 10043, Italy	mauro.sgroi@crf.it
Thomas	Zadrozny	NfA	Rue de la Presse, Brussels 1000, Belgium	t.zadrozny@mail.be

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GUIDEnano

Assessment and mitigation of NM-enabled product risks on human and environmental health: Development of new strategies and creation of a web-based guidance tool for nanotech industries.

Contract Agreement: 604387 Website: www.guidenano.eu
Coordinator: Socorro Vázquez-Campos

Table 1 Consortium List.

No.	Beneficiary name	Short name	Country
1	Acondicionamiento Tarrasense	LEITAT	Spain
2	Torrecid Group	TORRECID	Spain
3	PlasmaChem	PCHEM	Germany
4	Innventia	INNVEN	Sweden
5	Lati Industria Termoplastici S.p.A.	LATI	Italy
6	Inotex	ITEX	Czech Republic
7	Servià Cantó	SVCT	Spain
8	Technical University of Liberec	TUL	Czech Republic
9	Consorzio Venezia Ricerche	CVR	Italy
10	Commissariat à l'énergie atomique et aux énergies alternatives	CEA	France
11	University of Gothenburg	UGOT	Sweden
12	Instituto Superior Técnico	IST	Portugal
13	Institute of Occupational Medicine	IOM	UK
14	TNO Netherlands Organization for Applied Scientific Research	TNO	The Netherlands
15	Instituto Tecnológico del Embalaje Transporte y Logística	ITENE	Spain
16	Institut Català de Nanotecnologia	ICN	Spain
17	GeoChem	GEOC	The Netherlands
18	Utrecht University	DEI	The Netherlands
19	National Institute for Public Health and the Environment	RIVM	The Netherlands
20	Finish Institute of Occupational Health	FIOH	Finland
21	Uppsala University	UU	Sweden
22	National Environment Research Council	NERC	UK
23	Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria	INIA	Spain
24	University of Exeter	UNEXE	UK
25	Honeywell	HWELL	France
26	German Institute for Standardization	DIN	Germany
27	Nanoservices B.V.	NSER	The Netherlands
28	ThinkWorks B.V.	TWORKS	The Netherlands
29	Pinturas Hempel S.A.U.	HEMPEL	Spain
30	Pinsent Masons LLP	PM	UK

Contents

1 Summary

Project Duration: 42 months

Project Funding: 8.150.000 €

GUIDEnano will develop innovative methodologies to evaluate and manage human and environmental health risks of nano-enabled products, considering the whole product life cycle: synthesis of nanomaterials (NM), manufacturing of NM-enabled products, use, and end-of-life phase (including recycling).

These developments will be incorporated into an interactive web-based Guidance Tool, which will guide the NM-enabled product developers (mainly industry) into the design and application of the most appropriate risk assessment & mitigation strategy for a specific product. The correct implementation of this guidance will ensure that the risks associated with a NM-enabled product, throughout its whole life cycle/ value chain, have been appropriately evaluated and mitigated to an acceptable level, according to the most recent knowledge at the time of implementation. The evaluation of a NM-enabled product using this Tool will also be useful for risk communication to regulators, insurance companies, and society.

2 Background

Current uncertainties on the safety of nano-enabled products need to be urgently and carefully addressed. Otherwise, public fears could end up blocking the benefits of nanotechnology. Sound scientific information must be generated to identify potential risks of nano-enabled products on human and ecosystems health and, when considered unacceptable, efficiently mitigate such risks. This has to be done in a holistic manner, taking into consideration all stages of the life cycle of these products.

Numerous guidance resources have been generated on isolated parts of the risk assessment process. However, most of these consist on extensive papers documents of difficult use by industry. Some web-based control banding tools are also available, but these mainly focus on the worksite and are mainly intended at the identification of hotspots rather than a complete risk assessment.

3 Scientific and technological challenges

The GUIDEnano project aims at generating a risk assessment web-based Tool, which incorporates as well guidance on the selection of risk management options. To reach these goals, the project will build upon the state-of-the-art on risk assessment and management by validating critical assumptions in the risk assessment process, generating new predictive models, and developing new testing methods and novel risk management solutions.

Release and exposure evaluation. The GUIDEnano project will categorize the possible processes that take place during the different stages of a NM-enabled product life cycle. Default worse-case release values will be assigned to these different processes that will be refined when specific models or experimental data are available for some of the processes. In order to generate release data during the use phase, methodologies will be identified or developed to simulate such types of processes in an accelerated manner. GUIDEnano will work on the adaptation of other standard methods or develop new aging devices to evaluate release and transformation of NM during other types of processes not yet considered in previous projects. Different recycling strategies of nano-enabled products (including incineration, NM inertization, and waste water treatment (WWT)) will be investigated to identify possible hotspots for release into the environment.

During experimental release evaluation, released NMs or the residues of degradation of the nano-enabled products, e.g. paint debris containing NMs, will be collected and a thorough physical-chemical characterization will be conducted.

In parallel to the release work, the most relevant human exposure scenarios will be identified. Previously developed exposure scenarios (e.g., in NANEX and MARINA) and existing tiered approaches, including available control banding tools, will be used as a starting point. Exposure (computational) models will be tested (see details below). Depending on the outcome of the initial exposure/release evaluation tiers, experimental monitoring of release and/or exposure may be required.

To support future development of exposure prediction models, lab scale experiments will be performed to fill in data gaps in the information available in the literature and previously collected data on exposure scenarios in other EU funded projects in which GUIDEnano partners participate or have participated. A test chamber prototype under controlled conditions (air flow, pressure, temperature…) will be used to simulate industrial processes and evaluate release and transformation of NM during these processes.

Environmental fate. Once the NMs are released to the environment, understanding their mobility and transformations during their environmental fate trajectory is critical to identify possible ecosystem effects, including long-term effects due to persistency or bioaccumulation. To this aim, the GUIDEnano project will identify the main processes that determine environmental fate and provide guidance to select the appropriate test methods. Among the parameters considered there will be solubility, dispersion stability in different aqueous matrices, mobility in soil/sediment matrices, and other transformation/degradation studies in different environmental matrices. A battery of experimental tests will provide model parameters to predict the transport of NMs in natural waters, sediments or columns of natural soils. Ultimately, these transport models will be used to identify environmental sinks of NMs and to estimate predictive environmental concentrations as well as the associated bioavailable NM-forms in each of the relevant environmental compartments at a local or regional scale.

(Eco)toxicity. Guidance will also be developed with the aim to help industrial partners identify the toxicological tests to be conducted on a case by case basis, considering the relevant target communities, exposure routes, and exposure durations. Such tests will then be undertaken by the hazard assessment partners within the scope of the project, or could be undertaken by specialized CROs once the guidance is implemented beyond this project.

Most of the existing nanotoxicology studies have been conducted on pristine NMs. However, it is likely that consumers and workers in later stages of the life cycle are exposed to processed (e.g. coated) or aged NMs (recycling) rather than pristine NMs. Therefore, understanding the hazard of these exposure-relevant NMs is necessary for a meaningful risk assessment.

The evaluation of the ecotoxicological hazard of the exposure-relevant NMs will have the final goal of deriving predicted no effect concentrations for the relevant environmental compartments (i.e., freshwater, marine, benthic, terrestrial, and microorganisms). In addition to the implementation of such testing strategies to the project case studies, research efforts of the project will focus on recognized data gaps for the prediction of ecotoxicity of NMs, particularly for the definition of worse-case default values and the validation of the assumptions used during the risk assessment process.

A similar approach will be taken for the evaluation of the human health toxicological hazard of the exposure-relevant NMs. The existing data will be taken into consideration to allow the derivation of worst-case default reference values for human health effects, which would depend, as far as possible, on the physicochemical properties of a NM. To this aim, whenever possible, read-across approaches using information from similar materials would be used to derive conservative reference values. When the GUIDEnano Tool requires the refinement of these hazard estimations, this will represent moving into higher Tiers, involving a palette of in vitro assays and in vivo studies with laboratory rodents, evaluating inflammatory response, genotoxicity and histopathology to evaluate local and systemic toxicity. In addition, ex vivo samples will also be used to assess biodistribution and bioaccumulation of NMs, and facilitate extrapolation to long-term exposures.

Some of the (eco)toxicity studies require considerable amounts of test materials. If the amounts of NM collected during the life cycle simulation processes are not sufficient to undertake these studies, transformed NMs will be engineered in the laboratory.

Risk management. The risk mitigation strategies that the GUIDEnano project will generate and evaluate will range from the definition of best practices for handling NMs and NM-waste, to the evaluation of the efficiency of existing practices and the development of new control measures (PPEs, as well as air and water treatment), and the redesigning of NM towards a safer profile. These safer-by-design strategies will be developed to modulate the main factors determining safety of NMs: Intrinsic toxicity, release/ bioavailability potential, and persistence. Finally, suitable strategies for the end-of-life steps will be proposed in order to minimize the potential for occupational or environmental exposure, taking into account the features of different nano-enabled products.

Web-based risk assessment and risk management tool. The GUIDEnano Tool, will be generated to incorporate the developed risk assessment strategies, scientific decision-trees, predictive models, data collection and evaluation templates, standardized methods, and databases, and other related technical information. The resulting GUIDEnano Tool will support end-users (e.g. industrial partners) during risk assessment and in the definition of effective risk mitigation plans for the effective application of the safety interventions. The GUIDEnano Tool will be iteratively improved during the project, incorporating new research findings and the feedback from the industrial partners (Figure 1).

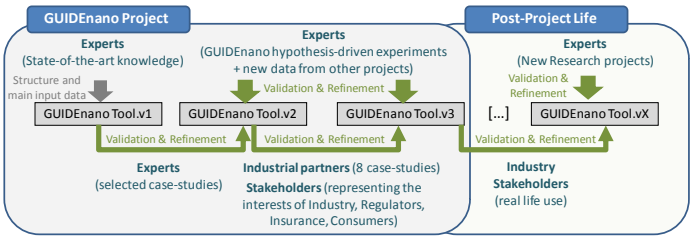

Figure 1. Main steps in the development of the GUIDEnano Tool.

A decision module that would either conclude on an acceptable level of risk, or on the contrary, would provide different options to refine the risk assessment (only in cases where a very conservative assessment was performed with large associated uncertainties) and/or to introduce risk mitigation measures. A sensitivity analysis of the risk assessment process would help identify the step(s) of the risk assessment where refinement would be most cost-effective. Similarly, this decision node would guide in the selection of the most appropriate risk mitigation options for each particular case (Figure 2).

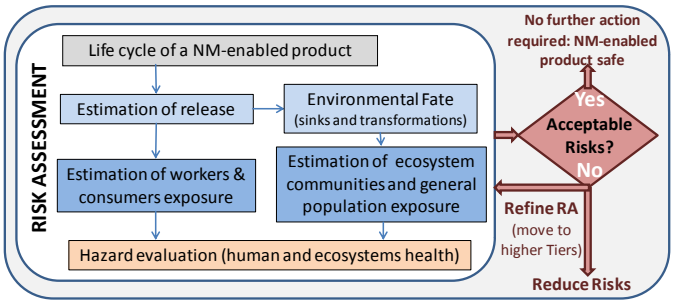

Figure 2. Risk assessment decision module.

Efforts will also focus on evaluating the needs of key stakeholders (industry associations, regulators, consumers, insurers) in terms of the information needed in the risk assessment report. This will facilitate that the outcome of the GUIDEnano Tool is accepted by such stakeholders.

4 Objectives

The main objective of GUIDEnano is to develop innovative methodologies to evaluate and manage human and environmental health risks of nano-enabled products, considering the whole product life cycle: synthesis of NM, manufacturing of NM-enabled products, use, and end-of-life phase (including recycling) (Figure 3).

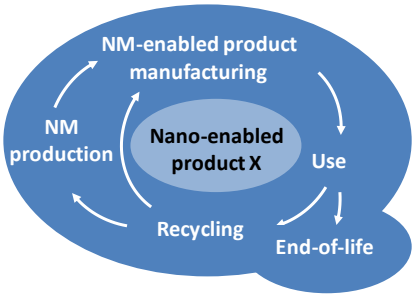

Figure 3. Target of the risk assessment in the GUIDEnano Tool.

These developments will be incorporated into an interactive web-based Guidance Tool, which will guide the NM-enabled product developers (mainly industry) into the design and application of the most appropriate risk assessment & mitigation strategy for a specific product.

Specific goals of the project:

1. To develop methodologies to evaluate the risks of a wide diversity of nano-enabled products on human and environmental health, throughout their life cycle.

2. To develop innovative solutions to reduce the identified risks. A wide range of risk mitigation strategies and guidance on the selection of the most appropriate measures for each scenario associated to an unacceptable risk will be provided.

3. To integrate the risk evaluation and mitigation strategies into the GUIDEnano Tool and to carry out an iterative process of performance testing, feedback and improvement steps to validate its suitability and applicability to real-case NM-enabled products, including a detailed plan for the hosting and maintenance of the GUIDEnano Tool after the life time of the project.

4. To efficiently communicate to consumers, regulators and insurance communities that, by following the GUIDEnano Tool, risks associated with an NM-enabled product have been adequately identified, evaluated and mitigated across the whole of their life cycle. Thus, ensuring that workers, consumers and environmental health have been appropriately protected, and facilitating social acceptance, regulatory control, and insurance activities related to nanotechnologies.

5 Organisation

GUIDEnano is structured into 11 work packages (Figure 4) arranged by four main blocks: the Coordination block (WP1 and WP2), the Knowledge block subdivided into different technological building sub-blocks (WP3, WP4, WP5, WP6, WP7 and WP8) that will generate the scientific input to the GUIDEnano Tool, the Software Development and Demonstration block (WP9 and WP10) that will create the Tool itself and will validate it in real life case studies, and the Dissemination, Standardization, and IPR block (WP11).

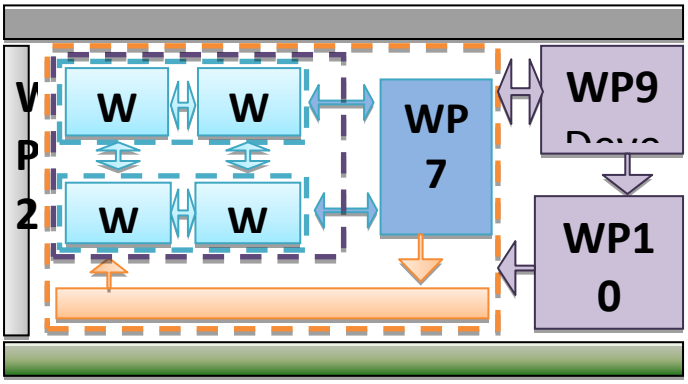

Figure 4. Organization of the project in work packages.

6 Expected Impact

GUIDEnano will build upon the state-of-the-art on NM risk assessment, which has not yet been translated into regulatory guidance, and will provide a Tool to be used by industry with the aim to complement existing regulations in different frameworks.

By using this Tool, industry will be able to evaluate and efficiently mitigate possible health risks for workers, consumers and the environment associated to the use of nanotechnologies.

The report generated by the GUIDEnano Tool will be designed to facilitate communication and acceptance of the Tool outcome by regulatory agencies, occupational safety and health agents, insurance companies, and consumer protection associations. Transparency in the risk assessment process, i.e. specific methodology and assumptions used, is crucial in such a new and scientifically-challenging framework that is constantly evolving and that does not yet benefit from internationally accepted risk assessment standards.

These steps are crucial in ensuring market and regulatory acceptance of NM-enabled products. A wider acceptance should benefit existing nanotechnology industry, which would increase market shares. In addition, it should also provide opportunities for a wide range of industrial sectors to incorporate nanotechnology in their processes and products.

7 Directory

Table 2 Directory of people involved in this project.

First Name	Last Name	Affiliation	Address	e-mail
Socorro Gemma Amro David Alex Joan	Vázquez-Campos Janer Satti González Vilchez Cabellos	Acondicionamiento Tarrasense	c/ de la Innovació, 2, 08225, Terrassa, Spain	svazquez@leitat.org; gjaner@leitat.org; asatti@leitat.org; dgonzalez@leitat.org; avilchez@leitat.org; jcabellos@leitat.org

First Name	Last Name	Affiliation	Address	e-mail
Carlos	Concepción	Torrecid Group	Partida Torreta, Alcora, Castellon, 12110, Spain	carlos.concepcion@torrecid.com; oruiz@torrecid.com
Alexei Leonid	Antipov Goldenberg	PlasmaChem	29 Rudower Chaussee, Berlin, 12489, Germany	antipov@plasmachem.com goldenberg@plasmachem.com
Ewa	Lie	Innventia	Drottning Kristinas vaeg 61, Stockholm, 11486, Sweden	Ewa.Lie@innventia.com
Loredana Cristiano	Mercante Citterio	Lati Industria Termoplastici S.p.A.	7 Francesco Baracca, Bedano Olona, 21040	lmercante@it.lati.com; ccitterio@it.lati.com
Jan Olga	Marek Chybova	Inotex	1208 Stefanikova, Dvur Kralove Nad Labem, 54401, Czech Republic	marek@inotex.cz; chybova@inotex.cz
Cristian Carolina	Cortés Rios	Servià Cantó	2 Calle Bonastruc de Porta 20, Girona, 17001, Spain	ccortesfu@fcc.es; crioslo@fcc.es
Miroslav Alena Ivana	Cernik Sevcu Chramostová	Technical University of Liberec	1402/2 Studentska Liberec 1, 46117, Czech Republic	miroslav.cernik@tul.cz; alena.sevcu@tul.cz; ivana.chramostova@tul.cz
Stefano Andrea	Zuin Massari	Consorzio Venezia Ricerche	12 Via della Liberta, Venezia, 30175, Italy	sz.cvr@vegapark.ve.it; massari.cvr@vegapark.ve.it;
Delphine Luana	Boutry Golansky	Commissariat à l'énergie atomique et aux énergies alternatives	25 Rue LeBlanc, Paris 15, 75015, France	delphine.boutry@cea.fr luana.golansky@cea.fr
Geert	Cornelis	University of Gothenburg	Vasaparken Goeteborg, 405 30, Sweden	geert.cornelis@chem.gu.se
Rute José Cristiana	Domingos Farinha Franco	Instituto Superior Técnico	Av. Rovisco Pais 1, Lisbon, 1049-001, Portugal	rute.domingos@ist.utl.pt; farinha@ist.utl.pt cristiana.franco@gmail.com
Martie Gordon	Van Tongeren Fern	Institute of Occupational Medicine	45 Research Avenue North, Riccarton, Edinburgh, EH14 4AP, UK	martie.vantongeren@iom-world.org; gordon.fern@iom-world.org
Derk Esther Jody Thies Roel Eef Rinke Joost Nienke	Brouwer Zondervan Schinkel Oosterwijk Bisselink Voogd Klein Westerhout Koeman	TNO Netherlands Organization for Applied Scientific Research	97 Schoemakerstraat, Delft, 2628 VK, The Netherlands	dick.brouwer@tno.nl; esther.zondervan@tno.nl jody.schinkel@tno.nl; thies.oosterwijk@tno.nl; roel.bisselink@tno.nl; eef.voogd@tno.nl; rinke.klein@tno.nl; joost.westerhout@tno.nl nienke.koeman@tno.nl
Carlos George Marga	Fito Boulougouris Santamaria	Instituto Tecnológico del Embajale Transporte y Logística	1 Calle Albert Einstein. Parque Tecnológico., Paterna, 46980, Spain	cfito@itene.com; george.boulougouris@itene.com; msantamaria@itene.com
Victor Martí	Puntes Busquets	Institut Català de Nanotecnologia	Campus de la UAB Edifici CM7, Bellaterra, 08193, Spain	victor.puntes@icn.cat; martibusq@gmail.com
Virginie Jacco	Heidweiller Hoekstra	GeoChem	28 Weesperzjide, Amsterdam, 1091 EC, The Netherlands	vmlheidweiller@geochem.nl; jaccohoekstra@geochem.nl
Hans Andries Celos	Gerritsen Meijerink Demello-Donega	Utrecht University	8 Heidelberglaan, Utrecht, 3584 CS	H.C.Gerritsen@uu.nl; A.Meijerink@uu.nl; c.demello-donega@uu.nl
Margriet Susan Petra Adrienne Henk	Park Wijnhoven Van Kesteren Sips Van Loveren	National Insitute for Public Health and the Environment	8 Antonie Van Leeuwenhoeklaan, Bilthoven, 3721 MA, The Netherlands	margriet.park@rivm.nl; susan.wijnhoven@rivm.nl; petra.van.kesteren@rivm.nl; adrienne.sips@rivm.nl; henk.van.loveren@rivm.nl

First Name	Last Name	Affiliation	Address	e-mail
Julia Hanna Helene Kai Hannu	Catalan Lindberg Stockmann-Juvala Savolainen Norppa	Finish Institute of Occupational Health	41 a A, Topeliuksenkatu, Helsinki, 00250, Finland	julia.catalan@ttl.fi; hanna.lindberg@ttl.fi; Helene.Stockmann-Juvala@ttl.fi; kai.savolainen@ttl.fi; hannu.norppa@ttl.fi
Natalia	Ferraz	Uppsala University	10B, St Olofsgatan, Uppsala, 751 05, Sweden	natalia.ferraz@angstrom.uu.se
Claus	Svendsen	National Environment Research Council	NERC	csv@ceh.ac.uk;
David	Spurgeon		CEH Wallingford	dasp@ceh.ac.uk;
Bill	Tyne		Crowmarsh Gifford	wit@ceh.ac.uk;
Elma	Lahive		Wallingford	elmhiv@nerc.ac.uk;
Daniel	Read		OX10 8 BB, Oxon	dasr@ceh.ac.uk;
Richard	Williams		United Kingdon	rjw@ceh.ac.uk;
Virginie	Keller			vke@ceh.ac.uk
Egon	Dumont			egdu@ceh.ac.uk:
Mª Luisa José Mª	Fernández-Cruz Navas	Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria	Carretera de la Coruna Km7.5, Madrid, 28040, Spain	fcruz@inia.es; jmnavas@inia.es
Tamara	Galloway	University of Exeter	Northcote House, The Queen's Drive, Exeter, EX4 4QJ, UK	t.s.galloway@exeter.ac.uk
Samuel	Ozil	Honeywell	33 rue des Vanesses, Roissy Charles De Gaulle Cedex 95943, France	Samuel.Ozil@Honeywell.com
Roman	Rüettinger	German Institute for Standardization	6 Burggrafenstrasse, Berlin 10787, Germany	roman.ruettinger@din.de
Dimmen	Breen	Nanoservices B.V.	13, Dr. van Deenweg, Dr. van Deenweg, 8025, The Netherlands	d.k.breen@online.nl
Ralph Lion	Vanhauten Traas	ThinkWorks B.V.	183 C, Rotterdamseweg, Delft, 2629 HD, The Netherlands	R.Vanhauten@ThinkWorks.nl L.Traas@ThinkWorks.nl
Markus	Hoffman	Pinturas Hempel S.A.U.	Carretera Sentmenat 142, KM3/8, 08213, Polinya, Barcelona, Spain	maho@hempel.com
Manoj	Vahela	Pinsent Masons LLP	Crown Place 30 Earl Street, EC2A 4ES, London, UK	Manoj.Vaghela@pinsentmasons.com

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INSTANT

Innovative Sensor for the fast Analysis of Nanoparticles in Selected Target Products

Website: http://instant-nps.eu
Coordinator: Günter Gauglitz, Institute of Physical and Theoretical Chemistry, Tübingen, Germany

No.	Beneficiary name	Short name	Country
1	Eberhard Karls Universität Tübingen	EKUT	Germany
2	Biametrics GmbH	BM	Germany
3	University of Umeå	UmU	Sweden
4	University of Vienna	UNIVIE	Austria
5	University of Cordoba, Department of Analytical Chemistry	UCO	Spain
6	Sitex45	SITEX	Romania
7	Sociedad de Investigación en Nanoestructuras S.L	SIANTEC	Spain
8	BAM Federal Institute for Materials Research and Testing	BAM	Germany
9	Corpus Datamining	CD	Sweden
10	Nanordic	NANO	Finland

Contents

1 Summary

Project Duration: 1 March 2012 – 31 August 2015

Project Funding: 3,772 Mio. EUR

INSTANT will face the challenge of the detection, identification and quantification of engineered nanoparticles (ENPs) in complex matrices such as cosmetic products and engineered food and drinks. Therefore, new detection methods and technologies are mandatory. This is completely in line with the Call FP7-NMP.2011.1.3-1 which deals especially with innovative, practically implementable and cost effective measurement approaches for ENPs in complex matrices. Recently emerging ENPs include Ag, SiO_2, TiO_2, ZnO, and organic NPs. The "Opinion of the Scientific Committee on the Potential Risks Arising from Nanoscience and Nanotechnologies on Food and Feed Safety" released by the European Food Safety Authority (EFSA) (2009) also highlights the urgent need for such a tool. Accordingly, the interdisciplinary project INSTANT will develop an innovative and integrated technology for monitoring the exposure of consumers to ENPs using a label free opto-electrochemical sensor array in combination with novel recognition elements.

The SME driven INSTANT will develop an innovative, cost effective, and easy to use analytical tool to extract, detect and identify ENPs typically used in cosmetic products (e.g. sunscreen, toothpaste, deodorant, ...) and engineered food (e.g. instant soups, ketchup, ice cream, ...) and drinks (e.g. fruit juice, energy drinks, bottled water, ...). A crucial point of measuring in these complex matrices is the sample preparation and extraction. Therefore, INSTANT will develop and integrate tailored extraction methods. Especially the size distribution of ENPs in the sample and the influence of the matrix on chemical and physical properties of the ENPs have to be taken into account. The INSTANT device will be designed to be used as a cost effective monitoring tool which is suitable for characterization and classification of ENPs for the future implementation of quantitative structure-activity relationship studies.

2 Background

In recent years, nanotechnology has been a hot topic in the scientific community due to the specific properties in the nanoscale and has become an enabling technology for numerous applications. Especially engineered nanoparticles (ENPs) have shown various beneficial properties. In many fields of application, these ENPs have left the scientific laboratories and made their way to consumer products[1]. Beside their advantages, ENPs are under discussion in the scientific community due to possible unforeseen hazards and an unknown disposition in living organisms and the environment. Nanoparticles (NPs) have drawn vast public attention due to their application in many consumer products (e.g. cosmetics, food and food packaging, drinks). Following the "Opinion of the Scientific Committee on the Potential Risks Arising from Nanoscience and Nanotechnologies on Food and Feed Safety" released by the European Food Safety Authority (EFSA) in 2009[2], the European Commission tackles this arising matter of public concern within the current FP7 NMP theme.

One of the key challenges is the detection, identification and quantification of engineered nanoparticles in complex matrices, such as products, food and the environment. However, currently none of the existing techniques allows for a holistic approach which is able to analyze all ENPs' properties in a single step.

3 What is INSTANT

INSTANT will face this challenge by developing a fully integrated tool for the extraction of ENPs from complex matrices and their subsequent detection and identification. The device will be tailored to be used as a cost-effective monitoring tool, allowing for analytics of food and cosmetics close to the point of need (Point-of-Product Testing POPT and Point-of-Food Testing POFT).

Accordingly, the project INSTANT is organized in a workflow using complementary expertise of well-known partners in their fields from all over Europe.

The detection and identification of ENPs in cosmetic products, food and/or drinks require an efficient sample preparation and extraction of ENPs from these complex matrices. Especially the size distribution of ENPs in the sample and the influence of the matrix on chemical and physical properties of the ENPs have to be taken into account. INSTANT will develop a generalized extraction protocol to isolate and pre-concentrate ENPs from food and cosmetic samples. An extraction protocol, as generalized as possible, will be developed for the extraction of ENPs from complex matrices. This generalized protocol allows for an extension in the future to a wider range of samples (e.g. for environmental monitoring).

After extraction and pre-concentration of ENPs, an innovative, cheap and robust sensor device is used for their detection. This sensor will be developed within INSTANT. For the detection of ENPs, recognition structures with a high affinity to ENPs are mandatory. INSTANT will use two different types of recognition elements (REs) with different selectivity combined on an array. On the one hand, technologies will be applied to for generating REs to distinguish between size, shape and material. On the other hand, REs will be chemically modified in order to generate a material for selective sorption of targeted ENP species.

Also, the sensor will combine two complementary transduction principles, an optical and an electrochemical one. Electrochemical sensing is sensitive to ENPs' speciation including conductivity, surface properties and chemical composition. Optical transduction will provide information on ENP size, size distribution and refractive index. Both transduction principles will be adapted to a sensor array, which allows for simultaneous detection of various ENPs.

By combining two transduction principles with two types of recognition elements on a single array, a huge amount of data will be produced. In order to reduce redundancies, to separate noise from signal and to extract relevant information, strong chemometric techniques for the detection, identification and quantification of these ENPs will be needed.

3.1 Summary of INSTANT`s key strengths

- Develop a simple and fully integrated sensing system, together with a suitable number of sensor elements for the detection and identification of ENPs in one device.
- Combine two complementary transduction principles to create a robust sensor system providing high-content information about ENPs.
- Implement innovative recognition elements (REs).
- Improve and modify sampling and separation techniques in regard to the complex matrices.
- Develop advanced chemometrics to extract information from the complex data sets.
- Provide characterized and standardized ENPs for the comparison of their properties as pure material used as product and food additives as well as during storage and processing.

4 Organization of INSTANT

INSTANT merges high ranked European partners with remarkable knowledge in each area of the proposed work. Abundant complementary expertise is provided by an interdisciplinary working group of researchers, whose contribution is essential for a successful outcome of the project. The combined resources mobilized completely fulfill all the requirements of the Programme in terms of facilities, equipment, personnel and resources. On a national level it would not have been possible to gather a consortium of this high quality and complementarity.

SMEs and research institutions are brought together to cover the various tasks by distributed expertise and to carry out

1 Tran, Lang; Chaudhry, Qasim, RSC Nanoscience & Nanotechnology, (2010), 14, 120-133

2 Scientific Opinion of the Scientific Committee on a request from the European Commission on the Potential Risks Arising from Nanoscience and Nanotechnologies on Food and Feed Safety. The EFSA Journal (2009) 958, 1-39.

complementary research which will lead to a highly innovative technology. SMEs are involved in all parts of the project that are interesting for future exploitation and will benefit from joint research activities of academia and industry.

Table 1 Workpackages (WP) of NanoImpactNet

WP	Title	Topic
1	Sensor development	In WP 1, the sensor array for the INSTANT device is developed. For this purpose, design and specific assembling of both transduction methods are elaborated.
2	Sample preparation and standard materials	WP 2 has two main objectives. Firstly, WP 2 synthesizes and characterizes reference ENPs. Secondly, WP 2 develops a generalized extraction protocol to isolate and pre-concentrate ENPs from food and cosmetic samples.
3	Recognition elements	WP 3 is the main platform for designing selective layers for the multivariate sensor platforms. The goal is to achieve as high "chemical" selectivity (i.e. directly on the measuring device) as possible.
4	Chemometrics and experimental design	WP 4 deals with data mining for extracting minute signals from the complex and probably noisy measurement data obtained by the sensor system(s).
5	System integration	The aim of WP 5 is to setup a fully integrated analytical tool based on the components provided by WP 1 (Sensor development), WP 2 (Sample preparation and standard materials), and WP 4 (Chemometrics and experimental design).
6	Management	This work package coordinates the overall financial, administrative, legal and contractual management of INSTANT.
7	Dissemination and exploitation of results	Dissemination activities beyond the consortium to the scientific community and towards a wider international audience through specialized events like conferences, fairs and exhibitions.

5 INSTANT Events and Reports

The project INSTANT targets three key issues of ongoing debate about nanomaterials, their safety, monitoring, and risk assessment:

- INSTANT will provide a powerful tool to researchers dealing with NP detection and characterization.
- With help of the INSTANT device, it will be possible to ensure that the consumer will feel safe that the product he or she is buying is either free of nanoparticles (if desired) or contains amounts and speciation of nanoparticles that are considered as safe by the EU.
- With the development of the INSTANT device, the consortium delivers a tailor-made instrument to the legislative organs of the EU. This will be the device of choice to gather enough data about nanoparticles in different products and exposure of the consumer to these nanoparticles to help the decision-making process within the EU.

In addition to these three points, the developed device will enable the participating SMEs to take a leading role in the production and distribution of the next generation device for the detection of nanoparticles in all fields of applications.

Beside this technological goal several events are planned in order to strengthen European knowledge and cooperation within the field of nanotechnology.

5.1 Events

In order to strengthen European knowledge and cooperation within the field of nanotechnology, INSTANT will join forces with other EC funded projects like SMART-NANO, NANODETECTOR and QNANO. Besides an ongoing exchange of samples and knowledge joint events as workshops and a public midterm seminar are planned.

5.1.1 Workshops

- INSTANT will conduct workshops where recent development in the detection of NPs will be presented to a wider audience. Representatives from EU projects will be invited as well in order to share knowledge and experiences with other EC funded projects dealing with the preparation of reference materials and the detection

of nanoparticles (e.g. NANODETECTOR, SMART-NANO, QNANO). This will draw the attention from customers to the INSTANT technology and their possibilities.

5.1.2 Conclusion of Results

The first half of the project led to following results that can be summarized as follows:

WP1 (Sensor Development):

- Microfluidic system allowing for maximal flexibility and performance was set up. This system includes pumps, valves, tubing and a flow cell, which can be used for the simultaneous optical and electrochemical detection of nanoparticles.
- Optical and electrochemical setup was built and combined in a complete assembly. Thus, it was merged with the fluidic system using an appropriate flow cell.
- Combined Electrochemical and Optical transducer is constructed.

WP2 (Sample Preparation and Standard Materials):

- Metal oxides nanoparticles are synthesized and well characterized, in order to obtain reference material for developing the sensor device.
- Synthesis and analytics for metal nanoparticles was performed.
- Different stabilization agents were tested and evaluated for the different kind of nanoparticles.
- The experimental parameters that affect sample preparation up to the stage of extraction and pre-concentration of different types of nanoparticles were investigated.
- Optimal conditions were developed for developing a general protocol for extraction and pre-concentration of NPs were developed.
- Implementation of substances added (like surfactants or quelating agents) to facilitate the extraction of NPs from matrix components and to pre-concentrate NPs.

WP3 (Recognition Elements):

- Recognition elements for different nanoparticle species have been successfully generated using an appropriate imprinting technique.
- Recognition elements could be characterized by QCM measurements. Sensor signals are reversible and reproducible.
- Optical and electrochemical measurements successfully demonstrated the ability of recognition elements to interact with nanoparticles specifically.

WP4 (Chemometrics and Experimental Design):

- Multivariate analysis of complex number data and the pre-algorithm was developed.
- The data from the electrochemical cell experiments are evaluated and a multivariate data analysis to optical and electrochemical data was performed.
- Using this data, the interactively design of flow-cell experiments was generated.

WP5 (System Integration):

- First integration concept of main hardware modules is defined.
- The design of interfaces, supply and display units and also possible housings are developed.
- Referring to optical components, concepts of sample preparation unit, microfluidics and treatment system are ready.
- At this stage the relevant interfaces and units for powering and supplying the units, interfaces and related accessories with housing, including the modular concept by special conditions are investigated.

6 Directory

Table 2 Directory of people involved in this project.

First Name	Last Name	Affiliation	Address	e-mail
Günter	Gauglitz	Eberhard Karls Universität Tübingen	Geschwister-Scholl-Platz, 72076 Tübingen (Germany)	guenter.gauglitz@ipc.uni-tuebingen.de
Günther	Proll	Biametrics GmbH	Auf der Morgenstelle 18, 72076 Tübingen (Germany)	guenther.proll@biametrics.com
Britta	Lindholm-Sethson	Umeå Universitet	901 87 Umeå (Sweden)	britta.sethson@chem.umu.se
Peter	Lieberzeit	Universität Wien	Dr.-Karl-Lueger-Ring 1, 1010 Vienna (Austria)	peter.lieberzeit@univie.ac.at

First Name	Last Name	Affiliation	Address	e-mail
Miguel	Valcárcel	Universidad de Cordoba	Avda. Medina Azahara 5 14071 Córdoba (Spain)	qa1vacam@uco.es
Dumitru	Ulieru	Sitex45 SRL	114, Ghica Tei Blvd, Bl.40, AP.2, DEPT.2, 023709 Bucharest (Romania)	dumitru_ulieru@yahoo.com.hk
Bartolome Miguel	Simonet Suau	Sociedad de Investigación en Nanoestructuras S.L	Plaza Escultor Ruiz Olmos 12 14005 Cordoba (Spain)	qa2sisub@uco.es
Andreas	Thünemann	BAM Bundesanstalt für Materialforschung und -prüfung	Unter den Eichen 87 12205 Berlin (Germany)	andreas.thuenemann@bam.de
Hans	Grahn	Corpus Data Mining Handelsbolag	Primusgatan 70 11267 Stockholm (Sweden)	grahnconsulting@telia.com
Geza	Toth	Nanordic Oy	Toth Kalikkakuja 2 D 25 90540 Oulu (Finland)	geza.toth@nanordic.com

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MARINA

MANAGING RISKS of NANOMATERIALS

Contract Agreement: 263215 Website: http://www.marina-fp7.eu
Coordinator: Dr Lang Tran, Institute of Occupational Medicine, Edinburgh (UK)

no.	Participant Legal Name	Country	Organisation Type
1	Institute of Occupational Medicine (IOM)	United Kingdom	SME
2	European Research Services GmbH (ERS)	Germany	Industry
3	Aarhus University (AU)	Denmark	University
4	BASF (BASF)	Germany	Industry
5	Commissariat à l'énergie àtomique (CEA)	France	Research Organisation
6	Das Institut für Energie- und Umwelttechnik (IUTA e.V.)	Germany	Research Organisation
7	Swiss Federal Laboratories for Materials Testing and Research (EMPA)	Switzerland	Research Organisation
8	Finish Institute for Occupational Health (FIOH)	Finland	Research Organisation
9	Fraunhofer-Institut für Molekularbiologie und Angewandte Oekologie IME-AE (IME)	Germany	Research Organisation
10	Freie Universität Berlin (FUB)	Germany	University
11	Gothenburg University (UGOT)	Sweden	University
12	Health & Safety Laboratory (HSL)	United Kingdom	Research Organisation
13	Institut National de l'Environment Industriel et des Risques (INERIS)	France	Research Organisation
14	Instituto Nacional de Investigación y Tecnología Agragia y Alimentaria (INIA)	Spain	Research Organisation
15	Joint Research Centre of the European Commission (JRC)	Italy	Research Organisation
16	Max-Planck-Institute for Molecular Genetics (MPI)	Germany	Research Organisation
17	Nanotechnology Industries Association (NIA)	Belgium	SME
18	National Physical Laboratory (NPL)	United Kingdom	Research Organisation
19	Stichting Dienst Landbouwkundig Onderzoek (DLO)	The Netherlands	Research Organisation
20	National Institute for Public Health and the Environment (RIVM)	The Netherlands	Research Organisation
21	Netherlands Organisation for Applied Scientific Research (TNO)	The Netherlands	Research Organisation
22	Universität Salzburg (PLUS)	Austria	University
23	University College Dublin (UCD)	Ireland	University
24	University of Leeds	UK	University
25	University of Wien (UVIE)	Austria	University
26	VTT Technical Research Centre of Finland (VTT)	Finland	Research Organisation

27	Westfälische Wilhelms-Universität Münster (WWU)	Germany	University
28	Technical University of Denmark (DTU)	Denmark	University
29	ACCIONA (ACC)	Spain	Industry
30	Venice Research Consortium (CVR)	Italy	Research Organisation
31	The REACH Centre Ltd (TRC)	United Kingdom	SME
32	Karolinska Institute (KTH)	Sweden	University
33	National Center for Nanoscience and Technology, Chinese Academy of Sciences (NCNT)	China	Research Organisation
34	Institute of Biochemistry, Russian Academy of Sciences (INBI)	Russia	University
35	University of Parma (UP)	Italy	University
36	Tor Vergata University Roma 2 (TVUR)	Italy	University
37	Edinburgh Napier University (Napier)	United Kingdom	University
38	Ludwig-Maximilians-Universität München (LMU)	Germany	University
39	University of Plymouth (UoP)	United Kingdom	University
40	The Food and Environment Research Agency (FERA)	United Kingdom	Research Organisation
41	University of Birmingham (BHAM)	United Kingdom	University
42	University of Tübingen	Germany	University
43	Nofer Institute of Occupational Medicine (NIOM)	Poland	Research Organisation
44	Institut universitaire romand de Santé au Travail (IST)	Switzerland	Research Organisation
45	NANOCYL sa (Ncyl)	Belgium	SME
46	National Institute for Materials Science (NIMS)	Japan	Research Organisation
47	Heriot Watt University (HWU)	UK	University

Contents

1 Concept

Nanotechnology is recognised as one of the most important new technologies of the 21st century. The global investment in nanotechnology from all public sources for 2008 exceeds $7 billion[1].

The market size for nanotechnology is expected to grow to over $3 trillion by 2015[2] and nanotechnology promises new materials for industrial applications by having new or enhanced physico-chemical properties that are different in comparison to their micron-sized counterparts. However, as in all industrial applications, the potential exposure of humans and the environment to these materials is inevitable. As these new materials go through their life-cycle – from development, to manufacture, to consumer usage, to final disposal – different human groups (workers, bystanders, users) environmental compartments, (air, soil, sediment, water), and species (e.g. worm, fish or human through secondary exposure), will be exposed to them. Emerging data show a range of toxic (hazardous) effects from engineered nanoparticles, suggesting that any exposure will result in a risk to human health or the environment, risk being the product of exposure and hazard. While standard methods exist for risk analysis, these tools need to be applied, modified and verified for nanomaterials. Previously used standard approaches to risk management, control and reduction need to be proven for the novel paradigm presented by nanomaterials. Thus, the development of nanotechnology-based products needs to be

complemented with appropriate validated methods to assess, monitor and reduce the potential risks of engineered nanomaterials (ENM) to human health and the environment. Public mistrust of any new technology is often high, and demonstrating 'safe' products of nanotechnology will enhance the confidence of consumers, workers and other stakeholders. Furthermore, these measures must be validated and integrated in an overarching, coherent strategy for regulators and industry to adapt them. Thus, a safe and environmentally responsible nanotechnology will safeguard current and future global investments and will be the key to the sustainability of this industry.

While there are standard procedures for product life cycle analysis, exposure, hazard, and risk assessment for traditional chemicals, is not yet clear how these procedures need to be modified to address all the novel properties of nanomaterials. There is a need to develop specific reference methods for all the main steps in managing the potential risk of ENM. The aim of MARINA is to develop such methods. **MARINA will address the four central themes in the risk management paradigm for ENM: *Materials, Exposure, Hazard and Risk*. The methods developed by MARINA will be (i) based on beyond-state-of-the-art understanding of the properties, interaction and fate of ENM in relation to human health and the quality of the environment and will either (ii) be newly developed or adapted from existing ones but ultimately, they will be compared/validated and harmonised/standardised as reference methods for managing the risk of ENM. MARINA will also develop a strategy for Risk Management including monitoring systems and measures for minimising massive exposure via explosion or environmental spillage**

2 Objectives

The specific objectives of MARINA are:

1. For *Materials*, to obtain reference nanomaterials for testing; to develop validated methods for characterising the physico-chemical properties of ENM as pristine materials, in biological matrices, in environmental samples and field detection; to isotope-label ENM for their use in bio-distribution studies
2. For *Exposure*, to conduct exposure assessment in the workplace throughout the life-cycle of a ENM, developing different exposure scenarios. To assess the fate and behaviour of ENM in soil/sediment/water. To characterize the actually released ENM (aged ENM) and compare them to the pristine ENM. To evaluate, as part of a performance assessment, different approaches to conduct exposure assessment for use in the MARINA integrated risk assessment.
3. For *Hazard*, to address the knowledge gap, especially in areas of non-genomic toxic mechanisms, toxicogenomics, proteomics and metabolomics by developing new test systems; to develop reference methods for in vitro toxicology tests (including and fully incorporating those developed in other FP projects) by means of a scientific validation strategy; to implement in vivo dose-response models of healthy and susceptible subjects exposed through repeated dosing to ENM via inhalation, ingestion, intravenous injection and dermal exposure; to develop and scientifically validate in vitro and in vivo tests for soil/sediment/aquatic toxicity and secondary poisoning.
4. For *Risk*, to combine phase (1), (2) and (3) in developing reference methods for assessing the health and environmental risk posed by ENM; to develop a strategy for Risk Management including onitoring systems and measures for minimising massive exposure via explosion or environmental spillage.

MARINA is to achieve the objectives described above in **48 months**.

3 The MARINA approach

The European Commission, to date, has funded some 15 projects relevant to health and safety issues regarding ENM. This commitment is set to continue in the future. At the national level, there are other similar efforts[3,4]. However, to date, the valuable results generated from these projects have in the main been unabled to generate concepts, methodology and data which have been practically used for risk assessment and management.. Thus, there is clearly a need to use the most up-to-date date available information and methodology for guidance on health and safety risk management to industry and regulators. To respond to this need, in MARINA, we have created a consortium consisting of first class scientists and organisations with a track records for research in Health and Safety Issues of ENM. Most importantly,

- we have representatives from more than ten FP projects[1]. Our aim is to take the beyond the state-of-the-art results from these projects and use them for creating validated reference tools for Risk Assessment and Management
- we recognise the relevance of our results to industry therefore we have involved the direct participation of the Nanotech Industries Association[2] and industrial key partners such as BASF and Nanocyl.
- we also recognised the geopolitical and economical importance of Third Countries such as China, Russia and Japan. The inclusion of the prestigious Academies of Sciences from China, Russia (for Toxicology) and the Japanese National Institute of Materials Science (for ENM synthesis, characterisation and Toxicology) as well as our existing US partners through current FP7 projects goes beyond the scientific excellence and will enable MARINA to reflect a true

[1] The FP projects are: **FP6** PARTICLE_RISK, NANOSH, NANOINTERACT, NANOSAFE2, **FP7** NANOMMUNE, NANOTEST, ENPRA, NEURONANO, NANODEVICE, NANOLYSE, NANOIMPACTNET, NANEX, ENNSATOX, NANOFATE, NANOHOUSE, ObservatoryNANO, LICARA, ITS'NANO.

[2] NIA is also involved in another proposal on the same call (NMP.2010.1.3-1: NanoREFORM); this involvement allows the establishment of added-value components to both projects and benefits the nanotechnology research and industries community.

global effort in addressing this important issue and to promote our strategy for risk management of ENM globally.
- we will interconnect all MARINA activites with other relevant ongoing activities such as the forthcoming EC European Technology Platform(s), Nanofutures, Infrastructure and cluster activities of FP7 projects, the ERAnet, the OECD WPMN sponsorship programme as well as National Research programmes, such as NanoCare2 and NanoNature.

Although the database that supports risk assessment and management continues to expand, the general approaches have not changed significantly. Risk assessment and management must be based on the best available science, which is continually progressing. These changes appearing in the nature and the interpretation of data prompt the MARINA approach: Specifically:

1. The likelihood of increasing restrictions and public acceptance of the use of animals for testing purposes in the EU drive MARINA to go for **integrated test systems (ITS)** targeting modules of hazard endpoints, fate and exposure, and monitoring.

2. The availability of data from new/rapidly advancing methodologies is fully acknowledged in MARINA - systems biology and early marker detection are used for **integrated assessment schemes (IAS)** for occupational and environmental exposure assessment and monitoring schemes.

3. Advances in mode of action research and in the understanding of effects/disease mechanistic processes in MARINA lead to addressing hazard more specifically and develop **interconnecting module systems (IMS)** for risk assessment and risk management as methodology for supporting decision making.

In summary, MARINA stands for integrated testing, integrated assessment and modular interconnection of knowledge and information for science-based risk management methods. The approach is to translate scientific advancements and methodology in contribution to shifting from toxicological studies of specific individual nanomaterials towards a more systematic health and environmental safety assessment and management that handle the overall risks for types or classes of ENM based on their intrinsic, e.g. physico-chemical properties.

4 Progress beyond the state-of-the-art

In the following sections, the scientific state-of-the-art is summarised and the many aspects of MARINA which go beyond the state-of-the-art to achieve the objectives listed above will be clearly described.

4.1 The state-of-the-art

Currently, the EC has funded many projects through their 6th and 7th Framework programmes. These projects generally cover the state-of-the-art landscape of health and safety issues related to ENM:

(a) *for Materials*, characterising the physico-chemical properties of ENM in bulk materials is generally accepted as essential to all toxicology studies. The detection and characterisation of ENM in biological matrices is being investigated in FP7 NANOLYSE. To date there is no attempt in harmonising and standardizing characterisation methods, although this has been widely debated.. Furthermore, the lack of reference materials to be used, means that there is difficulty in extrapolating the results between studies. Also, almost no study has characterized the ENM that are actually released into the envrionment. The new FP7 NANOHOUSE is going to do this but only for ENM released from paints.

(b) *for Toxicology*, it is well known that exposure to particles is likely to lead to adverse effects. Following exposure, some ENM have been shown to translocate beyond the portal of entry organ[5], the extent of translocation is dependent on the ability of ENM to cross biological barriers (blood-brain, blood-air, placental, and so on) which is a function of ENM size, surface properties, (e.g. surface charge[6]) or the formation of the protein or lipid corona[7] on the ENM surface. Inhaled ENM could be translocated to the brain via the olfactory bulb[8]. These ENM can also reach the terminal bronchial region and for high-aspect ratio ENM, they are likely to be further translocated to the pleura[9]. A small ENM fraction can reach the blood system where they can travel to secondary organs[5] like the heart, liver, kideny and beyond. The bio-distribution of internalised ENM follows a different pattern depending on the route of exposure: inhalation, ingestion or dermal[10]. For dermal exposure, there is some evidence that ENM can penetrate beyond the stratum corneum[11]. For oral exposure, TiO2 ENM have been shown to induce DNA damage and genetic instability in mice[12]. The paradigm for the adverse response is currently oxidative stress leading to inflammation[13]. Chronic inflammation could lead further to fibrosis, DNA damage and cancer[14]. ENM can also be genotoxic by direct by a totally new mechanism[15]. ENM driven oxidative stress also plays an important role in the immune response and apoptosis[16]. Cardio-vascular effects have been demonstrated in susceptible animal models exposed to ENM[17] although it is not clear whether these are caused by systemic inflammation or direct ENM interaction with cardio-vascular plaques causing plaque disruption. The ability of some ENM to cause a pro-thrombotic effect like platelet aggregation should also be noted[18] . Data on the adverse responses are generated from many FP projects. For example, the protein corona issue is being investigated in the FP6 NANOINTERACT project, the central nervous response is investigated in FP7 NEURONANO, the pulmonary and cardiovascular effects were investigated in FP6 NANOSH, PARTICLE_RISK and more recently in FP7 ENPRA, NANOTEST. Other target organ effects are also studied in ENPRA and NANOTEST and immunotoxicity is the research topic for FP7 NANOMMUNE. Taken together, a coherent toxicity profile of ENM begins to emerge. However, there are still many short-comings to be addressed. These include the proper validation of the test protocols developed in these FP projects, and the comparison of in vitro and in vivo results to reduce animal testing as part of the 3R strategy for toxicology testing.

(c) *for Eco-Toxicology*, relatively little is known about the environmental ENM toxicity, but toxicity has been reported from the molecular to the population level[19], including food chain effects. ENM uptake and tissue distribution within species are mostly unknown, except for in a few larger species[20]. It is however clear that ENM uptake mechanisms are different those for conventional chemicals (see *toxicology*). One of the early examples

of ENM effects in an environmental species was the study by Kerstin Hund-Rinke et al[21] who showed oxidative stress in algae and daphnids following TIO2 exposure. Since then data are emerging at increasing numbers every year. Almost all published ecotoxicological studies with ENM[33] have focused on the aquatic environment with little or no attention to the soil and sediment compartments, the latter even tested in aqueous suspensions or on filter paper soaked with the test suspensions[22]. Within the aquatic environment freshwater species, mostly pelagic have been tested[23]. The effects reported often differ depending on the method used to prepare the ENM for testing e.g. stirred ENM versus solvent carried ENM[24]. There is currently no guidance or guidelines for toxicological assessment of ENM, or guidance on how to adapt the guidelines used for conventional chemicals. This is probably one of the reasons for the diverse range of test conditions/protocols currently used and for the reported differences in effect levels. There is no clear pattern as to which ENM characteristics are important for toxicity, although surface area may be a candidate for certain ENM[25]. The lack of convincing patterns could be because the ENM characteristics reported are at best derived for primary particles and for ENM suspended in the exposure media, where ionic strength, pH and others changes to the primary ENM take place (e.g. agglomeration, aggregation and surface chemistry) i.e. media-specific factors that modulate effective exposure and hence toxicity.

(d) *for Human Exposure*, the workplace is still the most likely space where human beings will be exposed to ENM, although exposure is likely high throughout the entire life-cycle of the ENM from production to disposal[26]. At each stage of the life-cycle, there is potential for exposure to different groups of workers. Methods for measuring the ENM aerosol concentration in the workplace are currently being developed (e.g. FP7 NANODEVICE[39]), with measurements of as mass, particle size and size distribution[27]. Currently, there is no method for measuring the surface physico-chemical characteristics of the collected samples[28]. Also, dermal exposure from airborne ENM and secondary exposure to ENM from the environment are also not considered[29]. It has been recognised that models of ENM exposure will be important to counter the paucity of data. Workplace exposure assessment has been conducted in many national and european projects[30,31]. Of particular importance, is FP7 NANEX[32] which will establish exposure scenarios for the workplace throughout the ENM life-cycle.

(e) *for Environmental Exposure,* ENM are likely to end up in the environment, although uncertain estimates ranging from ng to mg per kg levels in various compartments[33]. Some ENM may be persistent while others rapidly dissolve. Soils, sediments and surface waters are complex matrices with many possible different exposure and interaction scenarios[34]. The environmental behaviour of ENM can be particularly complex with a high propensity for aggregation, agglomeration and deposition, along with dis-agglomeration and re-partitioning into the solution phase[32]. Formation of aggregates or agglomerates can take place between ENM or with natural organic and/or inorganic colloids[33, 34], and is influenced by environmental factors like pH and ionic strength [35-39] ; These factors combined with inherent the physical-chemical properties, structure and concentration/dose, contributes to the complexity of quantifying environmentally relevant and bio-available concentrations[40,41]. The physico-chemical distribution of ENM between dissolved, colloidal and a particulate phase is largely unknown[42-46], and remains a key unknown in regard to exposure of organisms (NANOFATE). The problems are also currently being addressed in FP7 ENNSATOX, which is specifically concerned with the environmental aqueous behaviour of ENM in relation to their toxicity. This underlines the need for detailed experimental work on the environmental fate of ENM in a coherent manner only possible in an integrated project.

(f) *for Risk*, there are two important elements: the assessment and management of risk. To assess risk is to compare the measured or predicted exposure level (PEC) from evidence in (d) and (e) with the derived-no-effect-level (DNEL) level from toxicology data or the predicted-no-effect-concentration (PNEC) in the case of the environment. To predict or estimate risk and consequently to manage risk is to implement procedures for the purpose risk mitigation. This includes, inter alia, establishing exposure control limit, controlling and monitoring exposure including accidental explosive or massive release of ENM into the environment, identifying risk scenarios, i.e. groups for health surveillance or geographical areas for health protection, communicating to key stakeholders and training about risk, including developing protective standard operating procedures and informing the regulatory process such as REACH. FP7 ENPRA is developing methods and tools for Risk Assessment. There are currently many Risk Management approaches, such as the HACCP (Hazard Analysis and Critical Control Point) for food safety control, but an integrated Risk Management Strategy specific to ENM is still to be developed

4.2 *Beyond the state-of-the-art*

It is clear from the summary above that there is still a considerable large knowledge gap in all the four major themes relevant to the risk management of ENM. MARINA will be able to go far beyond the state-of-the-art on all of the points above, because it represents the most comprehensive consortium on Nanosafety issues, with 46 partners merging knowledge into MARINA from numerous EU and large national projects.

To develop reference methods for risk management of ENM, we need to go beyond the state-of-the-art. The specific areas to be included in MARINA are (i) the development of reference materials; (ii) exposure assessment in human and environment settings; (iii) identification of key ENM parameters e.g. size, charge or coating important for describing dosimetry (iv) validation of existing (eco)-toxicology tests and development of new, relevant ones; (v) implementation of in vivo dose-response models of healthy and susceptible individuals exposed to ENM through repeated dosing via inhalation, ingestion, iv injection and dermal routes; (vi) combination of (iv) and (v) into an Intelligent Testing Strategy (vii) implementation of all relevant evidence generated by MARINA and from other projects into a rigorous Risk Assessment for ENM; (viii) development of an overarching Risk Management Strategy for ENM including exposure monitoring schemes and the management of rare events of massive exposure due to explosion or spillage. Most importantly, in all the themes stated above, we will emphasise the production of reference methods applicable ultimately in the Risk Management of ENM. In the text below, the specific beyond the state-of-the-art research in MARINA for each important Themes are described in more detail.

Materials

We will establish a panel of representative ENM of high volume production and of high economic importance (e.g. **TiO2** – in different size, shape and surface charge, **SiO2, Ceria Oxide, ZnO, nanoAg, Multi-Wall Carbon Nanotubes (MWCNT)** – in different lengths) as Reference Nanomaterials (RNM) for use in MARINA. Commercially relevant, fully characterised and quality-controlled ENM will be sourced from both industry partners (via NIA) and the JRC's repository for reference nanomaterials, which is already sub-sampling and distributing several commercially relevant ENM for other nanotoxicology projects, including the OECD Sponsorship programme. These RNM will be characterised, assessed for homogeneity, stability and described shelf-life according to the OECD WPMN SG3[67] endpoints and criteria. We will use these RNM to validate the metrology methods for measuring key physico-chemical ENM characteristics, which are suggested to drive the adverse effects. Important inputs to these activities will come from the nanometrology community (e.g. FP7 co-Nanomet and ISO TC229). We will harmonise and standardise these methods for the qualification/certification of these reference materials according to ISO Guide 30-35 and OECD Guide 34 as well as ongoing work at the OECD Sponsorship Programme for both risk assessment and nanometrology purposes. To date, there is no consistent method for labelling ENM, although knowing the target organ/cell dose is essential in understanding the nature of the dose-response relationship. In MARINA we will develop and validate methods for labelling ENM for studying the bio-distribution of ENM in body tissues. We will also develop and validate methods for characterising ENM in biological matrices and environmental samples from air/soil/sediment/water for field detection. MARINA will also characterise ENM released from products and aged under environmental conditions, as these are the ENM that the organisms are exposed to. Comparisons to the pristine ENM will be made.

Exposure

i. For Occupational and Consumer Exposure, In collaboration with the relevant industries, we will identify the relevant current and future occupational exposure scenarios and review available occupational/consumer exposure information and conduct exposure surveys to complement the occupational and consumer exposure data. We will review and revise models for predicting exposure to ENM in the workplace and from consumer products and implement these in an advanced control-banding tool. We will also develop and implement a strategy for occupational and consumer exposure monitoring including the charaterisation of workplace and consumer product samples; these strategies will be verified through industrial case studies, using both real-case exposure scenarios.

ii. For Environmental Exposure, we will review available environmental, identify and formulate the current and future environmental exposure scenarios, validated by monitoring. We will develop adapt and validated experimental guidelines for the fate and behaviour assessment of ENM in soil, sediment and water. This will be based on analysis ENM binding to and partitioning from natural components, including importance of agglomeration; besides distribution, availability and stability of ENM under standardised and real environmental conditions will be assessed. The data generated will allow parameterisation of the fate processes scientifically and permit the implementation of regulatory exposure assessment frameworks.

iii. For both spillage and explosion, critical parameters controlling risk, like concentration of agglomerates, the explosion severity, the minimal ignition energy and many others, will be identified experimentally, new reference evaluation methods of such parameters will be developed and quantified. using the unique expertise in pulse/intermittent exposure in our consortium. Moreover, for accidental release models, industry case-studies will also be used in support of the development of experimental models for massive accidental exposure from explosion.

Hazard

i. For Toxicology, we will develop new in vitro toxicology test methods on the following target systems: the immune, central nervous, cardio-vascular, pulmonary, hepatic, renal, reproductive, developmental and dermal systems. The adverse endpoints are target specific as well as oxidative stress, inflammation, genotoxicity, fibrosis. We will also investigate the ability of ENM to translocate across biological barriers such as the blood-brain, blood-air, endothelial and placental barriers and determine the ENM physico-chemical properties which facilitate this dynamics. Moreover, the interaction between ENM surface physico-chemical characteristics and body proteins and lipids is fundamental in how cells react to the presence of foreign entities. Thus, we will investigate this phenomenon in relation to the potential toxicity and translocability of ENM Most importantly, we will implement animal experiments dose-response and bio-distribution ADME models of healthy, pregnant and susceptible (to cardio-vascular problems) individuals exposed through repeated dosing to ENM via inhalation, ingestion, iv injection and dermal routes.

ii. For Eco-Toxicology, we will adapt and if develop in vitro and in vivo tests for soil, sediment and aquatic toxicity including secondary poisoning. Current test will be modified or if needed developed then standardised and validated for use with ENM. Key effect endpoints and dosimetry parameters directly specific to ENM will be identified, this will be done across all media benefiting on the size of the consortium. Data will be complemented by mechanistic information (see iii).

iii. For both Toxicology and Eco-Toxicology, we will develop methods for toxicological profiling using toxicogenomics, proteomics and metabolomics including some unique arrays that are being adapted for ENM available to the consortium and therefore identifying ENM specific Modes of Actions (MoA). We will adapt existing in vitro tests nominated by current FP projects, and harmonise toxicology and eco-toxicology endpoints into one unified framework for hazard assessment. The tests will be validated by reliability assessment and inter-laboratory round robin comparative tests and the selected ones will be implemented in High-Throughput Systems (HTS). Ultimately, we will integrate the validated tests into an intelligent Testing Strategy (iTS) and propose it as a Method Validation Framework for use by ECVAM in compliance with the 3R principles and we will update the OECD test guidelines with this iTS.

Risk

i. Assessment, we will implement a database for storing MARINA data and available data from other FP and national projects by using the existing NAPIRAhub database; We will implement and harmonise in silico models of exposure-dose-response (PBPK/PD) and QSAR models for both toxicology and eco-toxicology and to use them as tools for Risk Assessment (RA) (we will work with other successful projects in FP7 NMP-2010.1.3-2). Key differences from the present RA will be identified and ENM specific issues will be clarified. Based on the weight-of-evidence generated in MARINA and from other projects, we will implement a RA strategy for the humans and the environment and integrate both strategies into an Integrated Risk Assessment (iRA) Strategy for ENM.

ii. Management based on the results of the iRA, and in close collaboration with industries (i.e. via case-study verification), we will develop a Risk Reduction Strategy (RRS) in the form of a toolboxes for (a) the management massive release risk, (b) the assessment of monitoring systems for the control of occupational/consumer/environmental exposure, and (c) identification of susceptible groups (humans and other species) for future health surveillance. We will develop guidance manuals and SOPs and communicate them to all relevant stakeholders (e.g. research las, industrial manufacturing, prcessing and research labs). For both (i) and (ii), we will contribute the iRA and RRS as part of the development of the REACH process.

iii. O*ther issues* relevant to MARINA

MARINA will implement a strategy for (i) training of the next generation of researchers and relevant industry stakeholders through a series training schools and workshops and (ii) dissemination of MARINA approach and results targeted at policy-informing and –making bodies (e.g. OECD, EC Scientific Commitees, EC regulatory working groups, etc.), national public authorities, nanotechnology industries, and the wider nanotechnology research community and citizens by means of public forums, website and newsletter. Therefore enhancing the public awareness about the developments of sustainable nanotechnology through emphasis among the participants and encouragement of transparent and direct communication to the public. Most importantly, MARINA will collaborate with lead institutes of Nanosciences, the forthcoming INFRASTRUCTURE FP project, the existing nanosafety cluster activities promoted by the EC and also the very successful FP NANOIMPACTNET project for an effective dissemination effort. Through direct participation of Industry Associations and dedicated industrial partners, dissemination and uptake of RRS to key industry in different sectors including chemical industry, cosmetics and consumer products will be guaranteed. **MARINA strives for integrated testing, integrated assessment and modular interconnection of knowledge and information for science-based risk management methods.** The approach is to translate scientific advancements and methodology in contribution to shifting from toxicological studies of specific individual nanomaterials towards more holistic health and environmental safety assessment and management that manages overall risks. Finally, we are aware that for this large consortium to function efficiently, a rigorous management system must be implemented. For this reason, the management of MARINA is divided into two fundamental areas: The administrative and scientific management. We endeavour to manage MARINA using the latest techniques in project management and the expertise and experience of coordinating FP projects from the core MARINA members.

We present an overview of the project structure (see also Fig 2), with references to the WP that are summarised in the table below. The text here, together with the summarised WP, describes the MARINA project. The workplan covers both human health and environment and is comprised of the four main themes Materials, Exposure, Hazard and Risk.

For **materials**, WP3 and WP4 will obtain a panel of ENM including - TiO_2, ZnO, SiO_2, CeO_2, nanoAg, MWCNT – and characterise by measuring the physico-chemical properties of these ENM suspected of driving the adverse human and eco-effects. WP3 will also assemble an Industrial Case Study, consisting of the physico-chemical properties (in the pristine state and in different media) and the (eco)-toxicity profile, for each of the materials considered.

For **exposure,** WP5 address release scenarios, WP6 will develop a tiered human exposure assessment approach (Occupational/Consumer exposure scenarios) while WP7 and WP8 will do the same for the environment.

For **hazard**, WP9 and WP10 are specifically for the human and eco-toxicity of ENM. WP11 is for omics-based system toxicology approaches relevant to both humans and the environment.

For **risk**, the assessment of human and environmental risks is implemented in WP12 and 13. WP14 is dedicated to the management of accidental risk while WP15 is to develop and implement monitoring systems. WP16 is to develop a strategy for risk reduction which include the derivation of control limits, control banding and the exploration of new ENM synthesis which be used for substitution.

Finally, as a FLAGSHIP programme, we will devote two WP (17 and 18) for training and dissemination targeted at specific relevant stakeholders of NANOSAFETY. We are committed to providing long-lasting impact in the area of nanosafety and risk assessment, in Europe and at the international level.

5 Impact

MARINA is expected to make a significant and long-lasting impact on the European objectives for the safe, integrated and responsible approach to the development of Nanotechnology. Specifically, for ***the development of comprehensive understanding of the properties, interaction and fate of ENM in relation to human health and environment,*** MARINA is a multidisciplinary consortium of 46 organisations at the leading edge of European and world-wide research on ENM risk issues or industrial commercialisation of ENM and their products. As inputs we will incorporate state-of-the-art scientific findings, including those of over ten FP projects in the field and accessible national and international programmes and including the OECD WPMN sponsorship programme and transatlantic co-operations. Building on these inputs we will integrate into a web-based, comprehensive, searchable IT platform

to create an integrated resource to bridge the gaps caused by confidentiality, delays between findings and data access and limitations by search engines. Results generated within MARINA will serve to narrow the critical knowledge gaps in all of the principal areas in the risk management paradigm for ENM linking Materials, Exposure, Hazard and Risk. This in order to provide an overarching understanding of the interaction of ENM with humans and the environment towards a sound, scientifically based, coherent approach to assessing and managing the potential risk of ENM. MARINA is dedicated towards the development of validated reference methods for managing the risks of ENM and is the first project to also address monitoring of exposure of ENM and its contribution to RA and RMM. An integral part of the MARINA project is also to make available the findings and the methods to other users in an accessible format in order to maximize the benefit of the project. For ***support to policy and decision-making concerning nanotechnology in respect to various stakeholders***, MARINA will provide a unique resource of information and methodology. It will support shifting from case-by-case evaluations to holistic health and environmental safety assessment and management that addresses overall risks. By using frames and modules of integrated testing and integrated assessment, conclusions are set in a science-policy perspective. All information is provided web-accessible in real time. Decision makers will find information directly in a format, which allows analysis across endpoints, across material types or preparation forms. Industry depends on evidence-based safety assessment to safeguard market potential and sustainability of their products. MARINA will provide the industry with important data and tools to take decisions about products, processes, risk management measures and safety assessment. This includes validation, weight of evidence approaches, expressions of uncertainties, quantitative expression of risks and setting the risk in appropriate context in e.g. risk-sustainability or risk-benefit analysis. MARINA has a clear strategy for engaging the European and wider International Community. The design of the MARINA consortium and our dissemination strategy reflect our commitment to bring the MARINA results to the global community in general and to the international regulatory bodies and interested stakeholder groups and NGO in particular. For ***contribution to the future definition of appropriate measures, where needed***, MARINA objectives are to develop standard, reference methods covering a wide range of themes, from Materials to Risk. These reference methods will contribute actively to the much-needed global efforts in standardisation and harmonisation of methods and measures. The implementation of reliable reference methods will generate public confidence in the sustainable development of nanotechnologies. For ***support to good governance in nanotechnology*** MARINA will develop an overarching strategy for risk management and reduction and enabling the EU regulatory bodies, agencies and authorities to make informed decisions and policies to safeguard consumers while taking full advantage of the advancements that nanotechnology will bring to the economy and competitiveness of EU industry. MARINA will facilitate dialogue in the field via the advisory board and a dissemination plan adapted to international stakeholders in governance. We expect this to support European policy, specifically horizontal standardisation, harmonisation, as well as worker and consumer protection. For ***Support to pre and co-normative activities, such as with reference to the implementation of REACH***, MARINA is clearly committed to the REACH process through our integrated activities and iTS. MARINA will closely work together with the Commission services involved in development of adaptations of REACH guidance documents concerning nanomaterials. In particular, our definition developments, adequacy and supplementation of reference methods are of direct value for REACH, especially the focus on reliable methods and combined use of data including from modelling and monitoring. Noteworthy are new in silico approaches for cross-reading (QSAR-like) implemented in MARINA and the acceptance of approaches warranted by a communication strategy directed at relevant bodies including European Commission, European Food safety authority (EFSA), European Chemicals Agency (ECHA), ISO, CEN and OECD. For ***Support to the safe, integrated and responsible approach as laid down in "Nanosciences and Nanotechnologies: An action plan for Europe",*** the risk management recommendations will be developed in cooperation between the scientists and industrial stakeholders. It will identify conditions, challenges, and provisions to account for the impact on a responsible and flourishing industrial development of nanotechnologies within Europe. Decision-making by stakeholders will be supported in MARINA to enable risk issues to be addressed on the earliest possible level in order to improve assessment methodology and subsequently safe and responsible use of ENP. Contributions to validated reference methods for risk management will contribute to improve favourable conditions for innovation. MARINA will contribute to reinforcement of the international dimension of European research and collaboration between industry, researchers, NGO, authorities (at Member State and European level) and international standardisation bodies, such as OECD WPMN, WPN, WNT, ISO TC 229 and TC 24, CEN TC 352, and IUPAC.

MARINA has started on the **1st November 2011.** The Kick-Off meeting was held in Rome on the 16th to 18th November 2011 jointly with the project NANOVALD. The key activities implemented at the start of the project were:

1. To synchronise MARINA and NANOVALID activities;
2. To choose and procure ENM for a quick start of the MARINA experimental programme.
3. To train scientists on Nanosafety through the Nanosafety Autumn School in Venice.

A joint working group for MARINA/NANOVALID, consisting of key researchers from both projects, has been formed. The specific objective is to synchronise and optimise use of resources for the research activities with and between the two projects. This group meets regularly and has a teleconference every three months.

Much effort has been made to choose the relevant ENM for testing. We have identified TiO2-NM101, ZnO-NM110, Ag-NM300K and SiO2-NM200 and NM203, and a SiO2 from NANOVALID. This batch of ENM will be followed by MWCNT. The ENM has been delivered to the MARINA partner in early 2012.

Following the Kick-off meeting several WP meeting were held (e.g. WP5, 6, 7 and 8 in Hoofddorp, WP9 in Stockholm, WP12 and 13 in Copenhagen, WP9, 10 and 11 in Helsinki) to initiate the studies and ensure timely and appropriate collaboration.

MARINA has also made use of the NANOSAFETY Autumn School in Venice to initiate their training programme. The course covered the topics or Materials, Exposure, Hazard and Risk. Staff and students working in MARINA and NANOVALID as well as students from outside Europe have attended it.

In 2012, MARINA progressed steadily with the delivery of the ENM. The experimental WP have all started and generated both data and samples for further refined analysis, among others detailed omic analysis covering all levels. Several tasks have started before schedule, and results from other projects are incorporated. Management meetings are held both, weekly and at month 6 basis, i.e. May 2012 in Grenoble and in December 2012 during the Annual Meeting in Rome.

Several training events have taken place during 2012, both within MARINA and in collaboration with NANOVALID (i.e. Training School in Plymouth, January 2012; in Madrid, February 2012; in Leeds, September 2012). We supported the NANOSAFETY School in Venice in March 2013 and a workshop to implement exposure and hazard in risk assessment strategy for nanomaterials has taken place in Hoofddorp in April 2013.

In 2013, MARINA has reached the milestone of Month 18, with a mid-term project review, with the EC PO and PTA has taken place in June 2013. In WP1, the procedures for implementing the administrative and scientific management are running with regular meetings and meetings/teleconferences. The steering board, Advisory board and General Assembly are active. External collaboration and information sharing activities are running with many other FP7 projects, e.g. qua the extensive size of MARINA, the MARINA partners are attached to many other EU initiatives ensuring information transfer. There is a special focus on interaction between MARINA and NanoVALID as to produce harmonised Risk Assessment/ Management tools between the two projects. In WP2, the MARINA database is created and data collection templates were made available to the participants. We are currently populating the database with results from the other WPs. The ENM Reference Dossiers with industrial, scientific and regulatory relevance have also been compiled and updated. In WP3, the reference nanomaterials (ENM) have been distributed to all experimental partners, with the ENMs being characterised, labelled and verified in regard to homogeneity and stability. In WP4, MARINA partners are developing harmonised protocols for ENM characterisation in complex matrices. In WP5 and WP6, the assessment of the release processes taking place during production, processing and during handling (by consumer / workers) is progressing. To this the partners are developing a searchable database tool of occupational exposure scenarios, reviewed existing exposure models and evaluated protocols for workplace exposure measurement surveys. A tiered exposure assessment approach for human risk assessment has been introduced. In WP7 and WP8, MARINA partners are implementing the MARINA information database on ENM fate-determining parameters in environment and biota. Protocols are being established for assessment of the fate and behaviour of ENM in well-characterised model-systems. This work is complimented by the development of novel methods to actual measure ENM in complex environmental matrices. Within the hazard assessment (WP9), the characterization and exposure studies were matched with extensive human health results (from in vitro and inhalation experiments which are now completed) and environmental studies, measuring a wide variety of hazard (including bioaccumulation) parameters for different species. In WP11, elaborated pipelines for detailed system biology studies have been established for retrieving mode-of-action information. These -omics experiments are currently ongoing. In WP12 and WP13, we have developed an integrated risk-assessment strategy, incorporating risk management and risk reduction paradigms (in WP15) with focus on nano-specific issues e.g. how to handle NM size in a risk assessment and to monitor ENMs in situ (ongoing work in WP16). The current approaches are being evaluated and novel approach suggested, setting this in a regulatory perspective. Our findings, on accidental risks of explosion of Nanomaterials (WP14), which focus on relating NM parameters to risk of explosive behaviour of NMs, have also raised much interest in the research community and led to collaboration between MARINA and project FP7 Buonaparte.

To ensure fast and knowledge progress dissemination activities in WP18 includes both internal exchange of information and external information flow to the research community and to the public. Specifically, many MARINA partners have also participated in FP7 NanoREG. Their participation has created a conduit for MARINA results into NanoREG and therefore helped in shaping the regulatory processes addressed in NanoREG. We are currently preparing with our Japanese colleagues for the MARINA's "The Third Workshop on The Risk Management of Engineered Nanomaterials" to be held at NIMS in August 2014. For Training, we held a workshop on Risk Assessment with the participation of NANOVALID and NanoREG partners (April 2013, Hoofddorp).

Notably, in 2013, MARINA has taken the leadership role in the Nanosafety Cluster WG5 on Risk. The objectives of WG5 are now identified: In the short term, identify communication systems/strategies between projects and between WGs; in the mid term, draft of an overview of tools and methods (tools) available for Risk Assessment of Nanomaterials and in the long-term, draft of the concepts useful for the risk assessment of Nanomaterials.

MARINA and the Nanosafety Cluster Working Group 5 – Risk

In 2013, MARINA was asked by the EC to lead the Nanosafety Cluster (NSC) Working Group 5 on Risk. Currently, there is an urgent need for sufficient knowledge to allow reliable assessment of the risks associated with nanomaterials (ENMs). The formulation of a grouping/categorization concept that allows safety assessment across materials is required to overcome the current need of testing each nanomaterial on a case-by-case basis (see FP7 ITS-Nano), and that is useful for all stakeholders, though developing this grouping/categorization/modelling concept is highly challenging both from a scientific point of view as well as for the process. An Intelligent Testing Strategy (ITS) integrates data

from in vivo and in vitro tests, non-testing methods and physico-chemical properties for a specific material as efficiently as possible with regards to costs, the number of experimental animals and time in order to reach a conclusion on potential risks. Such concepts should be used in a risk assessment strategy for nanomaterials to come to a coherent approach.

A pursuit for a risk assessment and intelligent test strategy protocols is ongoing with various EU projects and in other projects worldwide. Based on these EU initiatives first attempts have been published (e.g. Oomen et al 2013) outlining a forward approach. However, we have a long way to go and this work must continue to get coherent, validated and implementable tools, which compass the expected future development for nanomaterials.

Given the worldwide implication of the risk work, the work of WG5 should be closely coordinated with the ongoing work in the risk group of the US-EU Community of Research (CoR). The communication systems/strategies between projects and between WGs are to be kept to the already established pathways i.e. emails, NSC-website and to face-to-face meeting in connection to NSC meetings or other international meetings. This approach was taken in order to avoid many parallel communication pathways, making progress difficult to follow. Thus, the CoR work will likewise follow the given communication strategies.

A questionnaire (by MARINA) has been send to all the project leaders regarding the tools used for risk assessment purposes within each project; this information is now being retrieved and analysed. Project contributing include MARINA where risk management tools are develop, SUN where long term tools are developed and GuideNano where a web-based tool is being developed. The WG5 participants[3] have also been asked regarding their progress in the risk related area, which has been reported.

The Risk Assessment methodology is ongoing with the MARINA project, and concepts from other projects has been included e.g. the NSC strategic research agenda and the ITS-Nano (see NSC website http://www. nanosafetycluster.eu/working-groups/5-risk-wg.html). Later in the process it is expected to get input from other projects e.g. SUN, NanoValid and GuideNano. The work is continuously fed into the NAnoREG project, to ensure coherency.

The risk related conceptual progress is also made within the CoR initiative, here the WG5 coordinators ensure that there is a continuous information flow between NSC-WG5 and US-EU Cor. MARINA has played a key role in the relation with our US colleagues through the US-EU CoR on Risk of Nanomaterials (organizing and participation to the US-EU meeting in December 2013).

EXPECTED IMPACT

MARINA is expected to make a significant impact on the European objectives for the safe, integrated and responsible approach to the development of Nanotechnology, notably for the development of comprehensive understanding of the properties, interaction and fate of ENM in relation to human health and environment. Results generated within MARINA in 2013 are providing an overarching understanding of the interaction of ENM with humans and the environment and therefore helping to assess and manage the potential risk of ENM. MARINA is continuing to develop validated reference methods for managing the risks of ENM.

For support to policy and decision-making concerning nanotechnology in respect to various stakeholders, MARINA is currently providing a unique resource of information and methodology. It supports the shifting from case-by-case evaluation of individual ENM risk to a more holistic health and environmental safety assessment and management that addresses overall risks decision makers and Industry will find information directly in a format, which allows analysis across endpoints, across material types or preparation forms.

For contribution to the future definition of appropriate measures, where needed, MARINA objectives continues to develop standard, reference methods covering a wide range of themes, from Materials to Risk.

For support to good governance in nanotechnology MARINA is contributing to an overarching strategy for risk management and reduction and enabling the EU regulatory bodies, agencies and authorities to make informed decisions and policies to safeguard consumers while taking full advantage of the advancements that nanotechnologies will bring to the economy and competitiveness of EU industry.

For Support to pre and co-normative activities, such as with reference to the implementation of REACH, MARINA is contributing to the REACH process through our activities and the ITS. MARINA also works with the Commission and global (e.g. OECD) services involved in development of adaptations of REACH guidance documents concerning nanomaterials.

For Support to the safe, integrated and responsible approach as laid down in "Nanosciences and Nanotechnologies: An action plan for Europe", the risk management recommendations are being developed in cooperation between MARINA scientists and industrial stakeholders.

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[3] Wg5 cover people outside EU projects and some (but not all) EU projects.

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6 Copyright

MembraneNanoPart

Modelling the mechanisms of nanoparticle-lipid interactions and nanoparticle effects on cell membrane structure and function

Contract Agreement: NMP4-SL-2012-310465 Website: http://www.membranenanopart.eu
Coordinator: Vladimir Lobaskin, School of Physics, University College Dublin, Belfield, Dublin 4, Ireland

Table 1 Consortium List.

No.	Beneficiary name	Short name	Country
1	National University of Ireland, Dublin / University College Dublin	NUID UCD	Ireland
2	Stockholms Universitet [Stockholm University]	SU	Sweden
3	Imperial College	Imperial	United Kingdom
4	Institute of Biology KRC, Russian Academy of Science	IB RAS	Russia

Contents

1 Summary

Project Duration: 1 January 2013 – 31 December 2015

Project Funding: 1 Mio. EUR

The goal of MembraneNanoPart is to develop physically justified models and computational tools to quantitatively describe and understand the molecular mechanisms of nanoparticle (NP)-cell membrane interactions, which we consider to be a crucial point in any predictive model of nanoparticle toxicity. We address the mechanisms of nanoparticle protein corona formation, the protective function of the membrane, nanoparticle uptake into the cell, and the effect of nanoparticles on the cell membrane. We are aiming to develop a consistent multiscale simulation scheme starting from nanoparticle-biomolecule interaction at the atomistic scale using molecular dynamics simulation, and then systematically constructing coarse-grained mesoscale models for simulating the structure and dynamics of the cell membrane perturbed by nanoparticles at the physiologically relevant time and length scales. We will develop and test a universal method for evaluating the rates of nanoparticle translocation through membranes. Based on the information acquired from the simulations and analyzed together with available experimental data, the toxicological impact will be deduced. We will apply our approach to a range of common engineered nanoparticles, relating their physicochemical properties such as size and shape, surface charge, hydrophobicity and hydrophilicity, to the toxicological effects and develop a test suite allowing one to make toxicity prediction on the basis of purely computational or limited in vitro screening tests.

2 Background

Development of nanotechnologies and nanomaterials, on the one hand, provide us with new opportunities for medicine (nanomedicine) in the form of the capacity to diagnose or treat many of the remaining intractable disease classes (viral, genetic, cancer) using the nanoscale agents or tools. On the other hand, it presents a variety of unforeseen risks, as the nanoparticles challenge the immune system of the human body at lengthscales where it is not well prepared to react. As of now, little is known concerning the health risks of synthetic nanoparticles, however rapid technological progress creating more (and novel) nanomaterials, with not always well understood biological effects, demands urgent action. The understanding of the potential hazards related to nanomaterials will enable manufacturers to quickly screen out particles with physicochemical properties related with a risk, and either develop new particles or re-engineer their products to modify their properties, thereby designing out the risk factors initially, and in the longer term potentially designing the nanoparticles to be safe.

In this project, we are addressing the issues of NP-cell membrane interaction by computer simulation. The role of computer simulation is now well recognized in many fields e.g. drug design and toxicology. It is equally possible to study the detrimental effect of NPs from physical considerations using molecular modelling methods. We can expect to make immediate progress

on many of the issues surrounding NP toxicity by modelling the interaction of NPs with biological matter at different time and length scales. Establishing a qualitative and quantitative connection between physicochemical properties of the NP and their effect on biological functioning of membranes will clarify the possible pathways leading to toxicity.

3 Scientific and technological challenges

Over the last decade, in vitro and in vivo experiments have produced significant amount of veritable information that can be integrated into theoretical models with the aim of predicting possible health and environmental effects of engineered nanoparticles. However, even the most systematic studies leave the question of precise toxicity mechanisms associated with nanoparticles wide open. An important finding arising from these studies is that the toxic effects can emerge either from membrane damage or from interaction of nanoparticles, once they are inside the cell, with the internal cell machinery. Therefore, an evaluation of possible risks should include an assessment of nanoparticle ability to penetrate, modify, or destroy the cell membrane. The cell membrane is the junction where foreign objects meet biological tissues, where they challenge the immune system and present a threat to the tissue function. Being selectively permeable, membranes participate in control of the transport of vital substances into and out of cells. Whereas some biomolecules may penetrate or fuse with cell membranes without overt membrane disruption, no synthetic material of comparable size has shown this property. Among the factors determining the outcome of NP-membrane interaction the surface properties of nanomaterials play a critical role, which can implicate the membrane glycans or plasma proteins in conditioning NP prior to cell penetration. In addition, the size and shape of the nanoobjects has been found to be important for their fate inside the living organism. Any predictive model for toxicological and biological impacts of nanomaterials should be based on accurate, physically justified understanding of the mechanisms of formation of the NP-biomolecule complexes, NP-cell membrane interaction, the protective function of the membrane, and NP uptake into the cell.

4 Objectives

The main technical goal of the project is to develop a quantitatively sound multiscale approach to modelling of the mechanism of NP uptake from blood plasma and NP interaction with the protective barrier of living cells, which results in particle penetration into the cell and/or disruption of cell constituents, with a careful treatment of molecular specificity and the long-time dynamics of both particles and the membranes. This approach will enable us to establish relationships between physicochemical properties of engineered nanoparticles and toxicity and suggest the descriptors for classification of engineered nanoparticles according to their toxicological action.

Our method is based on hierarchy of modelling elements, which connect together the NP-biomolecule interactions, NP-membrane interactions, NP uptake and translocation, going all the way from molecular specificity to the effects on a sub-micron scale. An overview of some critical steps of NP systemic transport, which we cover in our approach, is sketched in Fig. 1.

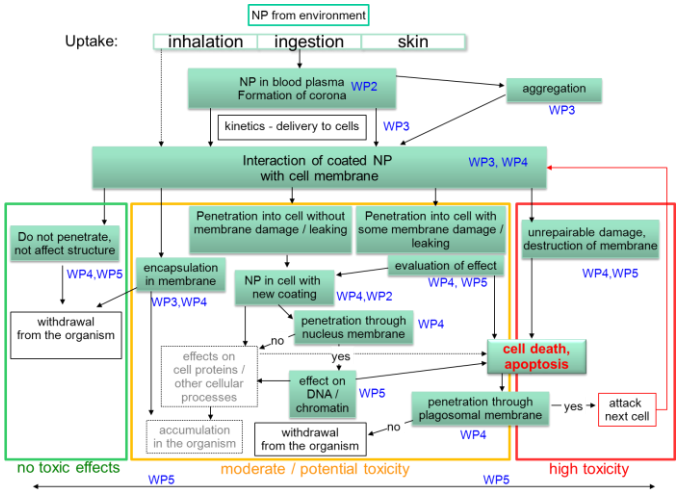


Figure 1. *Overview of the concept: some critical steps of NP systemic transport and Work Packages addressing the modelling of these steps. The questions within the competence of the project are shown by the shaded boxes and labelled by the corresponding work package codes.*

Our particular tasks involve:

- modelling the formation of NP corona from blood plasma proteins and lipids
- assessing the role of NP physicochemical properties such as size, shape, hydro-/lipophilicity, surface charge in stability of NP dispersions at physiological conditions, such as blood plasma, and in their systemic transport
- describing complexation of protein-coated or pristine particles with membrane lipids; particle interaction with membrane glycans; the probability of particle inclusion into the membrane
- describing the mechanisms of membrane-bound cytotoxicity: persistent change in the structural, chemical composition and mechanical characteristics of the membrane upon NP inclusion or translocation
- evaluating the probabilities of particle penetration through the membrane via membrane reorganization and disruption, or endocytosis
- developing an approach for systematic assessment of the degree of toxicity of engineered nanomaterials based on experimental biochemical data and molecular simulations.

5 Progress and Outcomes to date

During the first year of our project we following progress towards our objectives:

- Developed molecular and thermodynamic models for nanoparticle interaction with blood plasma and formation of NP corona based on coarse-graining of NP-protein interactions

- Prepared mesoscale models for NP-NP interaction in plasma based on DLVO theory of colloidal stability and coarse-grained united-atom models of biomolecules
- Prepared and tested a coarse-grained (united-atom) model of a cell membrane
- Modelled penetration of small NPs with size under 10 nm through the lung and cell lipid membrane

In Work Package 2:

1. Developed a new force field for TiO2 nanoparticles in contact with solvent and components of biomolecules.
2. Developed and tested a methodology for determination of adsorption free energy for protein side chain analogues and lipids.
3. Calculated binding free energies of all protein side chain analogues to TiO2. These data can be used for prediction of protein corona composition around TiO2 nanoparticles.

In Work Package 3:

1. Developed and partially tested a mesoscale model of nanoparticle-nanoparticle interaction in blood plasma including (i) united-atom simulation of the protein globule – NP interaction and calculation of adsorption energies with full account of the size and shape of both proteins and NPs; (ii) mesoscale modelling on NPs dispersed in blood plasma with adsorption energies and mobilities calculated in the united-atom model.

In Work Package 4:

1. Parameterized a coarse-grained lipid (DOPC, DMPC) and cholesterol model, which reproduces the key bilayer properties of atomistic model of the same system.
2. Modelled spontaneous NP translocation across DPPC monolayers at the atomistic level.
3. Developed a scheme for evaluation of the energy barrier for NP translocation through a lipid membrane.
4. Studied the relation between TiO2 NP size and the magnitude of translocation energy barrier for bilayers at the atomistic level. The barrier is found to grow with NP size.
5. Studied the role of cholesterol in NP translocation. Cholesterol is found to increase the energy barrier.

6 Expected Impact

Currently, most of the elements required to enable the NP toxicity assessment based on their characterization are unavailable or at their relatively early stage of development. We will attempt to build the missing parts within the time frame of the project. Upon completion of the scheme, we should be able for the first time to bridge between the NP physicochemical descriptors and the specific toxicity effects based on molecular level understanding of the underlying interactions and processes.

As the interaction of nanomaterial with human body is a complex process with multiple pathways and possible outcomes, we envisage that the most useful integral characteristic of the toxic action can be produced in the form of the risk levels. We plan to design a self-contained tool that would be able to evaluate the level of risk based on the physicochemical characteristics of the NPs and their aggregates. Note that we do not address the issue of critical concentration of NPs or the exposure but intend to solve the problem at a single NP/NP cluster vs single cell level so that the associated risk can be then subsequently scaled up according to the local NP concentrations calculated by other means. We envisage that final risk levels can be constructed from the probabilities of each of the anticipated toxic effects: production of ROS in the cell, loss of membrane polarization, leakage of cytosol material, and genotoxicity, or other, calculated in hierarchical way for specified nanoparticle types as described above. If successful, this will enable the subsequent formulation of expert Nano-QSAR systems based on key nanoparticle descriptors.

7 Directory

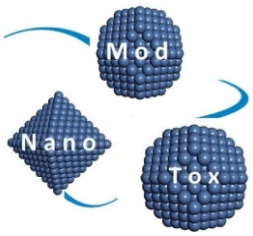
Table 1 Directory of people involved in this project.

First Name	Last Name	Affiliation	Address	e-mail
Vladimir	Lobaskin	National University of Ireland, Dublin / University College Dublin	School of Physics, Belfield, Dublin 4, Ireland	vladimir.lobaskin@ucd.ie
Alexander	Lyubartsev	Stockholm University	Department of Materials and Environmental Chemistry, Svante Arrhenius väg 16C, Stockholm, Sweden	alexander.lyubartsev@mmk.su.se
Nicholas	Quirke	Imperial College	Department of Chemistry, Exhibition Road, South Kensington Campus, London SW7 2AZ, United Kingdom	n.quirke@imperial.ac.uk
Alexander	Rabinovich	Institute of Biology KRC, Russian Academy of Science	Laboratory of Environmental Biochemistry, Pushkinskaya 11, 185910 Petrozavodsk, Russia	rabinov@krc.karelia.ru

8 Copyright

ModNanoTox

Modelling nanoparticle toxicity: principles, methods, novel approaches



Contract Agreement: NMP4-SL-2011-266712 Website: http://www.birmingham.ac.uk/generic/modnanotox/index.aspx
Coordinator: Professor Eugenia Valsami-Jones, University of Birmingham, Birmingham, UK

Table 1 Consortium List.

No.	Beneficiary name	Short name	Country
1	University of Birmingham	UB	United Kingdom
2	Roskilde Universitetscenter	RU	Denmark
3	Eidgenössische Anstalt für Wasserversorgung, Abwasserreinigung und Gewässerschutz	EAWAG	Switzerland
4	Eidgenössische Materialprüfungs- und Forschungsanstalt	EMPA	Switzerland
5	In-silico toxicology gmbh	IST	Switzerland
6	University of Nebraska Lincoln	UNL	USA

Contents

1 Summary

Project Duration: 1 November 2011 – 31 October 2013

Project Funding: 1 Mio. EUR

ModNanoTox, which was completed last October, developed a number of well-documented and technically advanced models describing the behaviour of engineered nanoparticles in organisms and the environment. The aim of these models was to construct a thoroughly documented database, based on: (1) an advanced evaluation of physicochemical properties of nanoparticles and in silico modelling of their reactivity; and (2) assessment of the characterisation methodologies as well as toxicity protocols used to develop biological responses in toxicological studies. Datasets were evaluated for a number of quality criteria and internal consistency as a condition for inclusion. The evaluation stage was followed by development of toxicity models based at the individual organism level, using statistical and mechanistic models, in parallel with models predicting environmental fate. The toxicity and fate models were integrated in mechanistic models to predict the long term risks of engineered nanoparticles for populations under realistic environmental conditions. The risk assessment framework was developed using results from the project's new models.

2 Background

The physicochemical properties of nano-sized particles are distinct from the properties of equivalent bulk substances and are also often unpredictable. As the use of nanomaterials increases, so must the research into any potential adverse effects on the environment or health. ModNanoTox was inspired by the realisation that many projects had begun generating large datasets of experimental results, which needed to be grouped and considered together. It was felt that it was critical to merge a number of available datasets and work towards generating models of toxicity. Such models would best be developed by the teams collecting the data, who also have a deep understanding of their limitations and relative quality and can influence the progress of ongoing experimental work.

3 Scientific and technological challenges

A number of influential reports that appeared since 2004 (when the Royal Society and the Royal Academy of Engineering in the UK, published the first such report) presented in technical detail concerns about the risks of nanotechnology, particularly with regard to free engineered nanomaterials. These concerns were also raised in the European Commission's Action Plan for Nanotechnology (2006), EPA's Nanotechnology White Paper (2005), Royal Commission on Environmental Pollution (2008) and in reports to most European governments, all expressing unease over the lack of urgency in identifying the extent of the potential risks. A major concern applying to the use and handling of nanomaterials is that their toxicity is likely to be due to physicochemical properties currently not included in standard toxicity screening tests. Of great concern is also that many of the commercial uses of such particles will directly result in their widespread dispersal and to human/ecosystem exposure in the workplace, via release of industrial effluents and via domestic waste streams. The environmental and human health implications of such wider dispersal are simply not known as yet.

4 Objectives

The overarching objective of ModNanoTox was to assimilate data from major EU and US funded projects on nanotoxicity (in many of which the ModNanoTox consortium has had direct links) and from this to generate models of the relationships between engineered nanoparticle (ENP) properties and toxicity. The project comprised 6 research workpackages, each of which addressed different aspects of model development with an overall environmental focus. The detailed objectives of the ModNanoTox project can be summarised as follows:

WP1

The overall objective of WP1 was to generate a computational fundamental understanding of nanoparticle reactivity, in order to inform other modelling activities within the project, particularly QSARs (WP3). Having reviewed and evaluated physicochemical descriptors (size, shape, phase, concentration, composition, surface modification, method of synthesis) and studied the effect of size and structure on nanoparticle reactivity the work was completed with a set of simulations of nanoparticle solubility as a function of size and structure.

WP2

The overall objective of WP2 was to generate a database that would support model development within and beyond the duration of the project and contribute to the nanotoxicology community needs for a reliable compilation of all relevant and up to date data. Having initiated the evaluation, classification and recording scheme for ENP toxicity data (data mining), and generated an initial database to support work in workpackages 3, 4 and 6, and having evaluated existing environmental exposure data for ENPs, in order to prioritise data for modelling in workpackage 5, the final objective of WP2 was to complete the database with studies up to the point of project end. A further important objective was to produce an analysis of data quality, address data gaps and identify challenges and next steps.

WP3

The overall objective of WP3 was to develop toxicokinetic/toxicodynamic models suitable for use in risk assessment. Having evaluated the data quality and availability from WP2 for use in toxicokinetic modelling and toxicodynamic analysis, and having made a selection of variables suitable for describing bioaccumulation as a toxicokinetic and mortality as a toxicodynamic model system, the final objective of WP3 was to assess the sensitivity of the models to different parameters identified across different organisms/cells/organs and exposure conditions to obtain generally applicable information, which could further guide environmental risk assessment for nanoparticles.

WP4

The overall objective of WP4 was to develop QSAR modelling of nanomaterial toxicity as far as current data availability allows. Having redeveloped the Lazar QSAR framework for nanotoxicological data, but realized current limitations in data availability for the model, the final objective of WP4 was to work on improving the accessibility and sustainability of the WP2 database by convert it into a format compatible with emerging standards (ISA-TAB NANO).

WP5

The main objective of WP5 was to advance and improve environmental exposure assessment models. Having reviewed and evaluated available models, work then progressed into the development of a model which combined nanomaterial flows into the environment with an environmental fate model, both connected on a local scale using geographic information on river flow and wastewater treatment effluents.

WP6

The main objective of WP6 was to model effects on ecosystems and then combine these with models from other workpackages to generate a risk assessment framework. Having produced a set of realistic worst-case scenarios for exposure of metal nanoparticles in freshwater, marine, pelagic and sedimentary environments and an assessment of species groups most likely to be at risk from engineered nanoparticles, WP6 also produced a population level risk model and a conceptual framework for risk assessment.

5 Progress and Outcomes to date

WP1 Physico-chemical properties assessment

Atomistic modelling has shown that NP properties such as size, surface roughness, fractal dimension etc. can all impact on transport (& uptake and likely toxicity) mechanisms and that interaction of the surfaces with water can affect the NP surface reactivity. Analysis of the 1st hydration layer of the water molecules around Ag NPs and the radial distribution functions facilitated prediction of water molecule residence times at

different surfaces and how these were affect by NP size, shape and structure. The size (number of Ag atoms) of the AgNP affects the shape of the particle, meaning that the size of the particle can affect the proportion of different surface configurations, which is important considering that some surfaces are more reactive than others purely as a result of their geometric configuration. The modelling was also able to estimate how attractive differently sized AgNPs are to water molecules. The residence time for water molecules in the 1st hydration layer around the AgNPs at different surface positions showed that certain sizes / morphologies are more attractive to water than others, and so they are potentially more liable to undergo dissolution – this is an area for future research.

Additionally, the process of Ag NP agglomeration was studied: Ag NPs in a simulated water box were monitored in terms of the changes in aggregate structure over time, focussing on the effect on different physicochemical parameters, for example, molecular roughness. The analysis software utilised in ModNanoTox was not designed to cope with such large systems and thus significant re-writing of the software was required; modelling approaches were therefore being pushed and expanded in terms of the software capabilities. However, now the code is re-written, it can be applied to other questions quickly and easily, and as such, the code can be shared upon request. The work on NP modelling aimed to address the lack of significant overlap currently between theory and experiment, and make the overlap more comprehensive. Three of the recommendations from the Intelligent Testing Strategy report support the importance of modelling of NP physico-chemical parameters:

- "F.6.1 The use of extrapolation and read-across between: materials of similar/related characteristics; species; human and environmental; as well as in vivo and in vitro should be more exploited.

- F.6.2 The impact of exposure route/pathways on PC characteristics (e.g. surface properties, protein corona etc.) and, therefore, the impact on toxicity, needs investigation.

- F.6.3 There is a need for better understanding of how NM interact with their environment, as factors such as dispersion, aggregations and agglomeration can influence NM toxicity".

Two publications are in final stages of drafting on this work. Data regarding atomistic modelling of the effect of NP size and shape on ligand stabilisation, using for example citrate, is being developed further within NanoTransKinetics and will feed also into the overall corona work there.

WP2 Data evaluation

The final form of the ModNanoTox database (DB) contains 99 studies dealing with the toxicity of nanoparticles (NPs) in aquatic organisms. Of the 99 studies included in the database 1% were published in 2007, 4% in 2008, 15% in 2009, 15% in 2010, 20% in 2011, 31% in 2012 and 14% in the first 10 months of 2013 (note that this includes data only to end October 2013 as the project ended then). This reflects the fact that ecotoxicity studies have traditionally lagged behind toxicity studies, and this was also observed for nanoecotoxicity studies compared to nanotoxicity studies: although the first nanotoxicolology papers were published in 1990s, the first nanoecotoxicology papers came out in 2005/2006. It is clear that the pace of research and publication in this arena (ecotoxicity focusing specifically on aquatic species and silver nanomaterials) is increasing rapidly, so a key goal now is to support researchers to ensure that the data they are generating is of sufficient quality/type for use in modelling studies to progress understanding rapidly and effectively.

Information extracted from each paper was grouped under 4 categories selected to describe the papers: Study Details, Particle Details, Assay Details and Study outcomes. Note that these were determined by the ModNanoTox consortium for the specific purpose of data quality assurance for subsequent modelling studies relating to NP interactions with the environment. One of the project deliverables (D2.4) contains the full list of publications included in the final database, as well as the list of publications assessed but not included in the database, along with the justification of their exclusion on the basis of insufficient characterisation.

During the last few months of the project, efforts were made to align the ModNanoTox database with the format of ISATab. The ISAconfigurator (a tool provided by the ISATab support community) creates experiment-specific configuration files in XML format that can be used to build spreadsheets in the ISAcreator. The ModNanoTox spreadsheet is separated into four tables, as explained above: Study Details, Particle Details, Assay Details and Study Outcomes. For each table we created a configuration file which maps the information from the ModNanoTox spreadsheet to the related ISA-TAB category/form and its fields and requests a data type or an ontology term (e.g. a species or a defined unit). Up to date ISAconfigurator files for the ModNanoTox database can be found at the public online repository and an example is included in ModNanoTox deliverable D3.3.

This ISAcreator tool creates and edits ISA-TAB files. Configuration files designed for particular assays help to map data from existing spreadsheets into ISA-TAB and can be reused with little modification for similar assays. ISAcreator provides a mapping tool to import spreadsheet files to deal with legacy data.

Challenges identified in terms of the conversion include the fact that ModNanoTox database has no common units as it is extracted from literature data and this is dependent on whatever units the studies reported. Significant additional manual effort would be required to convert all studies to common units, and the risk of introducing errors would be high. Additionally, ISATab uses a hierarchical ontology that our database cannot capture - we would have to move towards a database format with relational representation which would also require additional effort. Challenges exist on the ISATab side also as for the conversion, since the nano aspects are not yet included in the tools, and the fact that ISATab supports only single tables as input and the tables should be complete and the entries per column should have the a specific format (e.g. boolean, sting, integer, …).

Quality Assurance criteria / classification of degree of NP characterisation

The central purpose of the ModNanoTox database was to facilitate the modelling work packages to access good quality data with which to develop and validate their models. To achieve this ModNanoTox partners developed a set of criteria with which to rank the extent and appropriateness of nanoparticle characterisation in each study, leading to a quality score for each study. Thus, each study that passed the initial QC (based on their

source (industry or lab-synthesized) and the extent of in-house characterisation (none or some) with possible scores between 0 and 2; Combined scores of 0 meant exclusion from the database) was then scored for the completeness of particle characterization – note that this is specific for each particle chemistry, as not all descriptors are relevant for all particles. Studies were evaluated against a matrix of physico-chemical parameters as determined by specific methodologies and characterisation under relevant exposure conditions and over relevant timescales, giving a characterisation score for each, which is included as a field in the database. This score gives the database user an indication of the confidence they should have in the study from a characterisation perspective. The criteria per particle are a 'most informed guess' of what parameters will influence endpoints of interest: for silver NPs dissolution is obviously a key parameter, while it is not relevant for titania. Crystal structure is likely a relevant parameter for titania NPs, as it may be present as one of three structural configurations (polymorphs), but is not relevant for silver NPs, with one only possible structure.

The QC of the biological assays and end-points was based on basic scientific principles – the studies were too variable to standardize. Typical parameters recorded regarding the biological assays included species used, gender / life-stage, maintenance and preparation, media conditions (pH, ionic strength, temperature), exposure route, exposure duration, endpoints measured, endpoint method and controls included.

Future additions to the current characterisation matrix could / should include details of the coating characterisation (binding affinity, degree of coverage, displacement by other biomolecules in situ etc.) as well information regarding the ageing and/or transformation(s) in situ in a time resolved manner over timescales matching the exposure duration.

As the scientific community matures, and understanding of the importance of nanomaterials characterisation reaches the wider community, and is debated in the open literature and with journal editors, the degree of in situ characterisation reported in publications (i.e. characterisation in the relevant exposure medium) appears to be improving. Using the data in the ModNanoTox database, some analysis was performed to assess whether this is indeed the case: In order to test whether a significant correlation exists between advancing publication years and the characterization parameters, Two-Factor ANOVA without replication was performed. Results showed that when all parameters were taken into account there is significant difference in scoring change with time but that even more significance was calculated when comparison was made in terms of the different individual characterisation parameters (e.g. size, size distribution, shape/morphology, zeta potential, etc.).

Agglomeration is increasingly used to characterize NPs year on year, though initially (2008 studies) it was not assessed at all. The fact that agglomeration is correlated with the media in which the NPs are dispersed and that year-on-year the reporting of aggregation/agglomeration increased led to strong positive scoring correlation with time (rAP=0.804 and rAS=0.941) and significant variance with time (pA=8.56*10-11), which suggests that more and more studies follow a protocol of characterization both in situ and during the exposure time of the biotargets to NPs. Since non-linear correlation was found to be significant at the 0.01 while linear at the 0.05 level, exponential fitting was performed on the calculated data that provided highly significant values of $R2=0.992$ and reduced $\chi2=0.005$.

ModNanoTox has demonstrated that in situ characterisation of NMs in the exposure media and over the time-course of experiments is improving with time, as greater awareness builds in the community of the need for detailed characterisation, it is clear that there is still some way to go to generate data of sufficient robustness for modelling. Note that these studies related to NMs exposed in aqueous media, which for the OECD model systems typically contains salts only, making them much simpler from a characterisation perspective than river or sea waters which contained dissolved organic matter etc. NM characterisation in soil and sludge present further challenges, and are likely showing less improvement over time than aquatic ecotoxicity studies.

WP3 Bioaccumulation modelling

The primary aim of this WP was to utilise the ModNanoTox database of studies assessing impacts of Ag NPs on aquatic organisms to develop a statistical assessment to determine the influence of NP characteristics such as size, coating and concentration on NP bioaccumulation (as a toxicokinetic variable) and toxicity (as a toxicodynamic variable), ultimately in order to develop toxicokinetic/toxicodynamic models suitable for use in risk assessment. For the species considered, the team were also interested to understand whether feeding during exposure plays a role in modulating the bioaccumulation or toxicity.

For bioaccumulation, feeding and NP characteristics including surface coating appear to be most important, and for toxicity it is not yet clear if feeding is a significant factor, but hydrodynamic and nominal NP size and coating are drivers of toxicity. Non-significant factors are also assessed as it is also important to demonstrate those factors that don't influence bioaccumulation / toxicity in order to prevent unnecessary replication of studies in the future.

The most significant challenge for the bioaccummulation modelling resulted from the sparcity of the available dataset: following identification of data sets suitable for toxicokinetic (TK) and/or toxicodynamic (TD) modelling only 11 studies initially (M12 of the ModNanoTox project) were identified covering both waterborne and dietborne exposure to Ag NPs. Narrowing this down to only waterborne exposure of the organisms resulted in data for 14 different species from 20 studies in the database which resulted in just 143 data points in total, spread across these 14 species. Unsurprisingly, given its status as an OECD standard test organism, Daphnia was the species used in the most studies (4) and resulted in 57 data points in total, while the second most studied species was fish gills where internal concentration has been studied, but with only 11 datapoints. Within this also there were variations in the coating on the Ag NPs resulting in sub-groups of particles based on coatings – steric (PEG, PVP), electrostatic (carbonated, citrates, HA), and uncoated. This is a very clear limitation in the development of models and needs to be addressed as a matter of priority. Clearly there was virtually no redundancy in the dataset and no studies were replicated. Here also, it is clear that temporal data, and several datapoints for each measured endpoint would improve the amount of data available for the models dramatically without increasing the number of new studies needed.

In an effort to increase the number of data points in each category an effort was also made to group species according to broader criteria, e.g. Crustaceans & molluscs, Organs (gills, liver and intestines), fish cells and microalgae. Sub-groups of particles were also made based on coatings – steric (PEG, PVP), electrostatic (carbonated, citrates, HA), and uncoated. Media composition was also assessed as this can affect NP size and surface charge, thus a sub-grouping of these was made based on ionic strength (high, low, added biopolymers to disperse), while some studies only use dechlorinated tap-water.

The quantification of bioaccumulation and determination of a bioaccumulation factor for waterborne versus foodborne exposure illustrated that there was an approximately linear relationship for both such that internal concentration increases at same rate as the external rate. Comparing water versus foodborne exposure, a higher bioaccumulation rate was observed via waterborne exposure, possibly as a result of food-avoidance in organisms exposed via foodborne route as the food quality drops when spiked with NPs. The key finding was that the organism is highly important: higher bioaccumulation was observed in cells versus microalgae versus Mollusca etc. This was a much more significant impact than that of the particle characteristics, where only the hydrodynamic diameter affected bioaccumulation, but its impact was low.

The most important conclusions from this work were that organism-related characteristics are more important than particle properties to explain bioaccumulation (bioconcentration) and mortality. A slow elimination of AgNPs was observed for some species and much higher for others, as well as higher uptake from waterborne versus dietborne exposure, although ingestion of NPs is an important route of uptake.

WP4 QSAR models

The aim of WP4 was to develop QSAR modelling of nanomaterial toxicity using the lazar framework. Lazar (lazy structure–activity relationships) is a modular framework for predictive toxicology, whose approach is similar to the read across procedure in toxicological risk assessment in that lazar creates local QSAR (quantitative structure–activity relationship) models for each compound to be predicted. Model developers can choose between a large variety of algorithms for descriptor calculation and selection, chemical similarity indices, and model building. Within ModNanoTox, the open access OpenTox framework (www.opentox.org) for QSAR modelling was extended to include facilities for nanomaterials, which are much more complex that chemicals. Within ModNanoTox, the open access OpenTox framework (www.opentox.org) for QSAR modelling was extended to include facilities for nanomaterials, which are much more complex that chemicals. Work carried out as part of ModNanoTox included the regression of algorithms, which were extended to use quantitative descriptors; in addition, some chemical libraries were integrated to include descriptors, although most still relevant only to small molecules still. Ongoing challenges include the ability to compute NP properties, and this will continue to be addressed via projects such as eNanoMapper. More information on the lazar framework can be found at the In Silico toxicology webpage: http://www.in-silico.de/).

In terms of utilizing the ModNanoTox database as the basis for lazar modelling, similar challenges were encountered as those for bioaccumulation modelling, but here focused around the NP physico-chemical properties: Size, surface area/ charge (zeta potential), shape/ modification, dose, dissolution data are available for various studies in the database, but in most selections of studies, only a subset of the total dataset is complete which leads to missing values. The missing properties are hard to compare due to different methods of measurement and test environments. Additionally, the variety of properties is very sparse for modelling purposes. This is consistent with the fact that a single dataset (NPs in pancreatic cells) has been used to validate multiple different QSAR modelling approaches, and that papers are published without full statistical robustness (i.e. treating the replicates as separate datapoints) at present as a result of lack of datasets. Further evidence for this critical lack of data is apparent from the report on Intelligent Testing Strategies recommendation F.5.2: "There is an urgent need to conduct and verify structure activity relationships using an array of PC properties and hazard endpoints for different NM, so more work at all levels (characterisation, exposure, hazard and risk data) is required to generate and validate such systems".

Thus, at present the lack of substantial datasets in the field of NPs is presently the limiting factor for lazar QSAR modelling. Even if the models are valid, they will have an extremely narrow applicability domain, which makes them effectively unusable for any realistic application at present. However, as more and more datasets become available from the ongoing EU FP7 projects (and elsewhere) the lazar approach should eventually become applicable to nanotoxicity QSAR modelling. Full details are reported in Deliverables D4.1 and D4.2.

WP5 Exposure & Fate modelling

The main objective of WP5 was to advance and improve environmental exposure assessment models and support other WPs with data on likely environmental concentrations of nanomaterials. An important outcome from this study was the evaluation of existing qualitative and quantitative models and an assessment of how far these approaches can be utilised to understand environmental exposure, as well as the outlook for future modelling and experimental efforts. A detailed assessment and comparison of literature data regarding concentrations of NPs in the environment (focussing on surface water, waste water and sludge) was undertaken, which provided a comparison of measured, modelled and measured & modelled data regarding NP presence in surface waters versus in wastewaters. In all cases, an analysis of the assumptions used in the reported studies, as well as the challenges associated with background NPs and NP dissolution, was provided.

Mechanistic fate modelling of NMs in the environment was also performed (in collaboration with ETH Environmental Fate model). This approach attempted to combine two models, i.e. the model of flows of NMs during production, use and disposal into the environment and some environmental concentrations, with a model looking at agglomeration and heteroagglomeration to natural colloids etc. The team selected as a case study the Glut river in Switzerland, as they had access to detailed information regarding the number of treatment plants, the amount of wastewater output into the river, etc. and thus were looking at NMs coming into the river from households allowing a focus on impact from consumer products containing NPs. Using the model, the team looked at different fate scenarios and found that the

most important reaction is the heteroagglomeration of the engineered NPs with natural particles (colloids) in the river water. The study modelled free NPs in water, NPs in water attached to larger particles, and NPs-attached to larger particles in sediment. NPs were found adsorbed to suspended solids and in sediments. If the attachment efficiency was high then NP fate will be primarily influenced by sedimentation, whereas the opposite is likely if attachment efficiency is low. The key learning from this approach is that it is easy to couple the two models as they have compatible ways to calculate the parameters. This was the first time to include mechanistic models of NP concentrations in natural waters. The most significant problem or data gap is that currently it is only possible to do scenario modelling, as literature is missing the alpha parameter for heteroagglomeration, which must be determined experimentally.

Two publications have been submitted bases on this, one of which was highlighted as an editor's choice. An important outcome from ModNanoTox (which MARINA is taking forward) is that with current exposure & fate modelling methods cannot yet do any validation of the PEC (predicted environmental concentrations) values obtained, and cannot say yet whether measured concentrations are relevant.

WP6 Population models and risk assessment

The two key aims for WP6 were to model effects on ecosystems and then combine these with models from other workpackages to generate a risk assessment framework. Focussing on Environmental Risk Assessment (ERA), current scenarios are based on predicted (PEC) or measured (MEC) environmental concentration, and effect measures divided by a safety factor (PNEC). Bioaccumulation potential (B) and persistence (P) are 'simple' cut off criteria.

Mechanistic modelling offers an integrated approach that builds on and complements existing ERA in order to enable:

- Testing of effects under relevant exposure scenarios;
- Incorporation of bioaccumulation (toxicokinetics) & chemical persistence data;
- Relation of toxicokinetics to individual effects, leading to prediction of effects on populations.

Experimentally, it is possible to measure effects at individual or below individual level, but what is required is the protection of populations. However, it is not possible to measure effects at the population level with the required resolution. The current approach is determination of predicted environmental concentrations and lab experiments to get a NOEC value, which is divided by the PNEC value. Biopersistence criteria tell us about persistence / accumulation which are used to determine a cut-off criteria. Thus, the current scheme does represent everything from release to effect. ModNanoTox is proposing a more effective modelling approach that enables determination of the worst case scenario – i.e. effects on populations. The proposal contains all the exiting elements, such as the NOEC/PNEC, but now with spatial and temporal resolution, and including exposure scenarios, and integrating toxicokinetics/toxicodynamics into the population models. In an ideal world where we have the necessary data, it then becomes possible to test population effects under the different exposure scenarios. This is perceived as useful in risk assessment of pesticides, which are recognized as high risk chemicals, but has much broader scope also. This approach also provides the information that comes from the standard approach, and can calculate all the "standard" parameters: PEC, "p" for PBT, "b" for PBT etc. so nothing is lost and much is gained using this approach.

Another important aspect for consideration is which species to test. While this might seems like a small step, in reality for risk assessment there will be a huge impact. ModNanoTox suggests that in order to have a realistic worst-case scenario there is also a need for qualitative assessment of biological traits that can increase exposure. If organisms are not exposed, toxicity is not relevant, i.e. new approach: that incorporates a better integration of exposure & effect measures during the testing planning phase, in order to address those populations most at risk and assess only those. The types of questions that would inform this would include: Where in the environment do we find the highest NP concentrations? Which species are most affected by these concentrations?

An example of the type of qualitative approach could be as follows: Is the NP particle bound, agglomerated or present in surface water, etc.? Depending on how it is found in the different compartments, different species will be affected. E.g. if the NP is primarily located in sediment, then sediment dwellers that feed in the sediment are the most at risk group. Others in close proximity but that do not feed in the sediment less affected. Even if only small amounts left in the water column, some species are efficient at extracting this – for example filter feeders. These organisms will have high exposure. Organisms that do not filter will have low exposure. This is a practical approach, focussing on what is appropriate to test and forms the basis for a simplified risk assessment framework based on a "decision & testing" principle linked to likely environmental behaviour.

The next step is then to add on bioaccumulation (multiple timepoints) & long term testing of effects on survival, reproduction and somatic growth (note timepoints for both should be aligned). These can all be included in a population model (e.g. Dynamic Energy Budget (DEB) approach), which directly relates observed effects to predicted exposures. The approach can be tailored to either dynamic (spatial and/or temporal) exposure scenarios or static worst-case PECs. Integrating all the information into the population model can be used to assessment of population level risks. A clear advantage of the approach is that once developed it is not time-consuming to test different exposure scenarios, and potentially also different species (although this requires some re-coding).

Interestingly, the approach developed within ModNanoTox addresses several of the recommendations from the Intelligent Testing Strategy report whose recommendations for risk assessment include:

- "F.3.9 The most relevant species should be identified for specific scenarios (realistic exposure) and guidelines/standard protocols recommended.
- F.3.11 Protocols must be identified that can be applied at all stages of the NM life-cycle and should be carried out at life-cycle stages that best represent realistic exposure scenarios."

ModNanoTox developed a model for organism starvation using DEB and assessed how starvation varies with the size of animals. The sub-models involved in IBM are feeding rate; functional

response; assimilation rate (animals ingest depending on body length, or availability in environment); and assimilation efficiency from good in their gut. Once energy is obtained it can be spent on growth or reproduction. Within WP6, ModNanoTox partners modelled growth and reproduction versus nutrient availability for Daphnia magna initially and assessed how the animals deal with starvation and how this is distributed across the population. The same parameterisation was applied also for *Capitella teleta* with the result that the outcome at population level exhibits a boom-bust life cycle. Modelling a sediment dilution experiment, whereupon population behaviour in sediments with different carbon contents were assessed, demonstrated that the model predicts well. Sensitivity analysis of the parameters included in the model produced a qualitative rank in terms of importance: In both species parameters related to feeding and survival were most importance – this means then that when applying the approach to other species, there are the parameters to be most careful with.

Two publications are in final stages of preparation from this work, one on the population models and the other on risk assessment.

6 Expected Impact

As part of the ongoing dissemination and exploitation activities from the ModNanoTox project, the following list of exploitable outcomes was identified, and linked with the stakeholder groups for which they are deemed to be of most relevance:

1. Database (Excel and ISATab formats) of studies relating to Ag NP toxicity to aquatic organisms (99 studies from 2008-2013): eNanoMapper (FP7), NSC WG4 (databases), ISATab community, EU-US CoR Databases & ontology.

2. Quality Assurance criteria / classification for degree of NP characterisation in literature studies: NSC WGs 1 (materials), 2 (hazard), 3 (exposure) and 4 (databases), EU-US CoR Databases & ontology, Scientific community.

3. Adaptions to OECD protocols for aquatic assessment to include temporal information and facilitate determination of internal dose: Scientific community, QualityNano (FP7) for Inter-laboratory comparison, OECD and CEN, Regulatory organisations, MARINA, NanoValid, NanoMILE, FutureNanoNeeds & NanoREG.

4. Modelling approaches and outcomes (including what didn't work & why) on fate & exposure, bioaccumulation, QSAR models and population models: NSC WG6 (Modelling), EU-US CoRs on modelling, Modelling cluster of projects.

5. Atomistic modelling data and outputs regarding effect of size on particle stability, and NP dissolution: EU FP7 NanoTransKinetics & FutureNanoNeeds, NSC WG1 (Materials) and 6 (Modelling), NP producers / SMEs, Regulatory agencies.

6. Quality and reproducibility issues for studies related to aquatic toxicity of NPs: Scientific community, QualityNano (FP7), MARINA, NanoValid & NanoREG.

7. Proposed revision to current risk assessment approach to integrate predicted exposure levels with predictions of likely ecological effects: Risk assessors / regulators including ECHA and EMA, Policy makers, Industry, including pesticides, NSC WG5 (Risk assessment), NanoREG.

These outputs cover a diverse range of activities, and address a number of different stakeholders. Many of the identified stakeholders are accessible via the NanoSafety Cluster (NSC), including the NSC Working groups (WGs) and the EU-US Communities of Research (CoRs) as well as specific projects mentioned (e.g. NanoTransKinetics, QualityNano, MARINA, NanoValid, NanoMILE, eNanoMapper, FutureNanoNeeds, SUN and NanoREG). In addition, for each of the listed projects, specific plans are already in place / currently being implemented to ensure direct sharing of knowledge and incorporation of ModNanoTox findings into the ongoing work of these projects.

7 Directory

Table 1 Directory of people involved in this project.

First Name	Last Name	Affiliation	Address	e-mail
Roman	Ashauer	University of York	Environment Department University of York York, YO1 5DD, (UK)	roman@ecotoxmodels.org
Valery	Forbes	University of Nebraska Lincoln	School of Biological Sciences 348 Manter Hall, Lincoln, NE 68588-0118 (USA)	vforbes3@unl.edu
André	Gergs	Roskilde University	ENSPAC Universitetsvej 1 4000 Roskilde, (Denmark)	gergs@ruc.dk
Christoph	Helma	In Silico Toxicology GMBH	Altkircherstrasse, 4 CH-4054, Basel (Switzerland)	helma@in-silico.ch

First Name	Last Name	Affiliation	Address	e-mail
Jamie	Lead*	University of South Carolina	Department of Environmental Health Sciences, University of South Carolina, Columbia, 29208 (USA)	JLEAD@mailbox.sc.edu
Paul	Martin	University of Birmingham	School of Geography, Earth & Environmental Sciences, B15 2TT , Birmingham, (UK)	p.martin@bham.ac.uk
Bernd	Nowack	EMPA-Swiss Federal Laboratories for Materials Science & Technology	Technology & Society Laboratory Environmental Risk Assessment & Management Group Lerchenfeldstrasse 5 CH-9014 St. Gallen (Switzerland)	Bernd.Nowack@empa.ch
Michala	Pettitt	University of Birmingham	School of Geography, Earth & Environmental Sciences, B15 2TT , Birmingham (UK)	m.e.pettitt@bham.ac.uk
Annemette	Palmqvist	Roskilde University	NSPAC Iniversitetsvej 1 000 Roskilde, (Denmark)	apalm@ruc.dk
Kristin	Schirmer	EAWAG Aquatic Research	Überlandstrasse 133 Postfach 611 CH-8600 Dübendorf (Switzerland)	kristin.schirmer@eawag.ch
Eugenia	Valsami-Jones	University of Birmingham	School of Geography, Earth & Environmental Sciences B15 2TT , Birmingham (United Kingdom)	e.valsamijones@bham.ac.uk
David	Vorgrimmler	In Silico Toxicology GMBH	Altkircherstrasse, 4 CH-4054, Basel (Switzerland)	david.vorg@gmail.com

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* Also an adjunct Professor at the University of Birmingham.

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

Project number: 604347 Website: www.nanodefine.eu
Coordinator: RIKILT – Wageningen UR, Wageningen, The Netherlands
Contact : coordinator@nanodefine.eu

Table 1: Consortium List

No.	Beneficiary name	Short name	Country
1	Stichting Dienst Landbouwkundig Onderzoek	RIKILT	Netherlands
2	NordMiljö AB	NOMI	Sweden
3a	JRC – Joint Research Centre European Commission IHCP	JRC-IHCP	Italy
3b	JRC – Joint Research Centre European Commission IRMM	JRC-IRMM	Belgium
4	Universitaet Wien	UNIVIE	Austria
5	DanmarksTekniskeUniversitet	DTU	Denmark
6	BundesinstitutfüerRisikobewertung	BFR	Germany
7	Eidgenoessische Anstalt für Wasserversorgung, Abwasserreinigung und Gewaesserschutz	EAWAG	Switzerland
8	Commissariat a l'Energie Atomique et Aux Energies Alternatives	CEA	France
9	Technische Universitaet Dresden	TUD	Germany
10	Centrum VoorOnderzoek in Diergeneeskunde en Agrochemie - CODA	CODA-CERVA	Belgium
11	The University of Birmingham	UoB	United Kingdom
12	Fachhochschule Dortmund	FHDO	Germany
13	Bundesanstalt fuerMaterialforschung und - pruefung	BAM	Germany
14	Deutsches Institut fuer Normung e.V.	DIN	Germany
15	BASF SE	BASF	Germany
16	ClariantProdukte (Deutschland) GmbH	Clariant	Germany
17	SOLVAY SA	SOLVAY	Belgium
18	MBN Nanomaterialia SPA	MBN	Italy
19	L'Oreal SA	L'OREAL	France
20	NanoSight LTD	NanoSight	United Kingdom
21	RAMEM SA	RAMEM	Spain
22	Superon GmbH	Superon	Germany
23	Thermo Fisher Scientific GmbH	THERMO FISHER	Germany
24	EurofinsWEJ Contaminants GmbH	Eurofins	Germany
25	Institute of Nanotechnology	ION	United Kingdom
26	Nanotechnology Industries Association AISBL	NIA	Belgium
27	Verband der Mineralfarbenindustrie e.V.	VdMi	Germany
28	Cosmetics Europe – The Personal Care Association	Cosmetics Europe	Belgium
29	Laboratoire National de Metrologie et d'Essais	LNE	France

Contents

1 Summary

Project Duration: November 2013 – October 2017

Project budget: 9.3 Mio € (7 Mio. € EC contribution)

Nanotechnology is a key enabling technology. Still, existing uncertainties concerning EHS need to be addressed to explore the full potential of this new technology. One challenge consists in the development of methods that reliably identify, characterize and measure the size of nanomaterials (NM) both as substance and in various products and matrices. The European Commission has recently recommended a definition of NM as a reference to determine whether an unknown material can be considered a 'nanomaterial' (2011/696/EU). NanoDefine will explicitly address this question. A consortium of European top RTD performers, metrology institutes and nanomaterials and instrument manufacturers has been established to mobilize the critical mass of expertise required to support the implementation of the definition. Based on a comprehensive evaluation of existing methodologies and a rigorous intra-lab and inter-lab comparison, validated measurement methods and instruments will be developed that are robust, readily implementable, cost-effective and capable of reliably measuring the size of particles in the range of 1–100 nm, with different shapes, coatings and for the widest possible range of materials, in various complex media and products. Case studies will assess their applicability for various sectors, including food/feed, cosmetics etc. One major outcome of the project will be the establishment of an integrated tiered approach including validated rapid screening methods (tier 1) and validated confirmatory 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 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 Scientific / industry needs and challenges addressed

Nanotechnology is expected to stimulate industrial growth, innovation and development in many fields such as medicine, electronics, automotive, energy, construction, food, and cosmetics. But uncertainties about its safety are currently hampering more widespread exploration and exploitation. The recommendation of a definition of nanomaterial as suggested by the European Commission (EC) (2011/696/EU) is an important step to clearly determine if a material is a nanomaterial, or not. The definition concerns natural, incidental or manufactured particulate materials and focuses on particle size. According to the given definition, a material is a nanomaterial if 50% or more of the constituent particles (by number) have one or more external dimensions in the size range 1 - 100 nm. Recent research has shown that the reliable detection, characterization and quantification of nanomaterials – as raw material as well as in final products - is one of the big challenges in implementing nanospecific regulatory legislations. Another challenge is that the definition goes beyond advanced materials and encompasses e.g., pigments for paints, coatings and cosmetics, minerals and fillers for composite plastics, paper and packaging, construction materials or catalysts, and so has a broad impact on industry since most types of particulates now need to be re-assessed. Indeed, proper implementation of the definition may need to adapt already existing sector-specific provisions, such as Regulations on Cosmetic Products, Biocidal Products, Food Information and Plastic Food Contact Materials and the review and amendment of the European Chemicals Regulation REACH, to ensure a consistent approach across legislation which helps industry, regulators and consumers alike. It requires, in particular, validated detection, measurement and monitoring methods for nanomaterials because producers must be able to reliably classify an ingredient as a "nanomaterial" and control authorities must be able to test final products for the appropriateness of the labeling. To implement the definition in a legally clear and enforceable way, analytical tools and measures need to be developed for monitoring compliance with the definition.

This means that the EC Definition needs clear guidance on which methodology may be best suited to classify a material according to the definition. Such guidance should be flexible and easily extendable to allow a sector-specific implementation and adaptation for changing regulatory environments and the

continuous integration of new scientific and technological progress, but also provide method-independent performance criteria to ensure the applicability of methods for next generation (nano) materials as well.

The possible economic implications of the definition may also be far-reaching, as European industry has portfolios of 100,000s of conventional particulate products that a priori may fall under the definition. Only their size characterization may generate costs that are considerable and demand a cost-efficient integrated approach.

But just measuring average size or number size distribution for most nanoparticles in the range 1 - 100 nm, in particular in mixtures with other particles and substances, is challenging as the definition:

- is based on a "number size distribution"
- includes "particles in an unbound state or as an aggregate or as an agglomerate"
- size ranges below 20-30 nm are not adequately covered by most available techniques.
- demands application to nanomaterials in mixtures and complex matrices.

Only a few of the currently available methods measure the size distribution in particle numbers as required by the definition, but most are very labour-intensive. Even worse, there is no single method available that is applicable to all materials to determine whether they comply with the definition, or not, creating a huge uncertainty for industry, control authorities and consumers. The read-out of most widely-used methods, such as dynamic light scattering (DLS) or centrifugal liquid sedimentation (CLS), still does not allow to use these techniques alone and requires back up by more sophisticated techniques. Another challenge arises from the fact that the definition implicitly mandates that constituent particles within agglomerates and aggregates can be counted, although most modern techniques for particle size determination will size aggregates and agglomerates as individual particles. This implies that the use of such methods needs to be validated on a case-by-case and will depend on sample form and properties. De-agglomeration will be a crucial step that needs thorough evaluation and validation by suitable techniques. Only electron microscopy (EM) reliably covers the entire size range down to 1 nm under optimal conditions and for pure materials, which is why cost-efficient alternatives and statistically sound extrapolation methods need to be considered. Also no nanoparticle counting reference materials exist to cross-correlate and validate methods, and reference materials certified for size and mass concentration are rare, in particular for polydispersed materials. Implementation of the definition is even more challenging in mixtures and complex matrices, also due to the presence of other (natural or incidental) particulate materials and interfering matrix components. For this reason it is of paramount importance to develop an intelligent and integrated testing strategy that combines cost-efficient methods for the majority of cases, with more sophisticated methods for more complex cases.

To successfully implement the EU definition and associated legislations, all relevant stakeholders (industries, authorities, standardization bodies etc.) must have access to robust, validated and accepted methods and tools that are based on sound scientific data. This is why NanoDefine will develop suitable measuring techniques, reference materials and validated methods available by using an integrated and interdisciplinary approach and foster close international cooperation and networking between these end-users.

3 Scope and objectives

The main objective of NanoDefine is to provide the relevant industries and regulatory agencies with the tools that support the implementation of the definition in a regulatory context. To achieve this, a complete solution will be developed that is:

- ✓ easy to implement, as it integrates current practices/facilities/expertise available at end-users with new developments
- ✓ cost efficient, as it offers a tiered approach to straightforwardly identify the most adequate analytical route for a classification according to the definition
- ✓ flexible, as it defines criteria to include novel technologies and can be easily adapted to changing regulatory requirements
- ✓ sustainable, as the developed approach and performance structure can be implemented beyond the duration of the project.

The NanoDefine working concept is based on three pillars:

1. Development of a decision framework and classification procedure based on a tiered set of rigorously validated methods --> **The NanoDefiner e-tool**

2. Systematic evaluation of current methodologies and development/optimisation of key methods required for the classification procedure --> **The NanoDefine Method Manual**

3. Inter-laboratory validation and standardisation of key methods, including the provision of reference materials, case studies and technology transfer to end users --> **CEN/ISO standards**

The ultimate outcome of NanoDefine is an accepted and tested standardised procedure that allows industrial and regulatory stakeholders to classify particulate materials and products containing such materials according to the definition.

S**pecific objectives** are:

1. Development of a decision framework and classification procedure

NanoDefine will establish a tiered approach of measurement methods as it is expected that the majority of materials can be classified by comparatively simple, robust and cost-efficient methods (tier 1). Only in cases where these methods are not sufficiently reliable, one then proceeds to the next tier 2 including more sophisticated methods to reliably assess the size of different types of nanoparticles of different shapes. This concept allows one to (i) align with cost-efficient methods that are currently available/used in stakeholders laboratories, and (ii) limit the use of more labour intensive methods and high-end instrumentation to the cases where tier 1 methods fail.

The classification procedure we will develop will guide the user to select the most appropriate methods for a specific case by means of a uniform decision tree (see Fig. 1), taking into account already available information on the material as well as the requirements

on the quality of result and the availability of instruments and methods at the respective laboratory. At each decision node the system will evaluate the obtained information and data and guide the user through the next steps. This can either be an additional measurement by a tier 1 method or a tier 2 method, or may directly lead to the decision if a given material can be considered nano or non–nano according to the definition.

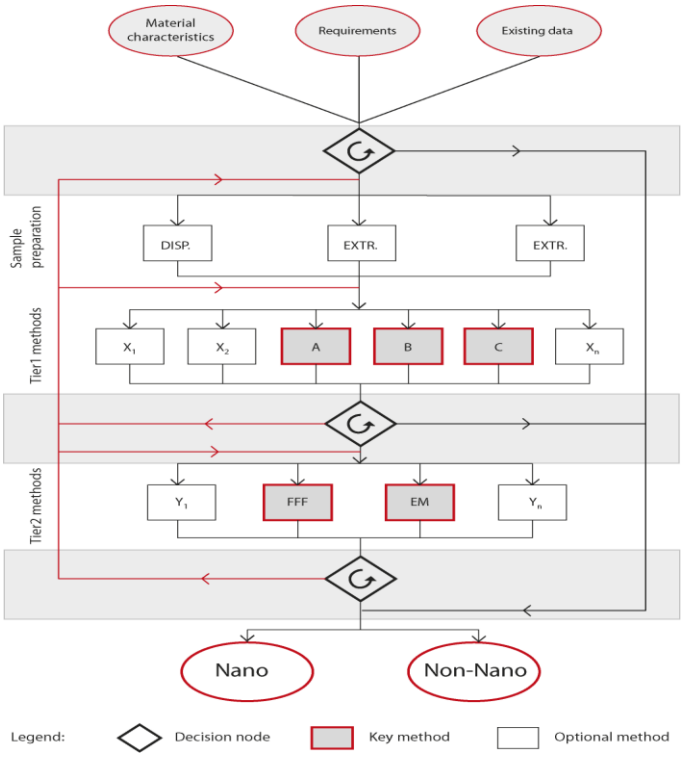

Figure 1: The NanoDefiner decision flow scheme that will integrate all project results and guide users by means of a decision tree. At each decision node the available data will be evaluated to guide to the next step until the classification into nano/non-nano according to the definition is achieved.

To make sure that the developed methods and procedures meet the needs of major stakeholders, NanoDefine has included various industrial sector associations in the project and has established a stakeholder panel to allow continuous input, interaction and exchange with all relevant end-user groups.

2. Systematic method evaluation and development

Currently available, broadly-used and promising novel methodologies will be systematically evaluated (with the same set of test materials) against specific performance criteria. The obtained data will be used in three ways: (i) they will be fed into the classification procedure to support the decision nodes on selection of appropriate methods, (ii) the method performance characteristics will be used in the NanoDefine method manual (which is a compilation of method descriptions with standard operation procedures (SOPs) and a guidance on their recommended use with strengths and limitations), and (iii) to support the selection of methods that will be developed into key methods. Key methods are deemed to be essential for the decision tree. Methods that are not yet validated or standardised will be developed/optimised and validated, which may include tailoring of instruments and software to the requirements in close collaboration with instrument manufacturers that are participating in the project. NanoDefine strongly focuses on actual particle counting techniques to avoid uncertainties from mathematical conversion.

3. Standardisation and implementation of methods

Selected key methods will be validated via inter-laboratory method performance studies based on standard operation procedures (SOPs) resulting from method development and further developed to work item proposals for submission to CEN/ISO. An early project liaison with CEN, the involvement of three metrology institutes with a strong focus on nanotechnology, and the strong representation of various project members in relevant standardization committees (e.g. ISO/TC229, CEN/TC352 Nanotechnologies) will ensure that method development can accommodate the needs and formats of standardisation.

NanoDefine will ensure the sustained availability of the developed measurement capacities after the end of the project to allow the durable implementation of the definition. To this end, the following measures will be taken during the course of the project: (i) posting of method SOPs on the project website and submission of work items for CEN/ISO standards, (ii) technology transfer to end-user laboratories (mediated i.a. via the involved stakeholder organisations), (iii) implementation of the methods in commercial contract laboratories, so that companies without suitably equipped laboratories can make use of the methods for their materials, and (iv) commercial availability of the required instruments/tools.

Selection of test materials:

The broad scope of the EC definition implies that all particulate materials need to be classified, starting from mined minerals and cements to pigments, fillers, catalysts and finally engineered nanomaterials with distinct novel properties, including further materials classes and various shapes (fibres, platelets, irregulars). The developed "NanoDefiner" and the Method Manual must be applicable to about 100,000 highly diverse materials. Test materials will be selected to represent this diversity and to establish the ranges of applicability of screening (tier 1) and confirmatory methods (tier 2). Conversely, the selection of test materials must remain sufficiently restricted to ensure that all participating laboratories work on identical materials, so that benchmarking and selection of methods is unambiguous.

The final **criteria for materials selection** will include:

- **Economic relevance**: impact on industry by economically prioritized application areas
- **Morphology**: representation of different shapes (3D (irregular) particles; 2D platelets; 1D fibres) and size distributions
- **Chemical identity**: representation of different chemical composition and surface treatments
- **Manufacturing process**: representative of the most important manufacturing processes
- **Origin**: representation of different origins with a focus on manufactured candidates, and only exemplary involving natural and incidental nanomaterials
- **Different formulation forms**: materials as pure substances and incorporated into products.

4 Technical approach and work description

The planned RTD activities as outlined above have been organised into 10 dedicated work packages (WPs), which cover the 3 main themes of the project: Decision & policy support (WP7+3), measurement technologies (WP2, 3, 4, 5) and standardisation & implementation of methods (WP1, 6, 8), in addition to project management (WP9) and scientific coordination (WP10).

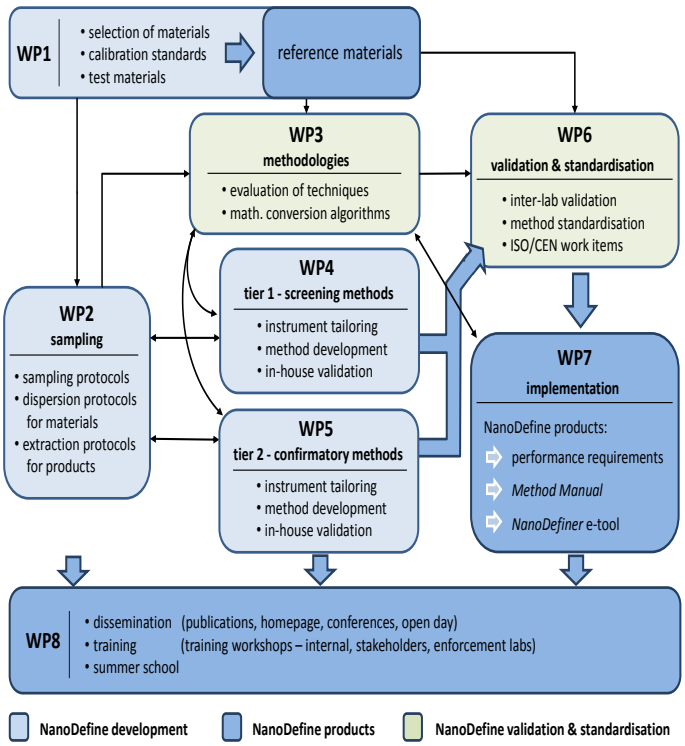

Figure 2: Interdependencies and interactions between WP1-8

All WPs are highly integrated with each other and will closely collaborate (Fig. 2). WP1 provides calibration standards and test materials to the method developers in WP2-5 as well as reference materials for the inter-laboratory validations in WP6 that are part of the standardisation procedure. WP2 develops dispersion protocols and sample extraction procedures for the materials received from WP1, which in turn are used in the tiered 1+2 methods that are developed in WP4+5. The developed methods, as well as the results of the thorough technology evaluation in WP3, are handed over for valorisation to WP6+7. WP6 takes care of the validation and standardisation, while WP7 develops a comprehensive decision-making framework for the implementation of the definition. All NanoDefine products (reference materials, method SOPs, NanoDefiner e-tool, NanoDefine Method Manual) are finally delivered to end-users and other stakeholders by WP8.

4.1 *Test and reference materials (WP1)*

Representative test materials will be selected, characterised and supplied in WP1 to the various WPs and tasks, and some of them will be further developed into calibration or reference materials.

4.2 *Sample preparation, dispersion and sampling methods (WP2)*

The aim of WP2 is to identify representative sampling strategies and to develop and optimize methods to disperse the nanomaterials in such a way that the resulting dispersions of nanoparticles from substances and products are stable and contain a maximum of mainly primary constituent particles. EM will be used to distinguish between large primary constituent particles and non-dispersable aggregates. An additional challenge is the possible dissolution and/or reactivity of some materials under certain conditions. The success of sample preparation techniques will be evaluated by a coordinated application of the methods of analysis that are developed in WPs4 and 5. WP2 will develop rather generic protocols that can deal with the majority of the most relevant products.

4.3 *Evaluation and selection of techniques and methodologies (WP3)*

WP3 is the NanoDefine methods evaluation hub. It provides continuous support for the method development in WPs 2, 4, 5 and surveillance of the developmental progress by the application of reference and test samples against unambiguous, quantifiable method performance criteria. A systematic critical review of the performance parameters of a broad set of suitable methods will be performed and results balanced against cost-per-analysis. This literature and expertise-based evaluation will be – for the first time - followed by competitive practical evaluation of methods on non-ideal, realistic samples from WP1. The quantitative establishment of method performance defines the required improvements and therefore directly affects the further work in WPs 4 and 5 where the NanoDefine key methods will be established. The two-step process of state-of-the-art based selection of candidate methods and continuous practical evaluation of the developed beyond-state-of-the-art methods in WP3 is tightly aligned with WP7 for development of the NanoDefine Manual. Evaluation will consider different categories: true counting methods (aerosol and liquid), non-counting ensemble methods, imaging methods, and separation methods.

4.4 *Screening methods (tier 1) (WP4)*

Methodologies selected in WP3 as rapid screening methodologies (e.g. NTA, CLS, stand-alone spICP-MS) will be improved and developed into validated methods (including determination of performance characteristics and measurement uncertainty) for the cost-efficient nanoparticle analysis according to the definition.WP4 delivers the most cost-efficient set of methodologies, gaining potentials and regarding limitations. Development efforts are separated in true counting methods and non-counting ensemble methods which require conversion of analytical data. If applicability ranges or cost-efficiency can be improved by advanced sample preparation, it will be recommended that these are developed in WP2. In-house validation and eventually a comprehensive inter-laboratory validation (in WP6) will establish the methods as fully quantitative in the sense of the definition.

4.5 *Confirmatory methods (tier 2) (WP5)*

WP 5 will deliver reference methods for an unambiguous, size and particle number based classification of materials and products by developing a set of confirmatory methodologies that are able to characterize difficult, broadly distributed, non-dispersible industrial samples, NM in products (cosmetics, food), in biota or environmental samples. Also, WP5 will develop reference methods to evaluate rapid screening methods of WP4 and any possible future methodologies. WP5 is using imaging techniques based on SEM and TEM, which are true particle number based methods, specific through elemental analysis and offer optical verification. They serve as high-quality references ("gold-standard") while the 'auto-TEM' toolbox will dramatically improve the cost-efficiency of EM techniques and will remove any operator bias. In addition, FFF based methods for particle separation in complex materials coupled to true particle counting techniques, together with conventional multi-detection techniques will be provided. Method development for both separation and imaging methods will be completed by a comprehensive intra-laboratory validation using the training and validation set of materials.

4.6 *Validation and standardization (WP6)*

This WP provides a harmonised platform for method validation and standardisation. Validation plans developed by WPs 4 and 5 will be checked for consistency and agreement with international guidelines. In-house validation studies will be harmonised as much as possible to provide common quality standards throughout the project. The most promising methods based on the results of the in-house validation in WPs4 and 5 will be subjectto inter-laboratory validation according to ISO 5725. This task shall be executed in close cooperation with other relevant projects, such as NanoREG, NanoValid and MARINA and wherever possible with VAMAS (Versailles Project on Advanced Materials and Standards) to cooperate with National Metrology Institutes outside the consortium. The project will also obtain project liaison status with the respective CEN technical committees early in the project to allow project representatives to participate in the meetings of the Technical Committees (TC) and relevant Working Group meetings as an observer and to propose new work items (standards) directly to the TC.

Selected methods developed in NanoDefine will be submitted as work item proposals to international standardisation bodies, e.g. under ISO TC 229 and/or CEN TC 352 in cooperation with WP6 and WP7. A close connection with CEN/TC 352/WG 1 "Measurement, characterization and performance evaluation" will be established.

4.7 *Implementation: NanoDefiner e-tool, manual and case studies (WP7)*

The NanoDefiner e-tool, a specific software-based classification approach and decision support framework, will pool results and conclusions together from method evaluation and development WPs with findings obtained from validation and case studies. This tool, with options based on material type, purpose, required data quality (including confidence level) and economic parameters, will guide the user to the most reliable and cost-efficient measurement method to identify/classify any substance or mixture according to the definition. The user will be provided with precise guidance which will allow the measurement to be extended to the widest possible range of substances, mixtures, and encompass also products and matrices. This will be accompanied by the NanoDefine Methods Manual comprising detailed information on the capabilities as well as strengths and weaknesses of each method addressed in the project. Both the NanoDefiner (decision support framework) and the NanoDefine Methods Manual will be available as software (e-tool) and as guidance documents, suitable for integration into the web platform on nanomaterials and nanotechnology announced by the European Commission. Case studies will finally demonstrate the real-world applicability of the developed and validated methods and the performance of the NanoDefiner by means of materials of industrial origin.

4.8 *Dissemination, training and technology transfer (WP8)*

This WP will provide the project results in a suitable format for the wider community to access and use; through regular news and information streams, reports, new standardised protocols, training events, and networking opportunities. It will provide the means by which to engage with different target groups: standardization bodies, industry, academia, regulatory agencies, policy-makers, relevant NGOs, and the wider public. It will create opportunities to exploit project results and assess their intellectual property value. It will also afford the wider community the opportunity to interact with the consortium and influence the development of the consortium's work. All public deliverables will be uploaded on the project website.

5 Status of the project

NanoDefine started on 1 November 2013 and was launched at a kick-off meeting in Wageningen, The Netherlands, on 19-20 November 2013. Work is being carried out in all work packages and first preliminary results have been achieved.

6 Expected impact

The outcomes of NanoDefine will have major positive impacts in the following areas:

- Smooth implementation of the EC definition of a nanomaterial in EU legislation
- Certainty for industrial and regulatory stakeholders on the methodology to be applied for the characterisation of nanomaterials
- Competitiveness of European industries
- International positioning of European RTD visibility in nanomaterial analysis.
- Confidence of EU citizens in the nanotechnology safety governance in Europe

6.1 Regulatory and legislative impact

There is still a broad uncertainty about the procedures and analytical technologies to be applied for the characterisation of particulate materials. This has in some instances caused the retention from dossier submission for approval of e.g. cosmetic ingredients. The uncertainty has even been increased by the introduction of the number-based criterion in the EC recommendation for a definition. NanoDefine will present the first systematic evaluation of potentially suited methods for the purpose of the classification of particulate materials according to the criteria of the definition. A clear technical guidance on which methods are suited for which applications will largely remove the mentioned uncertainties among both industry and regulatory agencies and the associated enforcement institutes. It is expected that the removal of this bottleneck will boost submission for approval of novel materials. Furthermore, enforcement laboratories will be able to take investment decisions for the most suitable analytical equipment. This will largely increase the capacities for the monitoring of nanomaterials in consumer products and food. In turn, this will support the safe implementation of nanotechnology in these areas and increase consumer confidence, which is a crucial pre-requisite for the general acceptance of this key technology.

NanoDefine will condense the gathered expertise on the possibilities and limitations of analytical methodologies into a recommendation to the EC with a view to the revision of the recommended definition. For different aspects of the definition it is currently unclear, if these can be covered at all by physico-chemical characterisation techniques, e.g. constituent particles in aggregates. The recommendations provided by NanoDefine will help to refine the definition (and/or some of the sector specific regulations to be based upon the definition) in a way that allows the actual implementation. This will be supported by recommendations for general method performance criteria that have to be met by methods that are to be applied in the classification of materials according to the definition. These recommendations can be adopted for an EC decision concerning the performance of analytical methods and the interpretation of results in a different regulatory context.

6.2 Economic impact and innovation

The EC definition of a nanomaterial has an immediate and broad impact on industry. This extensive impact is because European industry has portfolios of 100,000s of conventional particulate products that a priori could fall under the regulatory nano definition. Their size characterisation itself generates costs that are considerable. NanoDefine has the potential to cut these costs significantly. This has a huge impact on the competitiveness of European industries. In addition, the preparation of the NanoDefine guidance and classification procedure (The NanoDefiner) and the submission of developed and validated key methods as work items to international standardization organisations (CEN/ISO) as standard methods will remove existing uncertainties among industrial and regulatory stakeholders on which methodology has to be used in the regulatory approval of substances.

6.3 Scientific impact

The project will provide scientific tools that can be used to generate the necessary knowledge and understanding required to elucidate the complex nature of nanomaterials and the factors and mechanisms that control their stability, degradation and fate. The scientific advances achieved in the project, as well as the standard methods submitted to international standardisation organisations, will largely increase the capacities and visibility of the European analytical expertise in physico-chemical nanomaterial characterisation.

6.4 European and international integration

A number of efforts are currently underway both on the European and international levels to develop characterisation methods for nanomaterials. Consortium partners are already involved in these initiatives, providing a robust route for NanoDefine to carefully evaluate the results/progress of completed and on-going projects to avoid duplication of work and start at the most advanced state-of-the-art. In particular, the results from the FP7 projects NanoValid, NanoLyse, MARINA, and SMARTNANO will be taken into account. Close collaboration with WP2 of the NanoReg project will be established as this addresses similar topics to those in NanoDefine. Active involvement in the EU NanoSafetyCluster will help to integrate knowledge and experience gained from other relevant projects into NanoDefine in a timely manner, and also to disseminate results among the Cluster members. At international level, close cooperation is also foreseen with the North American NanoRelease project and within the Communities of Research (CoRs) of the US-EU collaboration on nanosafety.

7 Directory

Table 1: Directory of people involved in this project

First Name	Last Name	Affiliation	Address	e-mail
Hans	Marvin	RIKILT	RIKILT Wageningen UR, P.O. Box 230, 6700 AE Wageningen NL	hans.marvin@wur.nl
Stefan	Weigel	RIKILT	RIKILT Wageningen UR , P.O. Box 230, 6700 AE Wageningen, NL	stefan.weigel@wur.nl

First Name	Last Name	Affiliation	Address	e-mail
Wim	Beek	RIKILT	RIKILT Wageningen UR, P.O. Box 230, 6700 AE Wageningen NL	wim.beek@wur.nl
Anna	Undas	RIKILT	RIKILT Wageningen UR, P.O. Box 230, 6700 AE Wageningen NL	anna.undas@wur.nl
Ruud	Peters	RIKILT	RIKILT Wageningen UR, P.O. Box 230, 6700 AE Wageningen NL	ruudj.peters@wur.nl
Rudolf	Reuther	AB NOMI	Strandvägen 2, 671 29 Arvika Sweden	rudolf.reuther@enas-online.com
Rune	Karlsson	AB NOMI	Strandvägen 2, 671 29 Arvika Sweden	rune.karlsson@enas-online.com
Francois	Rossi	JRC-IHCP	Joint Research Centre, Nanobiosciences Unit, IPR-20i 008, I-21027 Ispra Italy	francois.rossi@jrc.ec.europa.eu
Hubert	Rauscher	JRC-IHCP	Joint Research Centre, Nanobiosciences Unit, IPR-20i 008, I-21027 Ispra Italy	Hubert.rauscher@ec.europa.eu
Douglas	Gilliland	JRC-IHCP	Joint Research Centre, Nanobiosciences Unit, IPR-20i 008, I-21027 Ispra Italy	douglas.gilliland@jrc.ec.europa.eu
Sandra	Vanhoovels	JRC-IRMM	Retieseweg 111, 2440 Geel Belgium	Sandra.vanhoovels@ec.europa.eu
Ana	Boix-Sanfeliu	JRC-IRMM	Retieseweg 111, 2440 Geel Belgium	Ana.boix-sanfeliu@ec.europa.eu
Robert	Koeber	JRC-IRMM	Retieseweg 111, 2440 Geel Belgium	Robert.koeber@ec.europa.eu
Thomas	Linsinger	JRC-IRMM	Retieseweg 111, 2440 Geel Belgium	Thomas.Linsinger@ec.europa.eu
Frank	v.d. Kammer	UNIVIE	Department of Environmental Geosciences, Althanstrasse 14 UZAII 1090 Vienna, Austria	frank.von.der.kammer@univie.ac.at
Erik	Huusfeldt Larsen	DTU	Technical University of Denmark, National Food Institute, Building B, MoerkhoejBygade 19, DK-2860 Soeborg, Denmark	ehlar@food.dtu.dk
Katrin	Löschner	DTU	Technical University of Denmark, National Food Institute, Building B, MoerkhoejBygade 19, DK-2860 Soeborg, Denmark	kals@Food.dtu.dk
Jutta	Tentschert	BFR	Berlin, Max-Dohrn-Strasse 8 -10 10589 Berlin Germany	Jutta.Tentschert@bfr.bund.de
Ralf	Kaegi	EAWAG	Überlandstrasse 133, P.O.Box 611, 8600 Dübendorf Switzerland	Ralf.kaegi@eawag.ch
Toni	Uusimaeki	EAWAG	Überlandstrasse 133, P.O.Box 611, 8600 Dübendorf Switzerland	ToniMikael.Uusimaeki@eawag.ch
Aurelien	Auger	CEA	Gif-sur-Yvette, Paris France	Aurelien.auger@cea.fr
Philippe	Capron	CEA	Gif-sur-Yvette, Paris France	philippe.capron@cea.fr
Frank	Babick	TUD	Technische Universität Dresden, AG Mechanische Verfahrenstechnik, Georg-Schumann-Bau, Raum 208 MünchnerPlatz 3, 01187 Dresden Germany	Frank.Babick@tu-dresden.de
Michael	Stintz	TUD	Technische Universität Dresden, AG Mechanische Verfahrenstechnik, Georg-Schumann-Bau, Raum 208 MünchnerPlatz 3, 01187 Dresden Germany	Michael.stintz@tu.dresden.de
Sven	Kreigenfeld	TUD	Technische Universität Dresden, AG MechanischeVerfahrenstechnik Georg-Schumann-Bau, Rm 208 MünchnerPlatz 3 01187 Dresden Germany	sven.kreigenfeld@tu-dresden.de
Eveline	Verleysen	CODA-CERVA	Groeselenbergstraat 99, 1180 Uccle Belgium	Eveline.Verleysen@coda-cerva.be
Jan	Mast	CODA-CERVA	Groeselenbergstraat 99, 1180 Uccle Belgium	Jan.mast@coda-cerva.be

First Name	Last Name	Affiliation	Address	e-mail
Eva	Valsami-Jones	UoB	University of Birmingham, School of Geography, Earth and Environmental Sciences, Edgbaston, Birmingham, West Midlands B15 2TT UK	e.valsamijones@bham.ac.uk
Christoph	Friedrich	FHDO	Emil-Figge-Str. 42, D-44227 Dortmund Germany	christoph.friedrich@fh-dortmund.de
Hans-Gerd	Lipinski	FHDO	Emil-Figge-Str. 42, D-44227 Dortmund Germany	lipinski@fh-dortmund.de
Marcus	Ingenfeld	FHDO	Sonnenstraße 98, D-44139 Dortmund Germany	marcus.ingenfeld@fh-dortmund.de
Monika	Schmidt	FHDO	Sonnenstraße 98, D-44139 Dortmund Germany	monika.schmidt@fh-dortmund.de
Thorsten	Wagner	FHDO	Emil-Figge-Str. 42, D-44227 Dortmund Germany	wagner@biomedical-imaging.de
Andreas	Thuenemann	BAM	BAM, Division 6.8 Surface Analysis and Interfacial Chemistry, Unter den Eichen 87, 12205 Berlin Germany	Andreas.Thuenemann@bam.de
Dan	Hodoraba	BAM	BAM, Division 6.5 Polymers in Life Science and Nanotechnology, Unter den Eichen 87, 12205 Berlin Germany	dan.hodoroaba@bam.de
Franziska	Emmerling	BAM	BAM, Division 1.3 Structure Analysis, Richard-Willstätter-Str. 11, 12489 Berlin-Adlershof Germany	Franziska.Emmerling@bam.de
Michael	Schmitt	DIN	Burggrafenstr. 6, 10787 Berlin Germany	Michael.Schmitt@din.de
Roman	Ruettinger	DIN	Burggrafenstr. 6, 10787 Berlin Germany	roman.ruettinger@din.de
Michael	Roeper	BASF	67056 Ludwigshafen, Carl-Bosch-Straße 38, Building M300, Room 202, Berlin Germany	michael.roeper@basf.com
Philipp	Mueller	BASF	67056 Ludwigshafen, Carl-Bosch-Straße 38, Building M300, Room 202, Berlin Germany	philipp.mueller@basf.com
Wendel	Wohlleben	BASF	67056 Ludwigshafen, Carl-Bosch-Straße 38, Building M300, Room 202, Berlin Germany	wendel.wohlleben@basf.com
Klaus	Kund	Clariant	Am Unisys-Park 1, 65843 Sulzbach am Taunus, Frankfurt Germany	klaus.kund@clariant.com
Antoine	Ghanem	SOLVAY	Rue de Ransbeek, 310 - 1120 Brussels	Antoine.ghanem@solvay.com
Alvise	Bianchin	MBN	via Bortolan 42, 31050 Vascon di Carbonera, TV - Italy	research@mbn.it
Paolo	Matteazzi	MBN	via Bortolan 42, 31050 Vascon di Carbonera, TV - Italy	matteazzi@mbn.it
Phillippe	Hallegot	L'Oréal	Pôle physico-chimie et microanalyse, L'Oréal, 1 avenue Eugène Schueller, BP 22, 93601 Aulnay Sous Bois Cedex, Clichy	phallegot@rd.loreal.com
Amy	Watt	NanoSight	NanoSight, Minton Park, London Road, Amesbury, SP4 7RT UK	amy.watt@nanosight.com
Iain	Wilson	NanoSight	NanoSight, Minton Park, London Road, Amesbury, SP4 7RT UK	Ian.Wilson@nanosight.com
Patrick	Hole	NanoSight	NanoSight, Minton Park, London Road, Amesbury, SP4 7RT UK	patrick.hole@nanosight.com
Phil	Vincent	NanoSight	NanoSight, Minton Park, London Road, Amesbury, SP4 7RT UK	Phil.vincent@nanosight.com
Emilio	Ramiro	RAMEM	Sambara 33, 28027 Madrid Spain Madrid	e.ramiro@ramem.com
Silvia	Lopez	RAMEM	Sambara 33, 28027 Madrid Spain Madrid	slopez@ioner.net

First Name	Last Name	Affiliation	Address	e-mail
Christoph	Johann	Superon	Hochstrasse 12, 56307 Dernbach Germany	Christoph.johann@superon.eu
Stefan	Boehm	Superon	Hochstrasse 12, 56307 Dernbach Germany	stefan.boehm@superon.eu
Ulrich	Roesch	Superon	Hochstrasse 12, 56307 Dernbach Germany	ulrich.roesch@Superon.eu
Lothar	Rottmann	THERMO FISHER	Hanna-Kunath-Strasse 11, 28199 Bremen, Germany	lothar.rottmann@thermofisher.com
Shona	McSheehy	THERMO FISHER	Hanna-Kunath-Strasse 11, 28199 Bremen, Germany	shona.mcsheehy@thermofisher.com
Philipp	Bruening	Eurofins	Neuländer Kamp 1, 21079 Hamburg Germany	PhilippBruening@eurofins.de
Andrew	Stewart	ION	University of Strathclyde Incubator, Graham Hills Building, 50 Richmond St, Glasgow G1 1XP UK	Andrew.Stewart@nano.org.uk
Lesley	Tobin	ION	University of Strathclyde Incubator, Graham Hills Building, 50 Richmond St, Glasgow G1 1XP UK	Lesley.Tobin@nano.org.uk
Mark	Morrison	ION	University of Strathclyde Incubator, Graham Hills Building, 50 Richmond St, Glasgow G1 1XP UK	Mark.Morrison@nano.org.uk
David	Carlander	NIA	101 Avenue Louise, 1050 Brussels, Belgium	David.carlander@nanotechia.org
Heike	Liewald	VdMi	MainzerLandstrasse 55, 60329 Frankfurt Germany	liewald@vdmi.vci.de
Florian	Schellauf	Cosmetics Europe	Cosmetics Europe - The Personal Care Association, Avenue Herrmann Debroux 40, 1160 Brussels Belgium	fschellauf@cosmeticseurope.eu
Georges	Favres	LNE	1, rue Gaston Boissier, Paris France	georges.favre@lne.fr
Tatiana	Mace	LNE	1, rue Gaston Boissier, Paris France	tatiana.mace@lne.fr
Charles	Motkus	LNE	1, rue Gaston Boissier, Paris France	charles.motzkus@lne.fr
François	Gaie-Levrel	LNE	1, rue Gaston Boissier, Paris France	françois.gaie-levrel@lne.fr

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Nanodetector

Ultrasensitive plasmonic detection of single nanoparticles

Contract Agreement: 280478 Website: http://www.nanodetector.eu
Coordinator: Vladimir Mirsky, Brandenburg University of Technology Cottbus-Senftenberg, Germany

Table 1 Consortium List.

No.	Beneficiary name	Short name	Country
1	Brandenburg University of Technology Cottbus-Senftenberg	BTU CS	Germany
2	Leibniz-Institut für Analytische Wissenschaften – ISAS- e. V.	ISAS	Germany
3	Imperial College London	IMPERIAL	UK
4	Fraunhofer-Institute for Biomedical Engineering	IBMT	Germany
5	Hybrid Integrated Technologies (HIT) Ltd.	HIT	Czech Republic
6	Phasis Sarl	PHASIS	Switzerland
7	Mivitec GmbH	MIVITEC	Germany
8	Optolita UAB	OPTOLITA	Lithuania
9	Upperton Ltd	UPPERTON	UK
10	MBN Nanomaterialia S.p.A.	MBN	Italy

Contents

1 Summary

Project Duration: 1 June 2012 – 30 November 2015

Project Funding: 2,968 Mio. EUR

The project is based on the new experimental phenomenon discovered recently by a project partner: single subwavelength-size objects give rise to optical signals in surface plasmon resonance microscopy. This provides a unique possibility for ultrasensitive on-line detection of engineered nanoparticles.

Within the project a development of the device for detection of nanoparticles and its application for a number of practically important tasks will be performed. The work includes the development of theoretical description of the new effect, optimization of main components of plasmonic microscope, development of sophisticated software for effective image analyses and isolation of nanoparticle signals from background optical signals and noise.

Preliminary experiments demonstrated a possibility to use surface modification to distinguish different types of nanoparticles. This approach will be used in the project to identify nanoparticles and to achieve this, an array with different receptor groups will be formed, and pattern recognition algorithms will be applied.

Measurements will be performed in aqueous media as well as in air. Inorganic, plastic and protein nanoparticles will be examined. At the final step of the project monitoring of nanoparticles in simple and complicated probes as well as monitoring of workplaces and waste during production of inorganic and protein nanoparticles and during aging of nanostructured materials will be performed.

2 Background

2.1 *Plasmonic detection of single nanoparticles*

A recent experimental discovery made by a project partner forms the basis for the novel approach to nanoparticle diagnostics proposed here. The proposed detector relies on exploiting a novel plasmonic effect that allows the detection and identification of single nanoparticles near metallic surfaces, and the determination of nanoparticle concentration in a sample.

The phenomenon of Surface Plasmon Resonance (SPR) determines the known effect of the attenuated total reflection of light from metal surfaces. In the well studied Kretschmann geometry for ATR experiments, the light travels through a prism which is bounded by a thin metal surface layer (typically gold), and is then reflected back from that layer (Fig. 1, on top). Near to a certain resonance angle (for a given wavelength of light) the collective oscillations of electron gas density in the film, known as surface plasmons, are excited. This gives rise to a sharp minimum in the reflection curve (Fig. 1, below). The position of this minimum is sensitive to changes in the refractive indices of the materials of the prism, the external medium and the metal layer. Species binding on the outer-side of the metal layer cause a change in the local dielectric properties of the system and hence a shift of the resonance position, which can be used for their detection and characterization. This effect is exploited in the well known SPR microscopy, frequently used in bioanalysis, e.g. for DNA/DNA, DNA/protein, protein/protein reactions. SPR microscopy applies imaging of the sensor surface onto a 2D detector (usually CCD camera), allowing the examination of up to several thousands surface processes simultaneously.

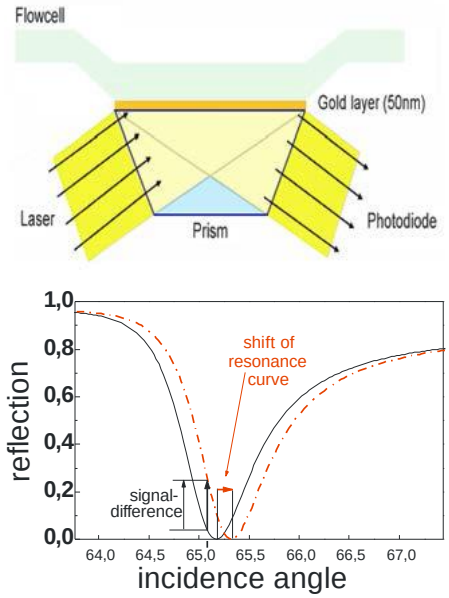

Fig. 1 The classical Kretschmann configuration mostly applied for SPR measurements in bioanalytical applications.

Limitations in the lateral resolution of SPR microscopy have been discussed since work in this area began. The large plasmon propagation length is usually seen as an obstacle, limiting optical resolution to approximately 5 - 20 µm thus preventing the observation of individual nanoparticles by this technique. However, it has been recently demonstrated by the project participant 2 that the plasmon propagation length in fact does not play the limiting role in detection of small particles bound to the surface. By precisely optimized SPR microscopy with digital subtraction of background it was demonstrated that particles with sizes down to the few tens of nanometers can be observed. Nanoparticles are detected by SPR microscopy using sensitive camera as bright spots with a size much smaller than the plasmon propagation length (Fig. 2). A precise physical model for this effect forms part of the current proposal, preliminary theoretical estimates suggest that these spots are caused by radiation of secondary plasmon waves around the particles. The spots are a few µm in size this is much larger than the particles that cause them. Illumination with a wavelength of 680 nm, allows dielectric particles with sizes starting from 40 nm and metallic nanoparticles starting from 20nm and upwards.

The suggested physical principle can be illustrated by the analogy shown in Fig. 3: a hindrance (a rock in the sea) interacting with the surface waves results in formation of secondary circular waves. Subtraction of the background (surface waves) would reveal an image with concentric waves similar the images obtained from nanoparticles absorbed to the metal layer given in Fig. 2.

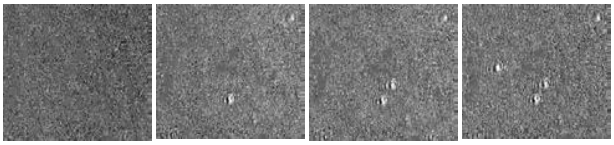

Fig. 2 Detection of 200-nm nanoparticles performed by SPR-microscopy.

Fig. 3.Illustration of the new method for detection of nanoparticles. The stone interacting with surface waves leads to the formation of secondary concentric waves. These waves can be detected with a spatial resolution which may be not enough to observe the stone itself.

This effect offers the potential new opportunities for measuring extremely low concentrations of nanoparticles. The feasibilities and limitations of this method will be studied during this project. In particular, the project will focus on optimizing this new principle of visualization and characterization of single nanoparticles of different materials. Coating the gold surface with corresponding receptors will allow for selective recognition of nanoparticles.

The selectivity can be improved by deposition of receptor spots with different coatings; this principle, well known in artificial noses, provides a possibility to make selective measurements using even poorly selective (but different) individual receptors.

2.2 *Quantification of nanoparticles*

The new method has already been validated by measurements using 50-200 nm polystyrene nanoparticles and protein particles. The particles were negatively charged; while the gold surface was

coated by positively charged compound. The suspension was pumped continuously through the flow cell. The number of bound particles was counted during the measurement interval. Measurements with the blank solution exhibited a negligible number of counts. The obtained dependence is shown in Fig. 4. The solid line is the linear fit of the data measured for 200 nm PS-nanoparticles. One can see that the slope of the line in the double-logarithmic scale is close to unity and the measured concentration dependence is well fitted by linear function over at least three orders of magnitude of particles concentration. At constant viscosity and flow the counting rate depends only on the particles size. This offers the opportunity for concentration measurements. This principle will be validated within the project and applied to quantification of nanoparticles in different samples.

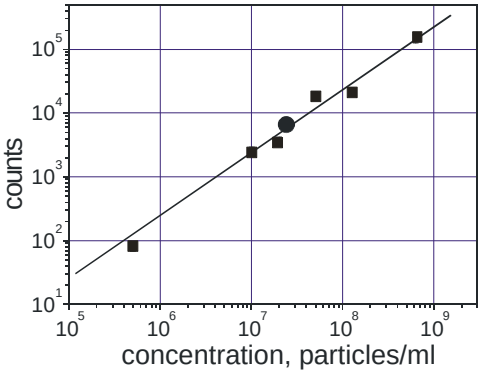

Fig. 4 Dependence of counts of nanoparticle signals on concentration of nanoparticles in the sample obtained for 200 nm polystyrene nanoparticles (squares) and for protein particles (circle).

2.3 Identification of nanoparticles

Identification of nanoparticles will be based on two principles: quantitative analysis of images from individual nanoparticles and modulation of nanoparticle-surface interaction.

Preliminary results demonstrate that the first approach provides information on size and effective refractive index of the material of nanoparticles. The second approach will be achieved by modification of the affinity properties of the receptor surface. Preliminary results have already demonstrated the potential of this approach for selective detection of nanoparticles by differently functionalized sensor layers. Selectivity of the second approach can also be improved further by the application of sensor array and by use of pattern recognition algorithms currently applied in artificial noses and tongues.

3 Objectives

The main objectives of the project are (1) the development and validation of technologies for the detection and analysis of low concentrations (10^7-10^9 particles/ml) of both engineered nanoparticles (ENP) and nanoparticles of biological origin in different environments and (2) the construction of a laboratory prototype of the device based on this technology.

The scope of possible applications for this new technology spans the entire spectrum of the nanotechnology industry. To confirm this broad utility, the current project will test for the selective detection of a broad range of different engineered nanoparticles, including plastic, metallic and metal oxide nanoparticles as well as nanoparticles of biological origin. The technology will be tested in both liquid and gaseous environment.

Ultimately, the technology will be applied by the end user for monitoring of nanoparticles in the work place to monitor the production waste in the immediate environment surrounding production facilities. This should provide a real time detection of nanoparticles and issuing of warnings in the event of a release of nanoparticles into a wider environment.

The table below summarizes the main current methods for detection of nanoparticles. The methods are broadly divided into two categories according to the detection principle.

Method		Drawbacks
integral methods	static or dynamic light scattering	low sensitivity; poorly compatible with qualitative analysis
	optical detection of plasmonic bands	low sensitivity; applicable only for plasmonic (gold or silver) nanoparticles
	using of labels (for example, radioactive, fluorescent)	applicable only for labeled nanoparticles
	surface enhanced Raman spectroscopy	requires a presence of Raman active moieties, applicable only for plasmonic nanoparticles
	photoacustic imaging	low sensitivity, time consuming technique
	optical coherence tomography	low sensitivity
methods based on detection of single nanoparticles	electron microscopy	time consuming technique, requires probe preparation, cannot be realized as a portable device for field applications
	scanning probe microscopy (AFM, STM, etc)	time-consuming, sophisticated and expensive techniques, cannot be realized as a portable device for field applications
	using of labels	applicable only for labeled nanoparticles
	spectroscopy of single nanoparticles using localized plasmon resonance	applicable only for plasmonic nanoparticles (silver or gold), time consuming and complicated, cannot be realized for field applications
	light scattering microscopy	non selective methods, size measuring requires sophisticated signal processing
	chemosensitive transistors based on nanowires	sophisticated technique, very small sensor area
	waveguide based detection	small sensor area, low concentration sensitivity
	Nanoparticle tracking analysis (NTA)	non-selective, limited concentration range

The new proposed technology combines a number of unique features which cannot be provided by any other detection technology (see table below). During the project these possibilities will be developed and characterized more exactly.

Expected Feature	Method of its realization
Extremely high concentration sensitivity	The method is based on detection of single nanoparticles bound to the sensor surface
Quantitative information on nanoparticle concentrations	Large number of nanoparticles can be detected, which enables a statistical analysis of the frequency of binding
Information on the size of nanoparticles (if material is known)	It can be obtained from the intensity of image. A calibration with reference materials may be required.
Particle size distribution	Difference in particle size can be distinguished.
Surface charge	The sign of the surface charge will be determined from interaction with charged surfaces.
Density	Correlates with refractive index.
Information on the refractive index of the material (if the size is known)	Can be evaluated from signal magnitude is the size is known.
Information on chemical content of the surface of nanoparticles	Will be done using chemical receptors and/or variations of chemical groups on the surface. Selectivity will be enhanced by using of an array with different coatings.
Possibility of on-line detection in native surrounding	On-line detection is possible with an appropriate software.
Continuous or (quasi)continuous operation mode	Even in the case of non-reversible adsorption, nanoparticles occupy only a very small part of the surface, therefore continuous operation can be realized by simple subtraction of the background.

4 Progress and outcomes to date

The goals of the first project period were focused mainly on the development of measurement technology, corresponding theoretical analysis and image processing software and the fabrication and testing of the first laboratory prototype.

4.1 General design of the measurement system

(i) Development of optimized optical system for SPR-microscopy of nanoparticles, computer simulation and optimization of this optical system, specification and test of single components of this system and a preparation for its manufacturing

This work is complicated by the fact that the optical requirements for the system are very complex and are not met by commercially available optics. Therefore, it was necessary to build a complete custom design for all the optical components of the system including illumination, collimation and imaging optics, and take into account optical distortions which are peculiar for SPR microscopy. The system was calculated for maximal field of view in focus and maximal possible resolution. It was first successfully tested in autumn 2013, since then it is being actively used in on-going research. The customarily designed optical system achieved much better optical resolution than it was possible in the laboratories of project partners using commercial optics.

(ii) General design, exact specification and experimental test of crucial electronic components, such as image sensor, embedded microcomputer and electronic drivers

Out of hundreds of readily available imaging cameras on the market, none fit optimally for NANODETECTOR. Detection of signals from a single nanoparticle is limited by the noise in the images, therefore, an optimal video-camera for NANODETECTOR-device should provide a possibility to capture images with extremely low noise while having the pixel resolution which corresponds to the optical resolution of system. After analysis of market situation it was decided to make a custom design of video imaging system, using optimal image sensor chip and a fast embedded microcomputer for the image preprocessing. Notably, it was necessary not only to develop an image sensor board to interface it to the microcomputer, but also to improve mechanical and optical design of this board too. The next parts of this task included a development of firmware for embedded microprocessor to provide fast image acquisitions, preprocessing of the captured images and sending results to PC for the storage and further image processing.

(iii) Development of sample delivery concept of the device

The concept should provide a possibility of operation in aqueous phase as well as in air, and to be compatible with the requirements of industrial partners. While this might sound simple, there are several major problems before one gets the leakage-free, contamination-free and air-bubbles free liquid sampling system. A number of other unknown questions, such as for example diffusion behaviour of nanoparticles in flow cell required to design several types of flow cell providing a possibility of future experimental test and optimization.

(iv) General design of the controlling system

It should include a thermostabilization, a control of the measurement setup parameters (e.g. of the light source, optomechanical actuators etc), a control of fluidic sampling etc. Possible complementary extensions of the measurement technology, based on new ideas and approaches which are now

under development, are also being taken into account. This work is completed, electronic controls printed circuit boards are designed and samples produced.
(v) Development of technology for highly reproducible preparation of very flat plasmonic (gold) layers on glass surfaces, optimization of layers thicknesses and coating conditions
An optimization of the resonant layer has direct influence on the sensitivity and therefore on the performance of the NANODETECTOR technology. The complete procedure for fabrication of gold coated sensor substrates on industrial scale was defined. This is an important step towards the commercialization of NANODETECTOR since these sensor substrates are consumables. Study of the influence of the coatings on the residual light reflection at the resonance angle gave scientifically interesting results too. A reproducible value of the residual reflectivity in resonance conditions below 0.5% was reached. Dozens of the coated glass prisms were produced and delivered to partners for the work on project, hundredths are in production.

4.2 Software development

The software for analysis of images, recognition of nanoparticles in the images and quantitative characterization of signals from nanoparticles was developed. It will be used in further work for analysis of experimental data, optimization of the input parameters and scenarios for automatic detection and verification of automatic detection software. Pre-processing routines such as grouping, normalization and standardization of captured image sequences, are followed by feature enhancement and static background elimination filters, then particle detection methods are applied. The nanoparticle SPR-signal recognition by blob detection methods and statistical intensity step analysis with Student's t-mean test were implemented. Particle detection is based on sudden change of intensity which is much stronger than noise and localized in the tight group of pixels. Beside the classic image processing approaches, the similarity concept based on the correlation coefficient value for recognition of image patterns from SPR-signals was developed. It consists of three steps: extraction of typical image patterns from the experimental images, construction of the etalon pattern and usage of resulting etalon pattern for further detection of the particles. Notably, the extracted resulting etalon pattern is very close to that obtained by theoretical analysis. Finally, the current version of the software can perform particle detection with a given set of initial parameters. It enables verification of the manual and numerical detection of the particles. Graphical User Interface operates as a sequence of dialog windows for convenient input and tuning of parameters, choice and combination of numerical methods for enhancement of image and detection of particles, visualization of output results. The goal of further software improvement is development of an instrument for completely automated image analysis without manually introduced parameters.

4.3 Design, fabrication and experimental evaluation of different planar surfaces providing selective binding of defined nanoparticles

The main part of the work was performed with protein nanoparticles. Receptor surfaces providing a strong selective binding of protein nanoparticles were developed. It was demonstrated that it is possible to use this classical approach of affinity sensors for detection of nanoparticles. At the same time, an essential difference in the required design of receptors for protein nanoparticles and for molecular proteins was evaluated. For detection and discrimination of metallic and plastic nanoparticles several functionalized self-assembled monolayers on the gold surface were investigated. While experimental results indicate on the viability of such approach, some anomalies in the binding of nanoparticles to functionalised surfaces were observed which cannot be explained by diffusion.

4.4 Theoretical analysis of physical nature of SPR-signals from nanoparticles

The mostly reliable approach for development of theory is based on analytical solution of Maxwell's equations. This approach was used to analyse an interaction of nanoparticles with surface plasmons wave. Then numerical solutions for particular set of parameters were done and an excellent agreement with analytical solutions was observed. The calculations were performed for a single nanoparticles and for their array. The refractive index and size of nanoparticles as well as their distance to the surface were varied. A far-field image of single nanoparticle was calculated, the image is close to that observed by filtration and averaging of experimentally obtained images of nanoparticles.

4.5 Experimental testing of the laboratory prototype

A laboratory prototype was developed and completed. Now further fine tuning and adaption of the device is in progress. Several inorganic and organic nanoparticles have been visualised and quantified successfully and the innovative device was introduced to the scientific community during a public workshop in November 2013.

5 Impact, Dissemination

The project NANODETECTOR has a high innovative impact because the ultrasensitive detection of single nanoparticles employing surface plasmon resonance is a novel application of SPR microscopy. No real image of the nano-object is generated because its size is below the resolution limit of this technique, but the measurable effects of its presence provide information concerning size, concentration, surface and possibly further properties (e.g., material). The combination of theoretical physics and nanoscience, analytics and measurement technology, surface and material science, microfluidics and microsensors, electronics, thin film technology, software development, precision optics and mechanics and designed organic and inorganic materials generates innovative knowledge. This combination is equivalent to a close and intensive cooperation between academia and SMEs for their mutual benefit. European science and industry is promoted beyond this project because the new measurement system is applicable to all other fields where the detection of nanoparticles is necessary, e.g. clinical diagnostics and biological research.

The project results are presented in the permanently actualized homepage, in international conferences and in a number of publications as well in local newspapers as in scientific journals. The project consortium organised an international public workshop on the "Detection of nanoparticles". Several European approaches to investigate and detect nanoparticles were introduced and the laboratory prototype of our project was successfully demonstrated to the scientific community. A close cooperation with another FP7 project focused on the development of pretreatment technology of complex nanoparticle containing probes (with the goal of combination of their pretreatment module with NANODETECTOR detection) is planned to generate and exploit synergies.

6 Directory

Table 1 Directory of people involved in this project.

First Name	Last Name	Affiliation	Address	e-mail
Vladimir	Mirsky	Nanobiotechnology, Faculty of Natural Sciences, Brandenburg University of Technology Cottbus-Senftenberg	01968 Senftenberg, Grossenhainer Str. 57 Germany	mirsky@b-tu.de
Alexander	Zybin	Leibniz-Institut für Analytische Wissenschaften – ISAS- e. V.	44139 Dortmund, Bunsen-Kirchhoff-Str. 11, Germany	alexander.zybin@isas.de
Alexei	Kornyshev	Department of Chemistry, Faculty of Natural Sciences, Imperial College London	SW7 2AZ London, Exhibition Road, South Kensington Campus UK	a.kornyshev@imperial.ac.uk
Thomas	Velten	Department of Biomedical Microsystems, Fraunhofer-Institute for Biomedical Engineering	66386 St. Ingbert, Ensheimer Str. 48 Germany	thomas.velten@ibmt.fraunhofer.de
Thorsten	Knoll			thorsten.knoll@ibmt.fraunhofer.de
Zdenek	Reznicek	Hybrid Integrated Technologies (HIT) Ltd.	68715 Nedachlebice 233, Czech Republic	hit@vcelarnet.cz
Jorge	Cors	Phasis Sarl	18 Chemin des Aulx, 1228 Geneva, Switzerland	jorge.cors@phasis.ch
Alex	Mirsky	Mivitec GmbH	81829 Munich, Wamslerstr. 4, Germany	am@mivitec.de
Dainius	Tumosa	Optolita uab	08412 Vilnius, Mokslininku 11,Lithuania	d.tumosa@eksmaoptics.com
Richard	Johnson	Upperton Ltd.	Cambridge Road 15, NG2 5NA Nottingham, UK	rjohnson@upperton.com
Paolo	Matteazzi	MBN Nanomaterialia S.p.A.	Via Bortolan 42, 31030 Carbonera, Italy	research@mbn.it

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NanoFATE

Nanoparticle Fate Assessment and Toxicity in the Environment

Contract Agreement: NMP4-SL-2010-247739 Website: http:// www.nanofate.eu
Coordinator: Dr Claus Svendsen (csv@ceh.ac.uk), NERC - Centre for Ecology and Hydrology, Wallingford, UK

Table 1 Consortium List.

No.	Beneficiary name	Short name	Country
1	Natural Environment Research Council	NERC	United Kingdom
2	VU University, Amsterdam	VUA	Netherlands
3	Oxford University	UOXF.DJ	United Kingdom
4	University of Aveiro	UAVR	Portugal
5	Faust & Backhaus	F+B	Germany
6	NanoTrade	NT	Czech Republic
7	Università degli Studi del Piemonte Orientale *Amedeo Avogadro*	UNIPMN	Italy
8	Institute of High Pressure Physics, Polish Academy of Sciences	IHPP	Poland
9	Cardiff University	CU	United Kingdom
10	Amepox	AXME	Poland
11	Gothenburg University	UGOT	Sweden
12	SYMLOG France	SYMLOG	France

Contents

1 Summary

Project Duration: April 2010 to March2014

Project Funding:

Concept: NanoFATE has been conceived to fill knowledge and methodological gaps currently impeding sound assessment of environmental risks posed by engineered nanoparticles (ENPs). Our vision is to assess environmental ENP fate and risk in for example high-volume products for which recycling is not an option, namely; fuel additives, polishing agents, personal care products and antibacterial products. To represent these products two commercial ENPs of CeO_2, ZnO and Ag (of varying size, surface and core chemistries) will be followed through their post-production life cycles, i.e. from environmental entry as "spent product", through waste treatment to their final fates and potential toxic effects. This will test the applicability of current fate and risk assessment methods and identify improvements required for assessment of ENPs at an early stage.

Objectives: Delivery of a systematic study of the environmental fate and toxicity of selected ENPs will entail addressing nine S&T objectives:

- Design, tagging and manufacture of ENPs
- Analysis of ENP interactions with abiotic and biotic entities
- Generating predictive models for ENP exposure in waters and sludge-amended soils
- Studying the fate and behaviour of ENPs through wastewater treatment
- Determining acute and chronic ecotoxicity

- Assessing effects of physico-chemical properties on ENP bioavailability
- Defining mechanisms of uptake, internal trafficking, and toxicity
- Developing spatial RA model(s)
- Improving understanding of ENP risks

Methodology: The work plan is designed to deliver research and progress beyond the state-of-the-art. While some objectives are focused in single WPs, others are cross-cutting, so ensuring the integration of the work plan to support delivery of novel ENP risk quantification methods.

Impact: NanoFATE will provide robust tools, techniques and knowledge needed by stakeholders to understand and communicate risks associated with ENPs of different physical or chemical properties, including their environmental interactions and toxicity.

Keywords: Nano, fate, exposure, bioavailability, uptake, toxicity, risk, environmental.

2 Background

The potential human health effects of ENPs are of obvious importance and a review of European research and national programs indicates that a number of ongoing projects are already addressing this issue (e.g. NANOTOX, CELLNANOTOX, IMPART, NANOSH, NanoReTox). In distinct contrast, there are as yet few studies that have focused on developing and refining methods to assess the fate of ENPs in ecosystems (e.g. soils and natural waters) and any resulting ecotoxicological effects. For this reason NanoFATE intends to focus on these neglected aspects and their integration.

To support the responsible development of the nanotechnology sector, it must be recognised that the development of environmental risk assessment methods should not lag too far behind those for human health. Past experiences highlight a number of other environmental issues such as organochlorine pesticide usage (Newton and Wyllie 1992; Newton et al. 1999; Sibly et al. 2000), endocrine disruptions (Jobling et al. 1998; Tyler et al. 1998), secondary effects of pharmaceuticals on wildlife (Oaks et al. 2004), and genetic modification (Haughton et al. 2003; Heard et al. 2003), where environmental impacts rather that direct effects on human health, emerged as the major area of concern. In each of these cases, the unexpected nature of these effects had a profound affect on public confidence in new technologies. This required that rapid regulatory action was put in place to control and mitigate risks. By ignoring effects on the environment, nanotechnology runs the risk that similar damaging and costly effects could occur.

Because of the initial and wholly understandable focus on direct risk to human health, knowledge of fundamental aspects of the environmental risks associated with ENPs is low in several key areas. These include:

- the post-production fate of ENPs from entry into the environment to final residence;
- how ENP-ENP and environmental interactions affect the biotic availability of ENPs and how different ENP properties (size, surface) affect exposure/uptake;
- how crucial ENP properties such as size distribution, surface chemistry, shape and optical properties influence toxicity;
- chronic aspects of ecotoxicity, which to date has mainly been assessed at environmentally unrealistic concentrations or in inconclusive studies where it was uncertain whether the co-solvent used for dispersal, impurities, or the ENP itself resulted in the observed toxic effect;
- the mechanisms of toxicity of ENPs when compared to the bulk chemical or free metal ion and how observed effects of ENPs on the expression of genes or proteins associated with particular pathways (e.g. such as oxidative stress in cell lines) relate to higher level *in vivo* effects;
- the fitness for purpose of existing risk assessment approaches designed for standard chemicals for use with ENPs and the modifications needed to allow existing frameworks and policies to be used in future for the risk assessments of nanotechnology products.

By studying the fate and behaviour of the selected ENPs and their effects on biota, NanoFATE will go beyond the superficial initial assessments that have been possible so far, thereby enabling a scientifically rigorous analysis in relation to each of the above aspects. The data gained in meeting each of the nine NanoFATE objectives will allow us to go beyond the current state-of-the-art as set out in the section below.

3 Scientific and technological challenges

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4 Objectives

4.1 *Current baseline of knowledge and points where NanoFATE will progress beyond the state-of-the-art in meeting project objectives.*

Obj.1: Design and manufacture of tagged ENPs for tracking in fate and toxicity studies.

Baseline. Differentiation of ENPs from the natural background has been a critical problem in understanding their fate in complex environmental systems. Even though some of the ENP core metals have low concentrations in the environment (Ce and to an extent Ag), approaches beyond simple elemental analysis using ICP-MS based methods are needed to study the partition process that determine the final destiny of ENPs. Furthermore, some types of labelled nano-sized particles (e.g. fluorescent silica NP) that have been used to track fate in the environment often lack the physical characteristics of production ENPs and so can not be expected to behave in a similar way to commercial ENPs. As a result, specifically designed ENPs that can mimic commercial particles, are needed to support the fate and effects work conducted in WP 2, WP3, WP 4 and WP 5.

NanoFATE progression beyond the "state-of-the-art". To undertake realistic real world fate studies, NanoFATE will design and fabricate ENPs "tagged" with selected ions that are detectable in bulk samples that will offer real advantages over the current state-of-the-art. ENPs tagged with ions of low background in the environment can under ideal conditions be detected by elemental analysis. Further, using cathodoluminescence spectroscopy it will be possible to detect the nanoparticles in small samples and investigate their degree of aggregation. Since the tagged ions will be inside the particles, they do not affect their behaviour and are also protected from chemical attack in the environment, hence preserving the tag:ENP ratios.

To provide tagged particles for use in NanoFATE, partners, IHPP, UOXF.DJ and UGOT will work together to identify any available uniquely identifiable ENPs suitable for off the shelf use that are relevant to the three product groups and incorporate particle types considered in NanoFATE. Where suitable tagged ENPs are not available, these will be synthesised by IHPP with input from UOXF.DJ. These two partners have particular experience in ENP design, production and characterisation. Acquisition or production of the tagged ENPs will be done with consideration to match the properties of the two variant ENPs of each type selected for NanoFATE. Studies will be conducted to validate the ability to track designed tagged ENPs within sewage treatment systems, environmental media and organisms. The resulting information will help the design of the targeted studies in WP 2 and WP 5 that will address these issues in detail. The detailed work to be conducted to meet this objective is set-out below.

1. NT and AXME will allow access to existing ENPs that are currently used commercially in our target product types (diesel additives, cosmetics, antimicrobial surfaces and products). These partners will also provide information on particle properties and characteristics to support detailed experimentation, to establish how closely tagged particles generated in our project match these commercially available ENPs.
2. IHPP will use their solvothermal process, in which a mixture of chemicals soluble in a water-ethanol mixture is enclosed in a pressure vessel and heated using microwaves to nearly supercritical conditions, to produce rare earth metal-tagged nanoparticles in volumes that can be supplied to all partners (Lojkowski, 2008; Cabanas et al., 2007). This manufacturing method allows ENPs of different core chemistries, sizes and coatings to be produced, with none of the disadvantages (poor ion concentration control, particle aggregation) associated with gas phase or wet chemical synthesis. Initial product particle characterisation (surface bonds, zeta potential, surface charge and particle size) will be undertaken.
3. UOXF.DJ will lead particle characterisation, measuring surface bonds, zeta potential and light scattering of ENPs will be determined by combinations of X-ray diffraction, electron microscopy, infra-red and Raman spectroscopy and dynamic light scattering to provide measurements of surface charge and particle size. When studies include work focusing on

properties in environmental media, UOXF.DJ and UGOT will collaborate.

4. UGOT will refine Flow Field-Flow Fractionation with high resolution ICP-MS (FLFFF-HR-ICP-MS), and if needed other in situ trace techniques (Stolpe and Hassellöv 2007), for detecting the interactions of the selected sets of tagged and untagged particles with environmental colloids in order to establish the methods for later detailed work targeted in Obj 3 that will be conducted in WP 2.

Obj.2: Generate models for predicting the likely levels and states of ENPs in receiving waters and soils.

Baseline. Current publicly available databases provide information on the use of ENPs within nanotechnology products (e.g. Project on Emerging Nanotechnologies) and this in turn provides information on the magnitude and nature of potential sources of ENP released into the environment. This identification of sources within consumer products has allowed initial risk assessments to be conducted to predict the potential levels of ENPs that may occur in environmental media at assumed levels of marker penetration. Combining the data with existing effects data has allowed initial estimates of potential risk to be conducted (Boxall et al 2007). So far, however, work to validate a number of the assumptions within these model predictions have yet to be tested and validated. These include the extent of potential market penetration of nanotechnology products, release rates of ENPs from products, how patterns of seasonal usage will influence concentrations reaching the environment under different scenarios, and the potential impact of the heterogeneous distribution of sources on realised environmental concentrations.

NanoFATE progression beyond the "state-of-the-art". To improve the current state of spatial and temporal exposure assessments, NanoFATE will, as a first step, compile source inventories and from this data derive plausible future scenarios of release (including median and extreme predictions) for the selected nanotechnology products and associated ENPs. This will be done through a stakeholder consultation led by F+B and involving NanoFATE's nanotechnology sector partners NT and AXME and other amenable companies. Additionally, information on the development of the nanotechnology field provided by other EU projects, within publications highlighted in the ICPCNANONET EU funded database and through the Inventory of Nanotechnology-Based Consumer Products Currently on the Market (http://www.nanotechproject.org/inventories/consumer/) will also be utilised.

In addition to acquiring usage information, industrial information on ENP usage rates in products and ENP properties associated with our focus products and information on release rates and states will be collated. This information will include data on particle sizes of CeO_2 associated with diesel exhaust fumes, ZnO concentrations and release from sunscreens, Ag loss from impregnated material during washing etc. This data will be used to support release scenario development. Initially, environmental concentrations of all the ENPs will be modelled with the current standard multi-media model, EUSES, based on the relevant release pathways addressed. This is important as it will allow linkage of the project's results with ongoing work on how ENPs can be adequately addressed within the REACH framework.

The developed release scenarios will provide a starting point for further modelling of the potential fate of ENPs in the environment using state-of-the-art approaches. This will allow a refinement of calculation of environmental concentrations and states of ENPs reaching particular environmental compartments. For modelling wastewater release for assessment of the fate of ENP, the process of disposal is visualised according to the schematic shown in Fig. 1. Modelling of deposition to soil will be the focus for CeO_2. Initial predictions will be generated based on worst case conditions.

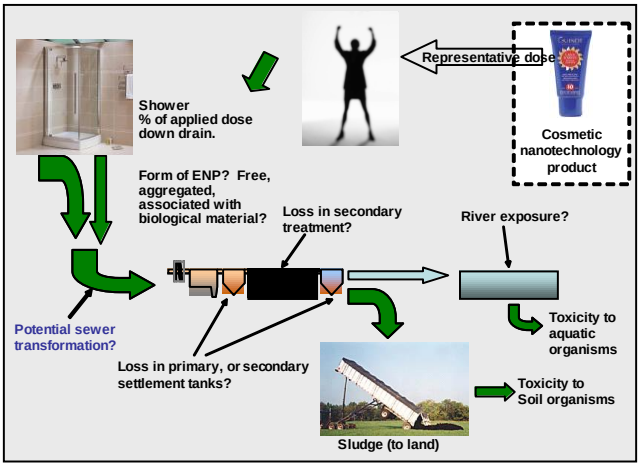

Fig. 1. Schematic illustrating key issues concerning the disposal, fate and environmental release pathways of an example "down the drain" nanotechnology product (e.g. ZnO ENP containing sunscreen).

This includes for example, assumptions of complete release from products, no removal during waste treatment, long persistence of ENP as free particles, and high traffic volumes. Since these are clearly unrealistic, predicted environmental concentrations will be iteratively refined to include information on fate available in the literature and also from model system studies, such as those on ENP removal efficiency in sewage treatment works and fate in WP 2. This takes us beyond what has been done to date either with "unit world" type fugacity models, or with simple dilution factor models for ENPs supporting prediction of multimedia fate and exposure (Hollander et al., 2006; Sumpter et al., 2006; Hollander et al., 2007). For modelling of environmental concentrations in different compartments for our set of six ENPs under different usage scenarios, simulation approaches relevant to each release pathways will be used.

1. For CeO2 the assessment will focus on direct deposition of particles to soil. This work will be conducted using air dispersion modelling tools available within the Cambridge Environmental Research Consultants ADMS modelling suite. During model derivation, the ADMS model will be used to provide geospatial predictions of CeO2 concentrations in air and deposition to soil surface in relation to rates of traffic flow. Information for air will be useful for human health assessment and so will be made available to human health focused projects. Within NanoFATE, the information on deposition will be used to calculate concentrations in soil based on simple assumptions regarding distribution through only the top 5 cm of the receiving soil surface. This is based on well established knowledge of metal deposition and distribution in soils subject to particulate metal deposition from smelter stacks (e.g. Martin et al 1983) (NERC, F+B).

2. For both ZnO and Ag ENPs the major route of release to the environment is likely to be through the wastewater stream. A simple wastewater process model for each ENP will be

developed to predict quantities going to effluent, or sludge. Information on rates of sludge application to soils across Europe will be used to estimate concentrations reached via this route. For that which partitions into effluent, realistic water levels will be modelled using the GIS water quality model LF2000-WQX Wales (Williams et al., 2009). Predicted environmental concentrations (PECs) will be generated for a representative set of river catchments in the Thames, Midland and Anglia regions of the UK, which are known to have the least dilution of sewage effluent across the UK. These catchment scenarios will be compared with catchments across Europe in the GREAT-ER model. The model will be driven by consumption and discharge values together with wastewater fate. With its underlying database of wastewater treatment plants (location, size and flow) together with river hydrological data (all discharges, abstractions and natural flow), the LF2000-WQX model provides unparalleled ability to predict concentrations that may reach real environments (NERC, F+B).

The predicted environmental concentrations in different compartments derived from the modelling work for our selected ENPs under different usage scenarios will used in the project both to inform the design of toxicity studies in WP 3, WP 4 and WP 5 and as input into spatially explicit risk assessment models in WP 6.

Obj.3: Analyse ENP interactions with environmental and biological entities using advanced microscope and physical analysis.

Baseline. NanoSafe II (FP6 - which involved NanoFATE partners) has defined the current state-of-the-art for characterising and measuring ENP interactions with each other and with different biological model environments. The project used industrially supplied ENPs in model systems (e.g. cells) to determine their toxicities and demonstrated that understanding the shape and composition of ENPs and how they behave in different media is critical to understanding their potential toxicity (NanoSAFEII, 2008). Currently a major barrier to extending this work to more complex environments is the ability to differentiate ENPs from naturally occurring NPs or clusters.

NanoFATE progression beyond the "state-of-the-art". The NanoFATE consortium will address ENP interactions with environmental and biological systems by specifically mobilising the expertise of researchers with extensive experience of preparing real environmental and biotic samples for analysis of the interactions of ENPs with, for example, natural colloids and bacterial cells in wastewater and soil pore water. A range of the advanced techniques suitable for detection of the commercially available and bespoke manufactured and doped ENPs will be used for the specific studies in NanoFATE. These will allow NanoFATE researchers to track the interaction of particle with colloidal and particulate matter, since these interactions are important determinants of particle bioavailability. Methods that also allow determination of the uptake and localisation of ENPs within prokaryotic and eukaryotic organisms will also be utilised. The major techniques that will be used in the studies in NanoFATE are as follows:

1. Raman microscopy for the detection of ENP behaviour both in waste water systems and in biological entities including the internalisation of particles in prokaryotic and eukaryotic organisms (Huang et al., 2004;Singer et al., 2005) (UOXF.DJ, NERC);
2. Light, X-ray and neutron scattering spectroscopy for detection of ENP-ENP and ENP-colloidal interactions in waters and assessing the role played by colloids in facilitating particle aggregation in waste and surface waters (Jarvie and King, 2007) (NERC);
3. Electron microscopy techniques such as scanning Electron Microscopy (coupled with Energy-Dispersive X-ray analysis (ESEM-EDX) and Transmission Electron Microscopy (TEM-EDX) and Energy Dispersive X-ray analysis for visualisation of ENP interactions with environmental media, aquatic colloids and biological entities in support of assessment of ENP bioavailability in soil and water systems and the detection and localisation of internalised ENP in organisms (CU, UOXF.DJ)
4. Matrix Assisted Laser Desorption/Ionization (MALDI)-Imaging mass spectrometry for detection of surface interactions of ENP with particulate matter and possibly also imagine of tissues for metal ENPs inclusions (UOXF.DJ, UNIPMN).
5. Flow Field-Flow Fractionation with high resolution ICP-MS (FLFFF-HR-ICP-MS) including use of a new detection mode. This detection method, called single particle ICPMS, built on an ultra fast (<1ms) scanning of the elemental signal for a single element of interest. For most of the time there is no signal during the short acquisitions but when there is a nanoparticle which homogeneously consists of the element of interest then there is a high signal spike. For dilute samples this method enables detection of single nanoparticles, and quantification of the number of nanoparticles by counting the number of spikes. The method has been successfully used as a stand-alone screening method for filtered samples and as a detection mode after FFF to derive number based size distributions. This method has been used for detection of metal ENPs in Gothenburg wastewater treatment plant effluent (UGOT).

The use of fluorescence labelling and detection by fluorescence microscopy is not at the present time a feasible option for ENPs relevant to the types that NanoFATE will focus upon. Work outside NanoFATE, using approaches such as incorporating a rhodamine dye in the silica shell of certain ENPs may provide new approaches for fluorescence detection and subsequently valuable information in due course. Such developments will be monitored by the NanoFATE consortium and exploited should they provide new methods that are an improvement over the developments made within NanoFATE.

Meeting this objective will allow us to study interactions through the post production life cycle of ENPs, and simultaneously assess how the properties of ENPs may change over their environmental lifecycle. The data obtained in these studies will be used to inform the design of studies that are intended to track ENP fate during wastewater treatment process or following the deposition of diffuse ENP directly to soil ecosystems in WP 2.

Obj.4: Study ENP fate and behaviour through wastewater treatment processes and in soils.

Baseline. Published studies on the environmental fate of oxide NPs have focused mainly on transport through porous media (groundwater/soils) and will be useful to an extent in NanoFATE. Despite the fact that wastewater discharges provide a major route for emissions of oxide NPs in cosmetic/personal care products to the environment, there has been very little attention focused on their fate during wastewater treatment (Chang et al., 2007).

Clearly such studies are vital to frame environmental hazard and risk.

NanoFATE progression beyond the "state-of-the-art". NanoFATE will improve current understanding in relation to ENP behaviour during wastewater treatment by providing the following information relating to ENPs post release fate that will support predictions of ENP concentrations delivered to waters via discharges and to soil via sludge disposal.

1. Examination of the colloidal behaviour of ENPs in real wastewater matrices using small angle neutron scattering to directly quantify, in real time, ENP partitioning during primary (settlement) treatment, between (i) non-settleable constituents which continue through the effluent stream to secondary treatment, and (ii) sewage sludge which settles out within typical residence times of approximately 2 – 6 hours in primary settlement tanks (NERC, UGOT).
2. Distribution of tagged ENPs in flow-through test reactors installed at a UK sewage works and using real activated sludge feed. Analysis of the aqueous and solid phases for the tagged ENP would be done by ICP-MS and fluorescence or SQUID magnetometry (IHPP, NERC).
3. Use of scanning and transmission electron microscopy and dynamic light scattering techniques to measure changes in aggregate size, shape and fractal dimension of ENPs to characterise the nature and mechanisms of ENP flocculation during wastewater treatment (UOXF.DJ). Also IHPP has excellent field emission scanning microscope Leo1530 that could be employed here.
4. Use of scanning and transmission electron microscopy and nanoparticle visualisation techniques (e.g. NanoSight) to measure changes in ENP size and aggregation in different soil pore water and wastewater extracts to provide estimates of ENP dissolution rates (UOXF.DJ, UGOT).

The data derived from the studies conducted above will be used to refine the estimates of exposure conducted in the risk assessment phase of the project. Additionally, the data on dissolution rates will be used to support later detailed measurements of ENP bioavailability as particles or as free, colloidal bound forms during ecotoxicity testing in studies conducted in different environmental media in WP 4.

Obj.5: Determine the chronic toxicity of ENPs of different properties, including co-exposures with other stressors (e.g. UV and combustion derived pollutants).

Baseline. To date, published data concerning the effects of ENP *in vivo* are principally restricted to acute toxicity tests (Handy et al 2008; Luoma 2008). Chronic toxicity data are mostly lacking. Furthermore, since the available studies each used a different ENP with different characteristic, it is difficult to compare these data directly. Another issue that is often highlighted (Royal Commission on Environmental Pollution, 2008; Luoma 2008), but to date remains poorly investigated is that of co-exposure of ENP with other pollutants and/or environmental stressors. Both have the potential to lead to greater than additive effects through processes, such as facilitating pollutant transport by ENPs (AKA piggybacking) and ROS generation (Baun et al. 2008).

NanoFATE progression beyond the "state-of-the-art". The knowledge gaps concerning ENP effects highlighted above indicate the pressing need to provide more detailed information on aspects of ENP toxicity. These include issues such as the relative sensitivities of species, acute-to-chronic ratios, the effects of ENP properties on toxicity, and the interactive effects of ENP with other co-stressors. NanoFATE will deliver such information by the following studies.

1. Literature review of data on ENP ecotoxicity for aquatic and terrestrial species. This will include information of the characteristics of the particles used for testing, the physicochemical properties of the test medium and the nature of the dose response relationship for different endpoints. The data set will be enhanced by our own studies of chronic toxicity on our selected set of ENPs in species from both aquatic (microorganisms as biofilm communities, algae, *Daphnia*, mussel) and terrestrial (nematode, springtail, earthworm, woodlouse) organisms (NERC, VUA, UAVR).
2. Establishing whether UV co-exposure affects toxicity in selected species *in vivo* for ZnO ENPs in *Daphnia*. This will build on work that has established that the cytotoxicity of some UV absorbing ENPs is mediated through radical oxygen species generation and is enhanced in the presence of UV light in mammalian cells (Sayes et al., 2006) and bacteria (Adams et al., 2006) (UAVR).
3. Assessing whether the ability of ENPs to bind and transport other molecules into biological systems modifies the toxicity of co-occurring pollutants, as shown previously for polycyclic aromatic hydrocarbon in the presence of sucrose polyester ENPs (Moore et al., 1997). While relevant to all the selected ENPs it is especially of concern for CeO_2 ENPs, which may serve to co-transport other combustion pollutants into biota. This will be addressed by taking a multiple exposure approach and analysing if the combinations of CeO_2 ENP with associated PAHs lead to higher uptake and effects than should be observed from the two components in isolation (UNIPMN, VUA, NERC).

The exposures to be conducted will utilize a range of environmentally relevant species in different exposure media and will measure a range of endpoints, thereby improving the current state-of-the-art. Variables such as aggregation and dissolution of ENPs will be monitored in the test media using qualitative and quantitative methods. Our experiences will also allow us to recommend refinements to existing ecotoxicity test protocols for ENP studies and will provide information that can be used to investigate approaches for calculating predicted no-effect concentrations in WP 6.

Obj.6: Establish and model how environmental physico-chemical properties in wastewater, natural waters and soil govern ENP parameters such as stability, soil–solution partitioning, downward transport and transformation (e.g. dissolution) that each may ultimately affect bioavailability to organisms.

Baseline. The properties of the selected ENPs will be characterised in detail (in WP 1); however, the consequences of these properties for behaviour of the ENPs in the natural environment (e.g. aggregation/dispersion, association with natural organic matter, binding to suspended sediments and soils, dissolution rates) have so far not been studied. Although knowledge of the behaviour of natural metal oxides suggests that chemical factors (e.g. dissolved organic matter, pH, ionic strength) should influence the stability of metal oxide ENP, the bioavailability of ENPs to organisms has only

been studied in simple or environmentally unrealistic systems, and it is unknown how these factors affect ENP uptake and toxicity. Work has been published showing that both pH and the presence of naturally occurring macromolecules can influence the dissolution and aggregation of ENPs and it is likely that these affects may change bioavailability (Baalousha, et al. 2008; Diegoli, et al. 2008).

NanoFATE progression beyond the "state-of-the-art". In NanoFATE we will address the role of water and soil physicochemical properties and particle characteristics by determining the magnitude of ENP effects for key organisms exposed to different particle types and under different environmental conditions. Specifically we will adopt the following approach.

1. Conduct tests to measure the toxicity of a selected set of ENPs in a set of soils and waters of known physicochemical properties (VUA, UAVR, CU).
2. Account for the role of dissolved metal in toxicity, by linking information on dissolution rates to predictions of free metal ion concentration using the Windermere Humic Acid Model (WHAM) (Tipping 1984) or empirical relationships with either the free ion activity model (Morel 1993), free ion effective dose model (Lofts et al. 2005, 2006) or biotic ligand model, as a prediction of available exposure and associated effect (DiToro et al. 2001) (NERC, VUA, UAVR).
3. Quantify additional toxicity (if any) beyond that predicted to be caused by the free metal ion.
4. Use multivariate statistical methods such as principal component analysis and partial least squares regression to investigate the relationships between ENP derived toxicity and soil and water chemistry (VUA, NERC, UAVR).
5. Investigate the use of rate transfer constants as a means to account for dissolution and the subsequent transfer of the causation of toxicity from ENP to free metal ion forms (VUA, NERC).

Meeting this objective will require integrative working among ecotoxicologists and environmental and physical chemists. We will need to quantify how physical properties of ENPs change with time in diverse chemical environments and how this affects ENP exposure. The information derived from these studies will allow us to modify assessments of risk in receiving waters and soils made in WP 6.

Obj.7: Establish the mechanisms of uptake, internal trafficking and toxicity of ENPs.

Baseline. To date, information on the toxicokinetics of ENPs is very sparse. Very little is known of their uptake, internal trafficking and distribution and the effects of ENP properties on these parameters. This is despite the fact that these aspects are important to understand mechanisms of action and long-term effects of ENPs.

In relation to mechanisms of toxicity, some observations do indicate that nanoscale materials used in biomedical and pharmaceutical research may modulate the expression of cancer genes (Omidi et al., 2003), and genes involved in cell signalling (Regnstrom et al., 2006). For ENPs, recent studies have indicated genotoxicity and cytotoxicity in cultured human cells and generation of pulmonary fibrosis and lung tumours in rats (Wang et al., 2007). Such effects have, however, only recently been studied in aquatic organisms (see review of Moore, 2006; also Klaper et al. 2009; Shinohara et al. 2009) and we know of no published genotoxic studies in terrestrial invertebrates (although NERC have submitted a paper on ENP immunotoxicity in earthworms) and only a single molecular toxicity study for terrestrial plants (Lee et al. 2009).

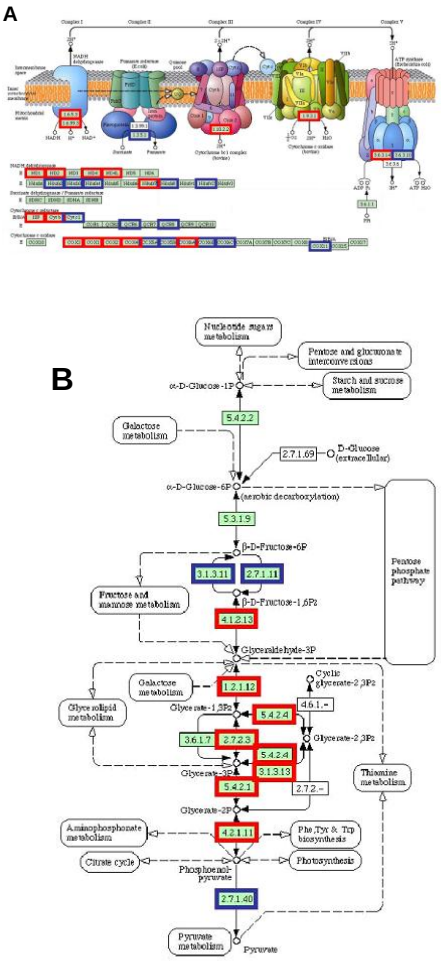

Fig. 2. Example analysis of the impact of ionic copper on oxidative phosphorylation (A) and on glycolysis/gluconeogenesis (B) of earthworms in a pathway based visualisation of the mechanism of toxicity. Transcripts outlined in bold are represented on the utilised microarray, those with < 2 fold change following copper exposure are outlined in blue.

NanoFATE progression beyond the "state-of-the-art". Since extensive studies on tissue and cellular localization and the mechanisms of action of ENPs remain lacking in aquatic and terrestrial species, NanoFATE will progress these aspects using a number of techniques that have been developed and used previously for conventional chemical assessment. To assess uptake and elimination, methods to both directly measure and also infer toxicokinetic parameters will be applied (see WP5.1 for details). Mechanisms of action will be investigated using a systems toxicology approach, which has proved valuable for the unbiased characterisation of the molecular basis of the toxicity of PM10 /

UFPs (Karoly et al., 2007) and ENPs in macrophages (Long et al., 2007; Xiao et al., 2003). This systems toxicology approach has never been applied for ENPs in organisms exposed to chronic ENP concentrations *in vivo*, although consortium members have applied the approach to assessing metal ion toxicity in a range of species (see Fig. 2 for example), which has the potential to reveal novel insights on the nature of chronic effects. Specific studies will comprise:

1. Time series studies of effects of ENPs on lifecycle parameters of species where full lifecycle data can be obtained (e.g. *Daphnia*, nematodes, springtails). This data will be used to parameterise the physiologically based model DEBtox (Kooijmann and Bedaux, 1996, Jager et al. 2003) to predict parameters relating to energy dynamics and ENP toxicokinetics (VUA, NERC, UAVR).

2. Electron microscopy of cryo-sectioned preparations from time series exposures to identify major uptake routes and gross tissue distributions of ENPs in earthworms using energy dispersive x-ray analysis (Cotter-Howells et al., 2005). This will provide information on the internal distribution of ENP in major organs (CU, UOXF.DJ).

3. The use of Raman spectroscopy to chart signatures of the interaction between ENPs in unicellular organisms (Huang et al., 2004;Singer et al., 2005) and also in the cells in body fluid samples from larger organisms (earthworms and/or mussel) (UOXF.DJ).

4. Measurement of biomarkers relevant to known modes of action of ENPs (e.g. genotoxicity, immune function and ROS production assays) (Long et al., 2006; Nel et al., 2006; Xia et al., 2006) to evaluate the cellular, organelle and molecular effects of ENPs in earthworms (Svendsen and Weeks, 1997; Svendsen et al., 1998) and mussels (Dagnino et al. 2007) (UNIPMN, CU).

5. Transcriptomics studies to directly compare gene expression responses following exposure to bulk material/ free metal ion and a variant ENP. Established microarray technologies for *Caenorhabditis elegans* (Reichert and Menzel 2005; Menzel et al. 2007) and *Folsomia candida* (Nota et al. 2008), along with a full genome earthworm (*Lumbricus rubellus*) microarray and extended feature *Mytilus* microarray developed, based on results of an ongoing sequencing programs will be used (Dondero et al., 2006; Owen et al., 2008; Svendsen et al., 2008; Viarengo and Dondero, 2006). Pyrosequencing initiatives currently in progress at CU will also allow the use of a digital transcriptomic approach using Solexa–based tag sequencing technology to probe the transcriptome more deeply to identify changes in expression of low abundance genes. Bioinformatic support given within these existing sequencing programs will assist in identifying the pathways associated with ENP toxicity and will also allow inter-species comparisons through web-accessible integrated systems developed by UNIPMN in EU FP6 IP NoMIRACLE for the storage, meta-analysis, and retrieval of toxicogenomics datasets (CU, UNIPMN, VUA).

Obj.8: Develop risk assessment model(s) that integrate ENP fate, availability, accumulation and toxicity over the full post production lifecycle including provision of data for use in full lifecycle assessment.

Baseline. The current state-of-the-art approach to risk assessment relies on the use of generic data to derive predicted environmental concentrations (PECs) and on the use of toxicity data from standard tests at best within a species sensitivity distribution (Posthuma et al. 2001) or otherwise merely in combination with uncertainty factors of between 10 and 1000, to derive predicted no-effect concentrations (PNECs). While possibly suitable for predicting generic risks, this approach is rather simple, deterministic and provides no information on the spatial distribution of risk.

NanoFATE progression beyond the "state-of-the-art". To develop and refine approaches for the risk assessment of ENPs that potentially may allow a more robust and detailed assessment, in NanoFATE we will evaluate the applicability of advanced risk assessment tools for use with ENPs. These include models for predicting no effect concentrations based on the species sensitivity approach; bioavailability models that develop the biotic ligand model to also incorporate ligand binding associated surface charge of ENPs to account for ENP mediate toxic effects; a GIS–based model such as the Air Dispersion Modelling Systems; and EUSES and LF2000-WQX hydrological model for visualising ENP risk in receiving ecosystems including river catchments.

1. For assessing risk, both generically and in a spatial context, we will first predict concentrations of the ENPs in different environmental compartments. As outlined previously these will be derived using two spatial based modelling approaches. ADMS and LF2000-WQX are two well established models that can be used to study the distribution of chemicals in air and surface water respectively. ADMS is an industry standard air pollution model that is well suited for modelling pollutant dispersion from road vehicle sources. EUSES and LF2000-WQX are chemical fate models, with EUSES being the current industry standard and LF2000-WQX an advance coupled hydrological and chemical discharge model that can be used to predict the spatial concentrations of chemicals in river systems. Each of these models has the potential to become established tools for predicting environmental concentrations of ENPs in air and water. Assessment for our selected ENPs with our different usage scenarios will start with a worst case assessment. We will progressively update PEC and PNEC values to the risk assessment model as we gain more data and understanding of ENP fate from the tracking studies conducted using particles synthesised and characterised in WP 1 and tracked within real systems in WP 2 (NERC).

2. To derive a suitable PNEC, we will examine the issues surrounding the application of the species sensitivity distribution approach for ENPs. Given that ENPs may have an infinite variety of physical properties it is not immediately clear that SSDs can be applied to ENPs even if only particles of the same core type are considered. Further it is not clear what exposure metric should be used (concentration, surface area, reactivity etc.). To establish the potential for applying SSDs and also to provide guidance on the selection of the exposure metric, we will analyse the collated data on ENP toxicity to identify patterns and trends within the data. Data can be retrieved from studies collated and available within the NAPIRAhub set of publicly available data resources. This will include studying correlations between ENP properties and toxicity, environmental properties and toxicity, and the influence of species–relevant traits including phylogeny and ecological traits (such as feeding mode, soft vs. hard bodied

organisms). On the basis of this analysis, we will seek to establish best practice for ENP PNEC generation, including identifying the most suitable dose metric. We will also define the operational limits of the SSD approach (NERC).

3. We will examine the relationship between PECs for receiving soils and an indicative PNEC derived from available toxicity data. From our studies of fate in soils (e.g. dissolution rates) in WP 2 and WP 4, information on the bioavailability and the relative toxicity and effect of CeO_2 in dissolved and nanoparticle form will be used to address issues relating to the relative contribution of ENP forms to toxicity. Information on bioavailability will be built using models developed in WP 4 that will build on the biotic ligand model and also information on particle properties including surface charge and dissolution. Such information will be of fundamental importance to the development of the concept of ecologically responsible design of nanotechnology products and is a key project outcome.

4. To visualise spatial risks for ZnO and Ag ENPs, usage scenario data, hydrological data, relevant literature information and experimental results on exposure and toxicity will be used to parameterise catchment based spatial risk assessments for a selection of UK river catchment and three indicative European catchments. The approach developed builds on that for endocrine disrupting chemicals to support the spatial assessment of risk (see Sumpter et al., 2006 and Fig. 3 for specific examples). Spatially explicitly risk maps for a range of catchments under normal and extreme flow conditions will be developed for a range of usage scenarios. If suitable insight is gained from studies of ENP physicochemistry, bioavailability and uptake mechanisms, the model will be updated to consider the effects of water chemistry on particle fate and on exposure and effects in organisms.

5. NanoFATE specifically addresses the fate, effects and associated risk of ENPs during their use phase. However, the consortium also recognises that the collected data is also highly relevant to studies that seek more comprehensive and high level lifecycle assessments for nanotechnology products. To allow researchers in the LCA community to utilise NanoFATE data, applicable project data will be collected within data holdings in a manner compatible for use in lifecycle analysis as set out in the International Life Cycle Data System (ILCD) Handbook. To support exchange of data with the LCA community, NanoFATE has included experts in LCA within the project advisory board. Prof. Sverker Molander from Chalmers Institute of Technology in Göteborg is a LCA expert who has been working in the area of nanotechnology LCA, with a particular focus on metal and metal oxide ENPs. Prof. Molander has been approached (and has agreed) to provide input into the development of LCAs based on NanoFATE data holdings and also to work within NanoFATE to ensure the compatibility of NanoFATE studies with national and international LCA guidelines and projects.

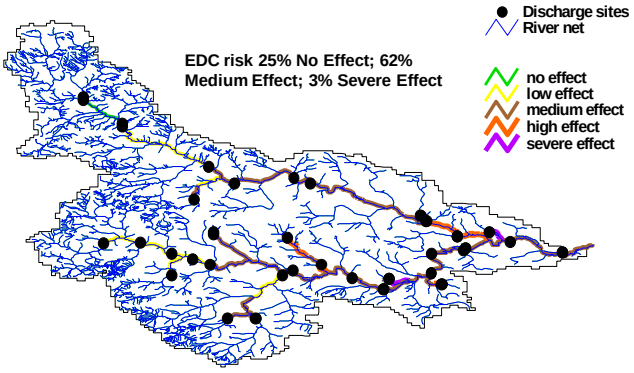

Fig 3. Catchment risk map of predicted endocrine disruption of fish from effects of oestrogenic chemicals for the Aire and Calder rivers, Yorkshire, UK.

Obj.9: Improve stakeholder understanding of ENP risks.

Baseline. Due to current uncertainties, public perception of the risks from nanotechnology could represent a barrier to the safe and sustainable development of the sector, even if ultimately the nature of such risks actually turned out to be rather limited. One thing that is missing from the nanotechnology debate is scientifically robust case studies that can be utilised as tools to communicate the real risk of potential adverse effects. Such studies can provide both a means to facilitate understanding within the regulatory community and also if correctly presented, effective platforms for discussion of actual risks for real world situations.

NanoFATE progression beyond the "state-of-the-art". By conducting a comprehensive scientific assessment of the fitness for purpose of existing risk assessment approaches and techniques for estimating ENP risks in real environments, NanoFATE will establish the state-of-the-art for evidence-based ENP risk assessment. Developed tools for assessment will be communicated to national and EU based responsible authorities and stakeholders to encourage adoption and exploitation through conference presentations, user-friendly reports and information (on WWW), webinars, and formal scientific outputs. A project newsletter will be produced biannually. For the regulatory and policy maker audience, we will prepare project briefing notes and offer presentations given by the Coordinator or appropriate selected partners to key international and national agencies. This material will be developed in collaboration with Advisory Board members from the regulatory community (National Environment Agencies) and also the Commission (as appropriate). Further the NanoFATE team will play a full and active part within the newly inaugurated NANOSAFETY cluster that has been developed at the EU level to establish a network of experts that are involved in (EU-) projects focused on the health and safety aspects of Nanotechnology. This will ensure NanoFATE is able to work with other EU projects to meet NANOSAFETY cluster objectives regarding consensus, effective communication and discussion, and avoidance of overlap in ENP studies.

To provide industrial stakeholders and the general public with appropriate knowledge on the risks of ENPs and nanomaterials for human health and the environment, we will also submit articles to the industrial press. Provision of information to the public in an easily understandable form will be an important part of the communication process. Because we will have data from

specifically designed and systematically conducted studies, we will be in a strong position to provide coherent information to the public on this debate. This will open up understanding not only of the nanotechnology area, but also of the risk assessment approaches, their inherent assumptions and their precautionary nature. Again, links with the NANOSAFETY cluster will ensure that consistent messages regarding these aspects are delivered to regulators, industry and the wider public.

5 Progress and Outcomes to date

Work on all nine of the main NanoFATE S&T objectives (see above), is being finalised in the run up to the end of the project. For more details than the summary below and for access to completed public deliverables, updates and subscription to newsletters, please use the website www.nanofate.eu .

WP 1. Characterisation and tracking of ENPs during processes involved in fate and toxicity.

The major deliverables here related to provision of high quality well characterised particles for the remaining project partners. A larger than planned range of commercial ENPs were characterised and assessed so that supply was consistent and without significant batch to batch variation issues. Consequently the final set of NanoFATE commercial particles were:

- The main ZnO particles are 30nm Nanosun from micronisers in Australia, with matching tagged ZnO ENP by IHPP, with some work on BASF z-cote and z-cote HP1 ZnO
- Amepox 3-8nm Ag ENP and a 50nm Ag NP from NanoTrade
- CeO_2 will be the Envirox or Antaria fuel additive and most likely a polishing agent from Umicore.

WP 2. ENP environmental behaviour and fate modelling

Have identified and prioritised specific properties that need principal consideration during the development, adaptation and validation of environmental fate models for nanoparticles (D2.1). They have, based on this, developed and validated fate models (with WP6) and supplied the CeO2 deposition in soils and the influent to and discharge (effluent and sludge) from sewage treatment works of nano ZnO and Ag (D2.7 & D2.8). Important parameter for soil and water PEC estimation have been identified (D2.6) and the behaviour of Ag particles in soil has been studied utilizing novel FAST spICP-MS-base approaches (D2.4).

WP 3 ENP Ecotoxicology

We have developed improved standard ecotox exposure protocols, principally adjusting properties of test media, media renewal frequencies and soil and food spiking methodologies, to ensure relevant and homogenous presentation of nanoparticles during toxicity testing. Employing these improved protocols the exposures needed for the hazard assessment has been completed. Chronic testing of our particles (D 3.2 and D 3.3) and the work to deliver the data for WP4 on bioavailability drivers has been completed. Samples have been archived for use in WP 5 (M 3.2) and data has been collated and used in ecotoxicological threshold estimation (D3.4 & M3.3). Mixture toxicity models have been used to determine combined effects of ENP, physiochemical and organic pollutant stressors (D3.5).

WP 4 ENP bioavailability - relations between soil and water chemistry and particle properties

Collected and databased all available information from literature, conferences and other projects and conducted a critical review of this available data and its quality. This identified which environmental factors have the greatest proven effect on the bioavailability and toxicity of nanoparticles to organisms living in soil and water. Based on this l bioavailability trials testing for pH, organic matter and cation effects have been implemented within WP3, plus additional long-term (12 months) exposures addressing ageing are on-going. A database of results for ENP exposure across soil and water types has been completed (D 4.2). Research reports on ENP property property-effect relationships to address confounder effects on bioavailability (D 4.3 and M 4.2) have been complete. Additionally, four manuscripts focussing on the interplay between soil and water chemistry with ENP properties and resulting effects on ENP physical presentation, bioavailability and toxicity (D4.4) have been produced.

WP 5 ENP toxicokinetics and toxicodynamics

A well thought through tired approach to the tracking of ENPs in tissues has been developed and applied, allowing us to make the most of our technical abilities (and tissue samples) by ensuring that the high-end expensive low throughput techniques only to be applied to samples where we have good evidence ENPs are present. Samples have been run looking at biological markers of ENP and dissolved metal effects to develop the knowledge of signatures of possible ENP tissue damage. An agreed data structure has been developed for this systematic data to allow later cross species comparison, and the format has been kept flexible to enable adaptation to the Cluster database when agreed. Toxicokinetic studies have been completed for ENPs in soil and aquatic invertebrates (D 5.5). Miami compliant dataset for gene expression in earthworm, nematodes and mussels in response to ENP exposure have been produced (D5.6) and a number of research manuscripts detailing systems toxcicology of ENPs have also been developed (D5.8). QualityNano facilities have been utilised to complete these studies (D 5.5 and D 5.8). The results of work package 5 have been presented and discussed in a well attended open workshop on the mechanistic toxicology of ENPs (D5.7).

WP 6 Integrated risk assessment

To assess ENP production and product incorporation estimation a report was produced based upon a review of the peer-reviewed as well as grey literature (reports from R&D projects, reports to governmental authorities, etc.) on production volumes of the three ENPs and reported (predicted) environmental concentrations in surface water, STP effluents, soils and sediments (D6.1) . Pan-European maps of predicted soil contamination (D 6.2), surface water levels (D 6.3) have been generated along with the first iteration of risk visualization for these habitats (M 6.4). These risk maps have been further refined using a species sensitivity distribution approach and incorporating different usage scenarios (D6.4, D6.5 & D6.6). A critical appraisal of available hazard and exposure assessment methodologies, the identification of vulnerable sp3ecies and environmental compartments and a

gap analysis of available data is due for completion in March 2014 (D6.7).

WP 7 Dissemination and Training

The NanoFATE web site and the interlinking of this with the e-based Newsletters has proven very successful in terms of bringing our work to the attention of the wider stakeholder community (see www.nanofate.eu and subscribe to the newsletters).

In terms of training NanoFATE has an inherent large training and capacity building element in that we directly have created 14 PhD or Post Doctoral positions across Europe. There are further indirectly associated students and fellows working closely with the NanoFATE partners. For the wider community two NanoFATE open PhD training workshops have been held (D7.14 and D7.15).

6 Expected Impact

NanoFATE provides robust tools, techniques and knowledge needed by stakeholders to understand and communicate risks associated with ENPs of different physical or chemical properties, including their environmental interactions and toxicity. Dissemination is done through a wealth of channels and activities, but centred around interactive e-Newsletters and the project website www.nanofate.eu.

In terms of direct engagement with stakeholders NanoFATE has organized with the fellow NanoSafety Cluster projects NanoRetox, Ennsatox, NanoTOES, NanoMILE, NanoImpactNet, Marina and QualityNano EU Cluster meetings and training events through the course of the NanofATE project. The focus of these activities has to link environmental fate and ecotoxicology aspects within EU NanoSafety Cluster projects. Additionally, there have been numerous presentations of the NanoFATE work to international conferences and workshops, and more than 20 peer reviewed papers have been published with a further 30 papers likely to come out. Furthermore, NanoFATE participated in a NanoRETOX regulator and industry focused workshop on the 11th of Sept 2012 in London and NanofATE progress was disseminated during an EU workshop on the 2nd Regulatory Review of Nanomaterials. Through the latter half of the project NanoFATE representatives have contributed to the OECD Working Party on Manufacture Naniomaterials through surveys, workshops and expert meetings. An ongoing bilateral dialogue between NanoFATE and nanoparticle team leaders within ECHA has been established to facilitate addressing the main questions of concern that ECHA have with regard to these materials. As a result of these discussions the NanofaTE coordinator has been invited to be a member of the Scientific Committee for a ECHA organised Topical Scientific Workshop on Nanomaterials; to be held in October 2014.

7 Directory

Table 1 Directory of people involved in this project.

First Name	Last Name	Affiliation	Address	e-mail
Thomas	Backhaus	Gothenburg University	Dep. Of Plant and Env. Sciences, Göteborg University, Carls Skottsbergs Gata 22B, Box 461, 40530 Göteborg, Sweden	thomas.backhaus@bioenv.gu.se
Mike	Bowes	Natural Environment Research Council	Centre for Ecology and Hydrology, Maclean Building, Benson Lane, Crowmarsh Gifford, Wallingford, Oxfordshire, OX10 8BB, UK	mibo@ceh.ac.uk
Filipa	Calhoa	University of Aveiro	Department of Biology, CESAM, Universidade De Aveiro, Campus Universitário De Santiago, 3810-193 Aveiro, Portugal	fcalhoa@ua.pt
Alison	Crossley	Oxford University	Department of Materials, Oxford University Begbroke Science Park, OX5 1PF, UK	alison.crossley@materials.ox.ac.uk
Maria	Diez Ortiz	Natural Environment Research Council	Maclean Building, Benson Lane, Crowmarsh Gifford, Wallingford, Oxfordshire, OX10 8BB, UK	marezo@ceh.ac.uk
Francesco	Dondero	Università degli Studi del Piemonte Orientale Amedeo Avogadro	Dept. Environmental & Life Science, Faculty of Science, University of Piemonte Orientale, Via Michel 11, 15100 Alessandria, Italy	fdondero@unipmn.it
Egon	Dumont	Natural Environment Research Council	Centre for Ecology and Hydrology, Maclean Building, Benson Lane, Crowmarsh Gifford, Wallingford, Oxfordshire, OX10 8BB, UK	egdu@ceh.ac.uk
Michael	Faust	Faust & Backhaus	BITZ (Bremer Innovations- und Technologie-Zentrum), Fahrenheitstrasse 1, D-28359 Bremen, Germany	faust@fb-envico.com

First Name	Last Name	Affiliation	Address	e-mail
Julian	Gallego	Gothenburg University	Dep. of Chemistry , Göteborg University, SE-412 96 Göteborg, Sweden	Julian.gallego@chem.gu.se
Emma	Gozzard	Natural Environment Research Council	Centre for Ecology and Hydrology, Maclean Building, Benson Lane, Crowmarsh Gifford, Wallingford, Oxfordshire, OX10 8BB, UK	emgo@ceh.ac.uk
Martin	Hassellov	Gothenburg University	Dep. of Chemistry , Göteborg University, SE-412 96 Göteborg, Sweden	martin.hassellov@chem.gu.se
Helen	Jarvie	Natural Environment Research Council	Centre for Ecology and Hydrology, Maclean Building, Benson Lane, Crowmarsh Gifford, Wallingford, Oxfordshire, OX10 8BB, UK	hpj@ceh.ac.uk
Andrew	Johnson	Natural Environment Research Council	Centre for Ecology and Hydrology, Maclean Building, Benson Lane, Crowmarsh Gifford, Wallingford, Oxfordshire, OX10 8BB, UK	ajo@ceh.ac.uk
Colin	Johnston	Oxford University	Department of Materials, Oxford University Begbroke Science Park, OX5 1PF, UK	colin.johnston@materials.ox.ac.uk
Monika	Juergens	Natural Environment Research Council	Centre for Ecology and Hydrology, Maclean Building, Benson Lane, Crowmarsh Gifford, Wallingford, Oxfordshire, OX10 8BB, UK	mdj@ceh.ac.uk
Kerstin	Jurkschat	Oxford University	Department of Materials, Oxford University Begbroke Science Park, OX5 1PF, UK	kerstin.jurkschat@materials.ox.ac.uk
Virginie	Keller	Natural Environment Research Council	Centre for Ecology & Hydrology, Maclean Building, Benson Lane, Crowmarsh Gifford, Wallingford, Oxfordshire, OX10 8BB	vke@ceh.ac.uk
Peter	Kille	Cardiff University	Main Building, PO Box 915, Park Place, Cardiff, CF10 3TL, Wales, UK	kille@cardiff.ac.uk
Pauline	Kool	VU University, Amsterdam	Department of Animal Ecology, Institute of Ecological Science, Vrije Universiteit, De Boelelaan 1085, 1081 HV, Amsterdam, The Netherlands	Pauline.kool@falw.vu.nl
Stephen	Lofts	Natural Environment Research Council	Centre for Ecology and Hydrology, Lancaster Environment Centre, Library Avenue, Bailrigg, Lancaster, LA1 4AP, UK	stlo@ceh.ac.uk
Witold	Lojkowski	Institute of High Pressure Physics	Instytut Wysokich Ciśnień PAN, Sokołowska 29/37, 01-142 Warszawa, Poland	wl@unipress.waw.pl
Susana	Loureiro	University of Aveiro	Department of Biology, CESAM, Universidade De Aveiro, Campus Universitário De Santiago, 3810-193 Aveiro, Portugal	sloureiro@ua.pt
Marianne	Matzke	Natural Environment Research Council	Centre for Ecology and Hydrology, Maclean Building, Benson Lane, Crowmarsh Gifford, Wallingford, Oxfordshire, OX10 8BB, UK	martzk@ceh.ac.uk
Claire	Mays	SYMLOG France	262 Rue Saint-Jacques. 75005 Paris, France	maysclairenanofate@gmail.com
John	Morgan	Cardiff University	Main Building, PO Box 915, Park Place, Cardiff, CF10 3TL, Wales, UK	morganaj1@cardiff.ac.uk
Andrew	Moscicki	Amepox	90-268 Łódź, ul. Jaracza 6, Poland	amepox@amepox.com.pl
Agnieszka	Opalinska	Institute of High Pressure Physics	Instytut Wysokich Ciśnień PAN, Sokołowska 29/37, 01-142 Warszawa, Poland	agnieszka.opalinska@gmail.com
Daniel	Read	Natural Environment Research Council	Centre for Ecology and Hydrology, Maclean Building, Benson Lane, Crowmarsh Gifford, Wallingford, Oxfordshire, OX10 8BB, UK	dasr@ceh.ac.uk

First Name	Last Name	Affiliation	Address	e-mail
Fabianne	Ribeiro	University of Aveiro	Department of Biology, CESAM, Universidade De Aveiro, Campus Universitário De Santiago, 3810-193 Aveiro, Portugal	ribeiro.f.@ua.pt
Ilenia	Saggese	Università degli Studi del Piemonte Orientale Amedeo Avogadro	Dept. Environmental & Life Science, Faculty of Science, University of Piemonte Orientale, Via Michel 11, 15100 Alessandria, Italy	ilenia.saggese@mfn.unipmn.it
Nina	Scheider	SYMLOG, France	262 Rue Saint-Jacques. 75005 Paris, France	Nina.schneider.nanofate@gmail.com
Darek	Smolen	Institute of High Pressure Physics	Instytut Wysokich Ciśnień PAN, Sokołowska 29/37, 01-142 Warszawa, Poland	dareksmolen@wp.pl
Amadeu	Soares	University of Aveiro	Department of Biology, CESAM, Universidade De Aveiro, Campus Universitário De Santiago, 3810-193 Aveiro, Portugal	asoares@ua.pt
Joanna	Sobczyk	Institute of High Pressure Pysics	Instytut Wysokich Ciśnień PAN, Sokołowska 29/37, 01-142 Warszawa, Poland	jsobczyk@unipress.waw.pl
David	Spurgeon	Natural Environment Research Council	Centre for Ecology and Hydrology, Maclean Building, Benson Lane, Crowmarsh Gifford, Wallingford, Oxfordshire, OX10 8BB, UK	dasp@ceh.ac.uk
Claus	Svendsen	Natural Environment Research Council	Centre for Ecology and Hydrology, Maclean Building, Benson Lane, Crowmarsh Gifford, Wallingford, Oxfordshire, OX10 8BB, UK	csv@ceh.ac.uk
Cameron	Taylor	Oxford University	Department of Materials, Oxford University Begbroke Science Park, OX5 1PF, UK	Cameron.taylor@materials.ox.ac.uk
Ladislav	Torcik	NanoTrade	Mozartova 178/12, 779 00 OLOMOUC, Czech Republic	torcik@nanotrade.cz
Paula	Tourinho	University of Aveiro	Department of Biology, CESAM, Universidade De Aveiro, Campus Universitário De Santiago, 3810-193 Aveiro, Portugal	paulatourinho@ua.pt
Jani	Tuoriniemi	Gothenburg University	Dep. of Chemistry , Göteborg University, SE-412 96 Göteborg, Sweden	Jani.tuoriniemi@chem.gu.se
Bill	Tyne	Natural Environment Research Council	Centre for Ecology and Hydrology, Maclean Building, Benson Lane, Crowmarsh Gifford, Wallingford, Oxfordshire, OX10 8BB, UK	wit@ceh.ac.uk
Kees	van Gestel	VU University, Amsterdam	Department of Animal Ecology, Institute of Ecological Science, Vrije Universiteit, De Boelelaan 1085, 1081 HV, Amsterdam, The Netherlands	kees.van.gestel@ecology.falw.vu.nl
Aldo	Viarengo	Università degli Studi del Piemonte Orientale Amedeo Avogadro	Dept. Environmental & Life Science, Faculty of Science, University of Piemonte Orientale, Via Michel 11, 15100 Alessandria, Italy	viarengo@unipmn.it
Lee	Walker	Natural Environment Research Council	Centre for Ecology and Hydrology, Lancaster Environment Centre, Library Avenue, Bailrigg, Lancaster, LA1 4AP, UK	leew@ceh.ac.uk
Richard	Williams	Natural Environment Research Council	Centre for Ecology and Hydrology, Maclean Building, Benson Lane, Crowmarsh Gifford, Wallingford, Oxfordshire, OX10 8BB, UK	rjw@ceh.ac.uk
Jacek	Wojnarowicz	Institute of High Pressure Physics	Instytut Wysokich Ciśnień PAN, Sokołowska 29/37, 01-142 Warszawa, Poland	wodorw@tlen.pl

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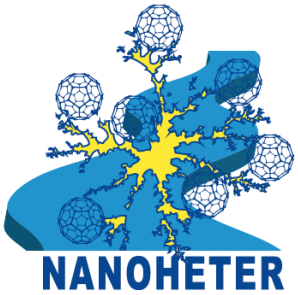
NANOHETER

Fate of engineered nanoparticles in the water column under natural conditions.

Role of the heteroaggregation with naturally occurring suspended matter.



Contract Agreement: NANOHETER ERA NET SIINN 2012
Coordinator: Dr. J. Labille, CNRS/CEREGE UMR 7330

Table 1 Consortium List.

No.	Beneficiary name	Short name	Country
1	Centre National de la Recherche Scientifique	CEREGE/CNRS	France
2	Eidgenössische Technische Hochschule	ETH	Switzerland
3	Bureau de Recherches Géologiques et Minières	BRGM	France
4	University of Wyoming	UW	USA

Contents

1 Summary

Project Duration: 36 months started on April, 1st, 2013

Project Funding: ERA-NET SIINN Call 2012

As part of the risk assessment of nanotechnology, this project deals with the exposure aspect of engineered nanoparticles (ENPs), focusing on their fate in surface water. Based on the trace concentrations expected to be present, the approach claims that the dispersion stability of ENPs in the water column is not a driving characteristic for their fate, but that their potential for interaction with the mineral and organic suspended matter occurring in surface water will be the governing factor. The aim of this project is to identify among these materials the potential carriers for ENPs. Mechanistic, holistic and model approaches are conducted together. The interaction of ENPs with surrounding materials are investigated, and the potentially induced heteroaggregation and/or sedimentation mechanisms are studied. The goal is to deliver a probability ranking of these potential scenarios that can be used to model the fate of ENPs in natural aqueous systems at the river scale.

2 Background

In assessing the risk posed by engineered nanoparticles and nanomaterials (ENPs), it is necessary to consider two related factors: toxicity and exposure. This project concerns mainly the exposure assessment of ENPs in surface water systems. This disciplinary field has to deal with the whole complexity of the natural systems that constitute potential receptacles for ENPs within their transfer through the environment once released.

Aqueous systems constitute a major way of transport for ENPs toward living organisms. An important first step in predicting ENP transport in complex aqueous systems, and thus accurately establishing the likelihood of exposure to these materials through the water column, is understanding their dispersion behaviour in these complex systems. Indeed, the colloidal stability for particulate matter largely determines its fate in the aqueous environment. We may classify possible scenarios into two ever-opposing tendencies, namely, dispersion of the nanoparticles

favouring their mobility, or aggregation, sedimentation and deposition, which limit their further transport. Many factors influence the balance between these opposing tendencies, both environmental and intrinsic to the ENPs themselves: (i) The intrinsic properties of the ENPs, and notably their surface properties, determine their affinity for the dispersing medium, and hence their tendency to agglomerate or disperse, depending for example on their hydrophilic or hydrophobic nature; (ii) The ionic strength and pH of the dispersing medium largely control the stability of the ENPs in suspension via interparticle electrostatic interactions; (iii) The interaction of the ENPs with surrounding dissolved, or particulate, matter is likely to modify their dispersion stability.

Some of these mechanisms have already been considered and studied in the scientific literature. To date, these fate studies considered mainly one limiting reaction for the ENP fate, which is the so-called homoaggregation of ENPs, i.e. ENPs colliding and sticking together. However, considering the effective, or potential, release of ENPs in the aqueous environment reported in the literature, the first estimations all agree that we have to deal with very low concentrations of ENPs in surface water. Predicted environmental concentration for Nano-TiO2 in Swiss surface water ranges from 2 to 1,623 ng/L, while the values for nano-ZnO and nano-Ag are by factors of 14 and 240 smaller. This is likely to decrease the probability for ENPs to meet each other.

3 Scientific and technological challenges

Mainly due to analytical limit detections of the metrology used, the fate researches have mostly used the studied ENPs in too high and non-realistic concentrations (> 10 mg/L) with regard to this potential ENP pollution scenario. Such over estimation of the ENP concentration completely alters the dispersion dynamic that may occur in real/complex environmental systems characterized by trace ENP concentrations. Indeed, in real aquatic systems, the probability that ENPs interact with each other is very low with regard to the likelihood of colliding with naturally occurring suspended matter present in significantly higher concentrations. Thus, the very low collision frequency of ENPs together becomes a limiting parameter, that prevents significant homoaggregation. On the other hand, this may likely be to the benefit of the collision frequency of ENPs with naturally occurring suspended matter. Due to the high specific surface area (m2 g-1) and reactivity of both suspended matter and ENPs, it is reasonable to expect that the natural suspended matter will act as a carrier phase for ENPs in the water column and strongly affect their fate. Such interactions certainly integrate ENPs into other, larger scale geogenic material transfer processes that this project proposes to investigate.

This consideration brings the past researches carried out with highly concentrated and model ENP suspensions out of the relevance for real scenarios occurring in the environment and discredits the risk estimations returned with that approach.

This project aims at studying the fate of ENPs in surface water in a realistic approach. This means dealing with ENPs present in trace concentration in an aqueous system of complex composition. The different scenarios involving interaction and heteroaggregation of ENPs with suspended matter will be studied in detail as a function of the system characteristics.

This project has to manage with different scientific and technical challenges. Working with trace concentrations of ENPs will bring them below the detection limit of number of the analytical tools usually used for their characterisation, e.g. size and surface charge measurement by light scattering will not be able to detect the ENPs when working below 1 mg/L. In fact, the SM will constitute the major part of the studied systems in a dry mass and volume consideration. Thus, studying the fate of ENPs therein will be mainly based on chemical analysis, using ICP-MS. Thus, an additional difficulty to deal with is the potential natural background of these chemical elements occurring in real surface water. Another challenging aspect of this project is its ambitious approach aimed at understanding physical chemical mechanisms in natural water with complex composition. Characterisation of the organic matter in natural water has been a recurrent problematic for a long time in diverse aspects of environmental sciences.

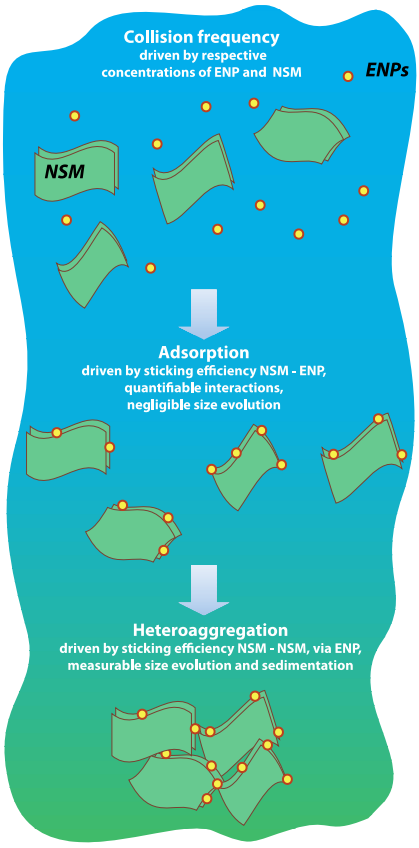


Figure 1 schematic view of the fate of ENPs in the water column, considering heteroaggregation with natural suspended matter (NSM)

4 Objectives

The approach of this project follows the assumption that ENPs present in trace concentrations in natural surface waters will interact with natural and manufactured suspended matter, and follow the colloidal dynamics of their carriers. The aim of this project is to identify, among the mineral and organic materials occurring suspended in surface water, the main potential carriers interacting with ENPs. Both of these minerals and organics will be considered hereafter under the name “suspended matter, SM”. In a second step, the induced altered transfer of the ENPs will be

investigated as a function of the physical chemical conditions of the natural system considered. A population balance of the ENPs will be established as a function of these conditions considering the different states or speciations encountered: free ENP, bound to SM, heteroaggregated, and sedimented. The final aim of this project is to deliver a ranking of these potential scenarios that will be based on both experimental results and model assessing the fate of ENPs in surface water. In order to carry these investigations under the most relevant conditions, a holistic approach will be also followed to better constrain the above mechanistic approach. Three real surface waters have been selected as field case studies.

The following key questions shall be answered: (WP1) Nature and characteristics of the SM occurring in real surface water? (WP2) Which types of SM interact with ENPs? (WP3) What is the induced effect on the ENP transport vs. heteroaggregation and sedimentation? (WP4) What is the residence time of the ENPs in the water column vs. in the sediment?

Two interconnected approaches will be followed to answer these questions: (i) A mechanistic physical chemical study will be carried with synthetic systems to better identify the preferential scenarios. It will consist in studying the relative affinity and interaction of ENPs with selected mineral and organic materials typically and potentially found in surface water. The induced effects on the fate of ENPs in the water column will be measured and modelled. (ii) A holistic approach, using natural surface waters, will enable us, after water characterisation, to better select the types of SM to focus on the mechanistic approach. Moreover, doping this natural water with ENPs, and studying their fate therein, will help to validate the global understanding brought by this dual approach.

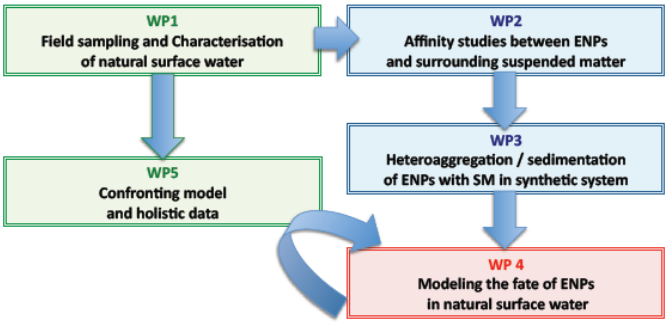


Figure 2 Nanoheter work plan

5 Organisation

The NANOHETER collaborative consortium consists of four partners: CNRS/CEREGE, ETH, BRGM and UW. It is coordinated by Dr. J. Labille (CNRS/CEREGE).

CNRS/CEREGE, in addition to the management charge, contributes scientifically to this project in terms of field sampling, physical chemical characterisations and heteroaggregation experiments described in WPs 1, 2 and 3. A post-doctoral person has been recruited for a two-year position thanks to NANOHETER funding, Dr. Danielle Slomberg. She is involved in most tasks of the project, closely collaborating with the other partners.

ETH Zurich, directed by Dr. Martin Scheringer, is responsible for the model development in WPs 4 and 5; it will provide feedback to the other project partners regarding the role of various processes in the models, which will help to set the focus for further experimental work. In collaboration with all other partners, ETH will contribute to the integration of all results obtained in the different WPs of the project into one coherent overall picture. a PhD student, Nicole Sani-Kast, has been recruited thanks to NANOHETER funding. She is in charge of developing the fate model in close connexion with the experimental data obtained in this project. ETH also benefits from the experience of Antonia Praetorius, who developed a conceptual framework for ENP fate models.

BRGM, directed by Dr. Patrick Ollivier, contributes scientifically to this project in terms of physical and chemical characterisations and heteroaggregation experiments described in WPs 1, 2 and 3. BRGM will coordinate works on Pesticides and, especially, on the influence of both ENPs-Pesticides and Mineral-Pesticides interactions on the fate and transfer of ENPs in the environment. To achieve these objectives, BRGM will provide up-to-date equipment (DLS, ICP-MS, Raman spectroscopy…) and facilities. A temporary researcher (Post-doc) will be recruited. A postdoctoral person will be recruited in mid 2014. He (she) will continue the work initiated in Nanoheter program on the different aspects of nanoparticle heteroaggregation, adding pesticides to the studied system.

UW, directed by Dr. Jonathan Brant, focuses efforts on the following areas: 1) conduct and interpret nanoparticle tracking analysis experiments with the goal of quantifying the nanoparticle number/mass concentration and the evolution of these values with changing environmental conditions and compositions, 2) carry out solid-liquid separations (e.g., tangential flow filtration, ultrafiltration, and nanofiltration) with the objective of separating the dissolved/particulate phases, and 3) provide water samples and analyses on those samples available to the CEREGE for a comparative analysis of colloidal/nanoparticulate composition. The University of Wyoming makes use of existing research funds from the National Science Foundation (NSF). In addition, UW and CEREGE also benefit currently from a PUF program entitled "Mechanistic Assesment of Manufactured Nanomaterial Behavior in Engineered Environments : Solid Waste Landfills and Drinking Water Treatment Plants", funding mobility and training of young scientists between these two partners.

In addition to this consortium stands an advisory committee. Dr. A. Bruchet (SUEZ ENVIRONNEMENT CIRSEE) was involved in some tasks of WP1 over this first year. He has expertise regarding the characterisation of the NOM occurring in the natural surface water. Dr. A. Thill (CEA) has expertise with modelling the aggregation kinetics of suspended particles.

The official starting date of the Nanoheter program is April, 1st, 2013. The kick-off meeting stood joint with the other funded ERA-NET SIINN programs in Berlin on June, 14th, 2013. The next yearly meeting will stand in Basel on May, 14th, on side of the SETAC Europe 24th annual meeting.

6 Expected Impact

Dissemination and/or exploitation in the scientific community

The novel and original approach proposed in this project resides in bringing the existing scientific community additional consideration

and experience with regard to the complex composition of the real aquatic environment. It follows a progression that goes from the study of simple model systems toward that of more complex and environmentally relevant ones, in order to better understand the physical chemical interactions that drive the fate of suspended materials. Within such a scope, this project constitutes a real opportunity for the community of Nanotechnology Risk Assessment to benefit from new and more relevant data. Such an approach being original within this community, it is expected to bring new consideration toward increased environmental relevance that will better answer pivotal questions arising with ENP exposure.

Moreover, in addition to the more relevant contribution brought to the exposure assessment of the ENPs, this project will also contribute more widely to further characterisation of the dynamics and transport of suspended matter in the water column at the river scale, that is largely driven by physical chemical interactions.

Consortia in touch

This project benefits of the French-US consortium involved in the GDRI iCEINT (International Consortium for the Environmental Implications of Nanotechnology), which is aimed at coordinating the researchers devoted to this field between France partners and US CEINT partners (Head M.R. Wiesner DUKE University, USA). This consortium meets annually to disseminate new results to the concerned scientific community, thus providing regular updates on the state of the art.

This project also benefits from the Rhone Sediment Observatory, that compiles and manages data on sediment flux and storage as well as pollutants associated with these sediments. A coordinator for this community is O. Radakovitch, member of CNRS/CEREGE partner. Its multi-partner platform for carrying out collaborative research between scientific researchers and managers will enable an easy dissemination of our results to inform managers and elected officials.

At the European level, the Nanoheter project sounds like visionary, fitting perfectly with the latest research priorities asked in the current EU Call H2020-NMP 28-2014 Assessment of environmental fate of nanomaterials. A common goal is evidenced in addressing the prediction of environmental distribution of nanomaterials, using laboratory, field and model simulations for exposure studies, to allow early assessment of potential exposure.

At this early stage of the Nanoheter project, mainly oral communications of the first results have been given to the community, already revealing the key consideration of the heteroaggregation phenomenon in our problematics. A larger impact is expected in the close future, with the first article published from the project. There is no doubt that the experience and data obtained from the Nanoheter project will provide a very useful basis for future funded research, and that a close connexion will be maintained with our consortium. Databases for Nanosafety and Nanorisk will be used for this purpose.

Impact to the Governmental and industrial partners

The project develops a novel approach for exposure measurements. This approach may be increasingly necessary for nanomaterial producers in the nanotechnology sector, to provide an assessment of the relative environmental and human health risks related to these substances. This will enable government agencies and industry representatives to assess the safety of these commercial substances using a sound scientific-based approach. The results will also be valuable to public utilities, risk management professionals, and environmental engineering firms working on various aspects regarding environmental risks of ENPs.

The expected results from the proposed project will assist both industry and governmental regulators in developing appropriate standards and techniques for minimizing the risk that is posed by manufactured nanomaterials, and thus facilitating the successful commercialization of novel nano-products. These obligations introduce the notion of sustainable production for the fabrication processes as well as for the knowledge of the life cycle.

It is worthwhile to note that such a perspective is exactly that aimed by the Labex Serenade (Safer Ecodesign Research and Education applied to Nanomaterials Development) in which Dr. J. Labille is in charge of coordinating Priority Research Actions around the Exposure and the End of Life of nanomaterials. There is no doubt that this context will strongly facilitate the impact of the Nanoheter project to the industrial partners collaborating in the Labex program (paint, sunscreen, cement manufacturing…).

7 Directory

Table 1 Directory of people involved in this project.

First Name	Last Name	Affiliation	Address	e-mail
Jerome	LABILLE	CNRS/CEREGE	CEREGE, BP80, FR-13545	labille@cerege.fr
Danielle	SLOMBERG	CNRS/CEREGE	Aix en Provence cedex 4	
Olivier	RADAKOVITCH	CNRS/CEREGE	France	
Jean-Yves	BOTTERO	CNRS/CEREGE		
Patrick	MASION	CNRS/CEREGE		
Jerome	ROSE	CNRS/CEREGE		
Mélanie	AUFFAN	CNRS/CEREGE		
Martin	SCHERINGER	ETH	ETH, Rämistrasse 101,	martin.scheringer@chem.ethz.ch

First Name	Last Name	Affiliation	Address	e-mail
Nicole	SANI-KAST	ETH	CH-8092 Zürich,	
Antonia	PRAETORIUS	ETH	Switzerland	
Konrad	HUNGERBUHLER	ETH		
Patrick	OLLIVIER	BRGM	BRGM, 3 av C. Guillemin,	p.ollivier@brgm.fr
Hélène	PAUWELS	BRGM	FR-45100 Orléans	
Nicole	BARAN	BRGM	France	
Guillaume	WILLE	BRGM		
Jonathan	BRANT	UW	DCAE, Dept. 3295	jbrant1@uwyo.edu
Ryan	KOBBE	UW	1000 E. University Av.	
Luke	RUFF	UW	Laramie, WY 82071	
			USA	

8 Copyright

NANOMICEX

Mitigation of risk and control of exposure in nanotechnology based inks and pigments

Contract Agreement: NMP4-SL-2012-280713 Website: http://www.nanomicex.eu
Coordinator: Carlos Fito, Packaging, Transport and Logistics Research Center, Valencia, Spain

No.	Beneficiary name	Short name	Country
1	Packaging, Transport and logistics research center	ITENE	Spain
2	LEITAT Technological Centre	LEITAT	Spain
3	Institute of Occupational Medicine	IOM	United Kingdom
4	Heriot-Watt University	HWU	United Kingdom
5	Yeditepe University	Y.U.	Turkey
6	University - Humboldt-Universität zu Berlin	H.U	Germany
7	Nanotechnology Industries Association	NIA	Belgium
8	Tec Star S.r.l.	TECStar	Italy
9	Ardeje	Ardeje	France
10	Pinturas Montó, S.A.U.	MONTÓ	Spain
11	Plasmachem GmbH	PLASMACHEM	Germany
12	Torrecid, S.A.	TORRECID	Spain

Contents

1 Summary

Project Duration: 1 April 2012 – 30 March 2015

Project Funding: 3.5 Mio. EUR

Nanotechnology and in particular, the use of nanoparticles in the pigment, ink and paint industry have a great potential for new applications, leading to products with new or enhanced properties, and opening new market opportunities. Consequently, many promising applications emerge nowadays, based on the use of nanoparticles such as Fe3O4, TiO2, ZnO, Quantum dots or Mixed-metal oxides at the nanoscale, which confer a wide range of properties to the final products, covering the most requested properties in pigment, inks and paints applications for the nearest future.

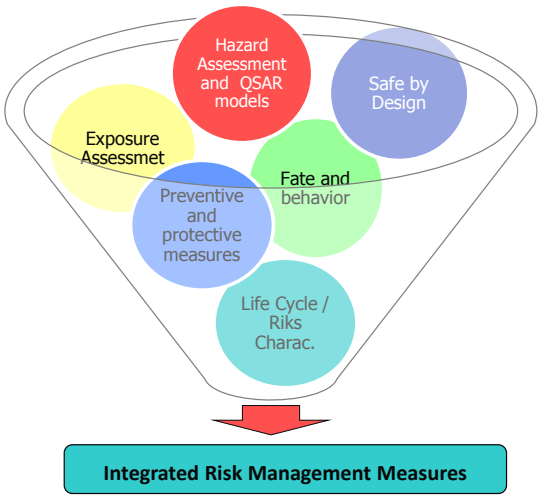
The possibilities for application of nanosized particles are rapidly increasing on the basis on the current societal needs and market trends; nevertheless there are number of issues that warrant concern about the mass commercialization of these nano-formulated products, considering mainly technical and safety concerns. The uncertainties are great because the properties exhibited by such particles are often exceedingly different to those demonstrated by bulk forms, affecting their physicochemical and biological behaviour, which results, in more toxic properties. In this sense, It is known that exits a causal association between exposure to NPs with human diseases, as well as environmental pollution, considering that NPs can be released to the ecosystem.

Despite such hazards, it is not possible to predict their impacts and there are currently no exposure limits specific to NPs, nor any national or international consensus standards on hazard assessment and measurement. In addition, there is a major debate

on nanotechnology future implications, including concerns about effects on global economics and consumers' acceptance.

In order to address these major concerns and considering the project concept, **the main objective of NANOMICEX project is to reduce the potential risk upon worker's exposure to engineered nanoparticles** through the modification of nanoparticles properties with effective surface modifiers and the characterization of practical and cost effective risk management strategies in the particular operative conditions of the inks and pigments industry.

To achieve such objectives, a panel of **7 ENMs** widely employed in the reference sectors of the project will be studied in detail in order to identify the mean parameters that may influence their chemical and physical properties. The hazard of these materials will be tested using both human and environmental models. Once characterized, the selected ENMs will be coated using different surface modifiers in order to obtain less hazardous and more stable particles. The methodologies used in the formation of less risk-posing nanoparticles will be relatively simple allowing them to be easily reproduced in a common laboratory in order to ensure the effectiveness of the methodology in the industry. In a second stage, levels of exposure for workers who are exposed when handling the nanoparticles will be determined in order to develop real exposure scenarios. In the third stage of the project, the exposure scenarios will be reproduced in the laboratory clean room, in order to assess the effectiveness of the risk management measures and engineered controls. To this end, the effectiveness of the personnel protective equipment, ventilation, filtration and other controls will be checked in the simulated conditions. As a result, the studies will determinate the most effective techniques to reduce and mitigate the hazard and exposure, and therefore minimize the risk, focusing on safe use. Finally, in the last stage of the Nanomicex project, the modified nanoparticles and cost effective risk management strategies will be tested in case studies with the aim of validating the strategies in the real operative conditions for preparing inks, where the nanoparticles can have a uncertain behaviour.



The colour industry all over the world is being driven by innovation, which allows manufacturers to develop new and innovative products for hundreds of industrial applications and billions of people who use them every day. The pigment industry has always been striving to improve application technology properties and the market demand properties such as dispersibility, color strength, light and weather fastness, migration resistance, color shade or hiding power. These properties depend on the chemical composition of inks and pigments and on the size and morphology of their particles. Therefore, nanotechnology and in particular, the **use of nanoparticles have a great potential for new applications,** leading to products with new or enhanced properties such as thermal stability, water repellence, scratch resistance, durability and antimicrobial properties. Consequently, many promising applications emerge nowadays, based on the use of nanoparticles such as FexOy, TiO2, ZnO, Quantum dots or Mixed-metal oxides at the nanoscale, which confer a wide range of properties to the final products, covering the current societal needs and market developments.

Along with the benefits there are also concerns that a variety of the characteristics possessed by nanomaterials, such as small size, high aspect ratio, shape, surface reactivity, solubility or dustiness, relate their potential hazard and risk.

However, despite such situation, due to the extraordinary possibilities derived from the application of nanotechnologies in different industrial sectors, the use of engineered nanoparticles is steadily increasing and the number of workers dealing with nanoparticles is also on the rise. For example, the organic pigment industry, in which nano-additives are used, employs more than 100,000 staff and achieve sales of 10 billion Euros. Similarly, the production of nano-structured inks represents both the largest and faster-growing market for advanced ink formulations.

On the other hand, significant regulatory concerns from the European Commission have arisen about unforeseen risks likely to arise from nanoparticles. In this sense, the communication from the commission to European parliament (SEC 2008, 2036) provides a description of elements of selected EU legislation that seems most relevant and likely to apply to nanotechnologies and nanomaterials. At the moment, the most important piece of legislation in the area of health and safety at work is the Framework Directive 89/391/EEC" on the introduction of measures to encourage improvements in the safety and health of workers", which fully applies to risks associated with nanoparticles. This Directive places a number of obligations on employers to take measures necessary for the safety and health protection of workers, considering also the risk mitigation as a recommendation when it is not possible to eliminate the risks. At the same time, the REACH regulation, which is the main legal instrument to ensure the safety use of chemicals in the European market, establishes the need to ensure the safety of substances, such and those included into mixtures (e.g. inks). Even if there is no specific regulation to nanomaterials, REACH regulation applies to all substances and mixtures supplied in the European Union, whatever size, shape or physical state. Nonetheless, in the absence of specific regulations, the precautionary principle should be first applied.

3 Concept and Objectives

3.1 Project Concept

The concept of NANOMICEX stems from the need to ensure the safety of workers dealing with the production or handling of engineered nanoparticles employed in the pigment/ink industry, as

well as the need to provide the workers with integrated, cost effective and appropriate strategies to control the exposure to engineered nanoparticles.

On the basis of this concept, the following activities will be conducted:

a- Application of the safe-by-design approach to reduce hazards caused by potential nanoparticle emissions during ink/pigment-based products life cycle.

b- Toxicological and ecotoxicological evaluation of nanoparticle impacts, selecting methods that are reproducible, simple, non-expensive and reliable.

c- Characterization of exposure scenarios in terms of REACH regulation, including exposure assessment

d- Assessment of the effectiveness of the personnel protective equipment, ventilation, filtration and other control systems in simulated conditions (cleaning room laboratories)

e- Validation and implementation of simple risk management strategies, involving industrial partners.

3.2 *Project Objectives*

The main objective of NANOMICEX project is to **reduce the potential risk upon worker's exposure to engineered nanoparticles** through the modification of nanoparticles properties with effective surface modifiers and the characterization of practical and cost effective risk management strategies in the particular operative conditions of the inks and pigments industry. New surface modifiers will be identified to obtain less hazardous and more stable engineered nanoparticles, suitable for their use in the industrial facilities. An exhaustive exposure assessment will be carried out in order to control the surface modified nanoparticles in the real operative conditions, including the evaluation of the current risk management strategies. Additionally, a practical and cost effective risk management strategy will be developed, which can be used in combination with the surface modifiers as a consistent and integrated approach for mitigation of workers risks. Additionally, NANOMICEX must cover these actions, including the fulfilment of the current regulation in terms of worker safety and consumer health, avoiding workers exposure to nanoparticles in the current industrial settings.

Related to the nanoparticles considered, the project is focused on those nanoparticles employed in large scale by pigments manufacturers, and ink/paint formulators, covering an extensive range of high-tech applications and added value properties (semiconductor, insulator, luminescent, catalytic, refractive and magnetic properties). Such criteria are satisfied by several **metal oxide nanoparticles** (ZnO, TiO_2, Al_2O_3 and Fe_3O_4), **Ag metal nanoparticles**, **CdSe Quantum Dots** and the mixed metal oxide **Cobalt Aluminate spinel**, therefore, these nanometer-sized particles will be studied within NANOMICEX project.

4 Overview of the Workplan

NANOMICEX consists of 9 complementary Work Packages (WP), summarised in Table 1. For each WP, a complete description is presented below, including the objectives; the WP leader (in bold) and team members; the Hypotheses and Methods; the Deliverables; and linkage to other Work Packages.

Table 1: Work Packages of NANOMICEX

WP n°	WP Title	WP Leader
1	Characterization of engineered nanoparticles	HU
2	Development and selection of functional modified nanoparticles	YU
3	Hazard Assessment	HWU
4	Exposure Assessment	IOM
5	Risk Management and Control Measures	ITENE
6	Nano SLCRA: Adaptive Streamlined Life Cycle / Risk Assessment of nanoparticle-based inks and pigments	LEITAT
7	Industrial Case Studies	ARDEJE
8	Project Coordination and Management	ITENE
9	Project dissemination and training	NIA

This work plan has been split into 4 types of activities and based on the combined experience of the consortium members. The activities are explained below:

1. Scientific and Technological development

These activities cover the scientific tasks to be conducted to achieve the project objectives. A detailed description of these tasks is described on table 2.

2. Validation and Demonstration activities

The main objective of these activities is to prove the viability of the solutions proposed in the industrials settings. It will be conducted under the scope of WP 7, checking the surface modifications in industrial case studies. The exposure scenarios and risk management measures will be implemented and monitored to ensure their correct application.

3. Project Management

This work includes the tasks to be completed by the project Coordinator and contains the tasks required to successfully manage the project. The coordination activities will be undertaken by the ITENE.

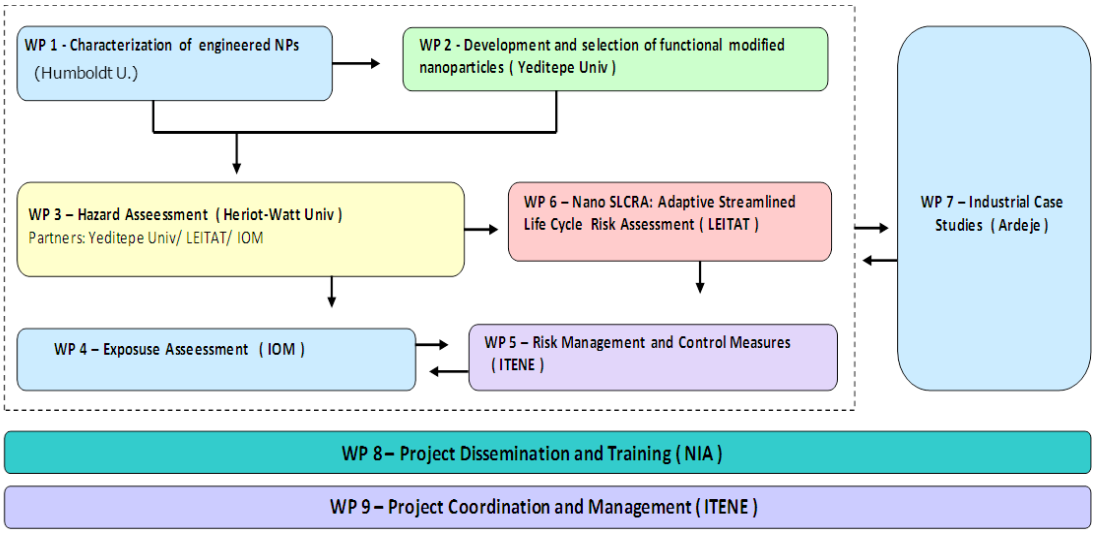
4. Dissemination Related Activities

In order to achieve an optimal use of the Project across the EU, dissemination, training and exploitation are essential to the success of the NANOMICEX project. These activities will be conducted within WP 9.

Table 2 Technical & Scientific Workpackages (WP) of NANOMICEX

WP	Title	Description
1	Characterization of engineered nanoparticles	Workpackage 1 (WP1) is focused on the characterization of the nanoparticle panel, identifying the specific types of the metal oxide nanoparticles, AgNPs, CdSe quantum dots and the mixed-metal oxide $CoAl_2O_4$ employed in the pigment and ink industry. Once identified, a full characterization, in terms of size, shape, mass, surface area, chemical composition, physical and optical properties, will be conducted
2	Development and selection of functional modified nanoparticles	Workpackage 2 (WP2) will select the surface modifiers and derivatize the nanoparticles with the selected modifiers. A systematic study will be carried out in order to design and develop surface modifiers, which will be custom designed from bimolecular structures, hydrophobic organic stabilizer and PEGs. The designed modifiers will be attached through several routes available in the literature or/and newly developed within the project. The new NPs synthesized will by characterized by imaging techniques such as SEM and AFM along with other characterization techniques.
3	Hazard Assessment	Workpackage 3 (WP3) will evaluate the current literature on the environmental fate of nanoparticles used in the ink and pigment industry, and will assess the toxicity and ecotoxicity of the NPs characterized in the WP1. The toxicity profile will be assessed by in vitro assays using a range of cell lines that represent significant exposure and target organs and cell types in the human body. This in vitro approach will be combined with a small number of organism studies to confirm key in vitro observations, as well as to assess ecotoxicity. In addition an analysis of relationships between nanoparticle properties and effects will be undertaken to allow collation of data and reduction in future testing requirements.
4	Exposure Assessment	Workpackage 4 (WP4) will be focused on the exposure assessment. At this stage levels of exposure for workers who are exposed when handling the NPs will be determined in order to develop real exposure scenarios. These scenarios will be studied at all stages of nanoparticles production, use and disposal, considering the nanoparticles as such or as a component of the ink/pigment formulations.
5	Risk Management and Control Measures	Workpackage 5 (WP5) will be focused on the assessment of the effectiveness of the Risk management Measures. The workplace controls as personnel protective equipment, ventilation, filtration and other controls will be checked in controlled conditions in order to determinate the most effective techniques to reduce and mitigate the hazard and exposure, and therefore minimize the risk, focusing on safe use.
6	Nano SLCRA: Adaptive Streamlined Life Cycle / Risk Assessment of nanoparticle-based inks and pigments	Workpackage 6 (WP 6) will assess the potential impact and evaluate the risk posed by NPs on workers. The risk assessment will be done at the different exposure scenarios defined on WP4. Furthermore, a novel methodology based on the combination of the life cycle assessment (LCA) and the risk assessment of nanoparticle-based inks and pigments will be conducted in order to establish their potential health and environmental impacts along their life cycle. This WP will include the development of novel strategies for the management of inks and pigments waste containing nanoadditives.

The workpackages and their interdependence are shown schematically below:



5 Advances beyond the state of the art

NANOMICEX project propose an **integrated approach to manage the risk posed by nanoparticles**, dealing with the current limitations in relation to the worker protection strategies, considering the risk assessment methodologies and risk management measures. The current handbooks, guides and reports of research projects are not focused on specific nanoparticles used in the current industrial setting of the ink and pigment industry, which can differ enormously from another industrial process involving the use of nanoparticles.

In relation to the progress beyond the current state of the art, NANOMICEX is working on the design of functional groups to modify the properties of the engineered nanoparticles employed in the pigment and ink industry in terms of toxicological profile, cell interaction and surface reactivity, but without causing significant changes in the nanoparticles properties, reproducible applications in real conditions and using modification techniques that are easy to implement by non-expert personal. In this sense, the surface modifications of metal oxide nanoparticles, AgNPs, CdSe quantum dots (QDs) and mixed-metal oxides ($CoAl_2O_4$) NPs are aimed for their inclusion into inks and pigment formulations, reduce their hazardous properties and adverse effects on living systems, without compromising their further application in their current industrial pigment/ink formulations.

Regarding the potential hazards posed by nanoparticles, NANOMICEX will assess the cytotoxicity (in vitro approach), sub-lethal toxicity and dermal effects, considering the relevance of such aspects in relation with the occupational exposure to nanoparticles. This work will allow the determination of the toxic responses in the worker place, and also allow a comparison of the effects of modified and unmodified particles. In addition, the work developed will provide the stakeholders with scientific and consensuated data to conduct regulatory actions (e.g. Occupational Exposure Levels- OELs based on LC_{50}/EC_5 data). Similarly, regarding the environmental impacts of the nanoparticles, NANOMICEX will provide new knowledge in relation to the environmental fate and behaviour of nanoparticles relevant to the pigment and ink industry. A comprehensive ecotoxicological study based on selected OECD test models will be conducted, including the assessment of acute toxicity, sub-lethal ecotoxicity and bioaccumulation. In addition, NANOMICEX project will work in the computational analysis of data obtained from hazard studies, with the aim of determining structure activity relationships in order to provide valuable data to improve the current QSAR tools or create new ones.

In terms of exposure assessment, the research activities within the NANOMICEX project will further develop real exposure scenarios in order to assess the exposure in the real operative conditions of workers dealing with engineered nanoparticles. Once developed, such scenarios will be modelled and reproduced in controlled conditions, improving the knowledge about the background effects and interactions between the engineered nanoparticles and their environment, and how such interactions modify the exposure patterns of the engineered nanoparticles in real conditions. Furthermore, the state of the art real time measurement devices will be tested in controlled conditions, improving the knowledge to interpret the data obtained.

In relation to the protection strategies, during the NANOMICEX project several protective measures will be assessed, evaluating the effectiveness of the existing technical and management exposure control strategies, providing the ink and pigment industry with the most appropriate measures to control the exposure to engineered nanoparticles and therefore to minimize the risk.

Finally, the NANOMICEX project will conduct a life cycle assessment combined with risk assessment, studying the health and the environmental impacts of NP-based inks and pigments at all the stages of their life cycle. The availability of data on the nanoparticles release to the environment, and consequently to humans at all the stages of their life cycle, which is one of the current most critical limitations to perform LCA of NPs, will allow the improvement of the accuracy of the LCA analysis in comparison with existing attempts. In addition, concerning the disposal of nanomaterial-based products, NANOMICEX will propose novel strategies for the management of the waste produced along the life cycle of inks and pigments containing nanoadditives.

6 Progress to date

The project started officially on April 1st 2012 and had its kick-off meeting at the Valencian Regional Office in Brussels on April 26 and 27.

At the moment, much of the research and development work is concentrated on the physicochemical characterization, the surface modification of the selected NMs and the toxicological assessment of both modified and unmodified NMs.

The overall work conducted since the beginning of the project can be summarized as follow:

1. Characterization of the chemical and physical properties of the selected ENPs before and after surface functionalization with different techniques such as SEM, AFM, DLS, UV/Vis Spectroscopy, IR or Raman spectroscopy (WP1)
2. Exploration of the surface chemistry alteration strategies for Al_2O_3, TiO_2, Fe_2O_3, $CoAl_2O_4$ and ZnO nanoparticles. A variety of ligands with biological origin such as carbohydrates and oligonucleotides has been tested (WP 2)
3. Ecotoxicity and Cytotoxicity screening for all unmodified NPs. Comprehensive literature reviews regarding the biological effects and the dermal effects of the nanoparticles involved in the project (WP 2-WP3)
4. Scoping visits to Plasmachem, Ardeje, TecStar, Torrecid and Monto. Gathering of data and critical information related to the conditions of use, risk management measures and exposure data throughout the life cycle of the ENPs (WP_4)
5. Construction of an aerosol testing chamber and evaluation of the barrier efficiency for skin protective equipment, leakage efficacy for respirators and filtration performance of protective clothing and ventilation devices (WP_5)
6. Cost effective analysis for industrial-scale implementation of risk mitigation solutions and strategies developed within the project

7. Participation in several international events related to the research topic of the project, mainly workshops and conferences.
8. Concerning dissemination materials, the project web site and the first project brochure is available. A number of newsletters has been also published by partners.

A more detailed explanation of the activities conducted since the beginning of the project is given below:

WP1. Characterization of engineered nanoparticles

The characterizations were conducted using state of the art techniques, including X-ray diffractograms, SEM, AFM, DLS, UV/Vis Spectroscopy, FT-IR-ATR Spectroscopy, Raman spectroscopy and surface-enhanced Raman scattering.

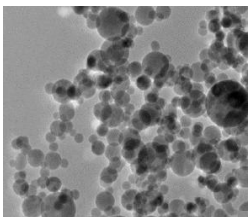


Figure. TEM images of ZnO

The results showed that most of the samples were highly polydispersed with broad range in size and shape. Furthermore, according to the XRD data, all samples were crystalline whereas TEM study showed that nanocrystals were mainly present as aggregates. FT-IR spectroscopy and elemental analysis studies indicated that the aggregates were formed mainly due to the lack of organic stabilizers.

Additional experiments will be carried out within WP1 and 2 related with the new coated NPs.

WP2. Development and selection of functional modified nanoparticles

An exhaustive literature review was performed regarding the current surface modifiers used for coating the ENMs. The toxicity of the possible coating materials and the NMs prepared from these coatings was also included in the literature search.

On a second stage, the Nanobiotechnology reseach group of the University of Yeditepe investigated a number of experimental strategies to attach hydrocarbon based polymers and macromolecules such as chitosan, starch, mannose, lactose and glucose, which are known with good biocompatibility.

The group confirmed that the crosslinking of a variety of ligands with biological origin such as carbohydrates and oligonucleotides bearing hydroxyl group to metal oxide NMs was possible, observing that Al2O3, TiO2, Fe2O3, and CoAl2O4 surfaces were successfully modified using peptide and oligonucleotide.

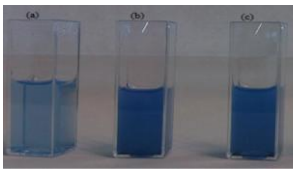


Figure. The color changes of the nanoparticles (a) CoAl2O4, (b) CoAl2O4 functionalized, (c) CoAl2O4 chitosan coated.

WP3. Hazard Assessment

According to current models of environmental exposure, it has been concluded that Ag, ZnO and QDs ENPs are the most significant ''nano-contaminants'' although this does not necessarily mean that they are hazardous. The ecotoxicity studies on P.Subcapitata, D. magna and L. Variegatues showed adverse effects in concentrations ranging from low µg/L to nearly 1g/L. The ranking of toxicity obtained to date for environmental species and cell lines is relatively similar:

Ag>>ZnO>Qdots>>$CoAl_2O_4$>TiO_2>=Fe_2O_3=Al_2O_3.

WP4. Exposure Assessment / WP 5. Risk Management and Control Measures

5 key exposure scenarios have been identified (Synthesis, Handling of powders, Formulation of inks/paints, cleaning and maintenance). The review of literature indicated that exposure to NPs may occur during the **synthesis and handling** of ENPs.

More precisely, the handling of pure, non-consolidated NPs was considered one of the most critical operations because it involves direct manipulation of the NPs. On-site measurements were also conducted. Analysis of real-time sampling data (CPC and NanoTracer) identified consistent releases in particle concentrations within 6-120nm range when sampling directly inside fume hoods or glove boxes.

Figure. On-site measurements

An in-depth literature review was conducted with the aim of describing the procedures for testing in accordance with the International Standards. Information included: experimental set-up, required equipment, test outcomes expected, critical parameters.

Half mask respirators and gloves/protective cloths have been tested. Average penetration levels (APL) for different masks tested were between 20 and 55 %, with a minimum penetration level in the case of Fe_2O_3.

Sample Nanoparticle	PPE Model	C Max outside	C Min inside	Average Outside	Average inside	PF_{pic} %	PF_{Av} %
ZnO	Half masks	21,379	1,1745	17,236	9,432	54,9	54,7
Fe_2O_3	Half mask	65,090	21,120	57,783	11,725	32,4	20,3
TiO_2	Half mask	59,813	29,214	48,368	25,786	48,8	53,3
Al_2O_3	Half mask	22,832	9,062	20,929	7,667	39,7	36,6
Al_2O_3	Half masks	7,942	3,289	6,019	1,972	41,4	32,8
Al_2O_3	Half mask	74,892	17,400	62,696	13,259	23,2	21,1
$CoAl_2O_3$	Half mask	12,104	6,170	10,164	4,747	50	46

Gloves and protective clothes were tested on the basis of the thorough diffusion method derivate from the ISO standard EN 374. The effectiveness values show a high degree of protection, with penetration factors below 0.5% and 15% in gloves and laboratory coats respectively.

ENPs	PPE	Average Outside	Average Inside	PF_{int} %
Al_2O_3	Latex	2,023,241	7011	0,35±0,16
Al_2O_3	Nitrile	435,284	5103	1,2±0,2
Al_2O_3	garment	551,353	5914	1,0±0,5
Al_2O_3	Lab coat	683,621	33806	9,9±0,9
TiO_2	Latex	1,876,907	679	0,04±0,03
TiO_2	Nitrile	122,514	45	0,2±0,5
TiO_2	garment	122,291	262	0,11±0,16
TiO_2	Lab coat	210,896	17982	11,1±1,9

Furthermore a testing device has been developed for testing the leakage efficacy and protection factors (PFs) of respiratory protection equipment (RPD) and filtrations performance of protective clothing and ventilation devices.

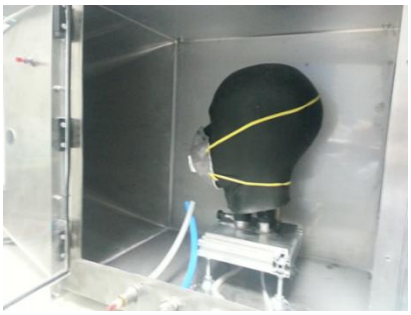


Figure. Testing device

WP 6. Nano SLCRA: Adaptive Streamlined Life Cycle / Risk Assessment of nanoparticle-based inks and pigments

The most critical stages and processes in the life cycle of the target ENPs have been established and data have been collected from Partners for the Life Cycle analysis.

Risk characterization is performed using the Stoffenmanager-nano software.

Number	Stage	Uses	Code
1	Ag nanoparticles production	Ag NP's synthesis	GES_{Ag} 1
		Ag NP's Functionalization	GES_{Ag} 2
2	Ag nanoparticles formulation	Manufacture of intermediates (blending/mixing)	GES_{Ag} 3
		Formulation (Incorporation in paints)	GES_{Ag} 4
3	Use of Ag nanoparticles	Industrial use	GES_{Ag} 3, GES_{Ag} 4
		Professional use	GES_{Ag} 5
		Consumer use	GES_{Ag} 6

WP 7. Industrial case studies

Application of the ENPs modifiers under industrial conditions

A glucose modifier was applied by Yeditepe University on ZnO nanopowders supplied by project partners. A performance comparison is currently being made between coated and uncoated ENPs. Tests have also been performed in order to evaluate the compatibility of unmodifierd silver and quantum dots inks with print-head modules.

Furthermore, the applicability of the ZnO and TiO_2 ENPs to the specific paint formulations manufactured by project partners is being validated.

Finally yet importantly, a development of new inks based on modified Quanum Dots is expected while the Cost-Benefit analysis for the implementation of the project results at industrial scale, is still in progress.

It is worth to note that manufacturing cost of nano-pigments and nanoformulated ink/paint is currently higher than that of conventional products manufacturing due to higher cost of raw materials the required RMMs that need to put in place. However according to current data on the market share and business opportunities, investments in nanotechnology applications are expected to be beneficial in the near future for the investors in terms of both cost savings and market share as well as in terms of environmental performance.

WP 8. Project Dissemination

The following table contains all the events that Nanomicex partners have participated during the last period.

Name of Conference	Date	Location	Partner	Target Audience[1]	Size of audience	Regions addressed
Safety issues and regulatory challenges of nanomaterials	05.2012	San Sebastian (Spain)	ITENE	- Industry - Public Authorities - International Organisations	150	EU
Safe Implementation of Nanotechnologies: Common Challenges	05.2012	Grenoble (France)	ITENE	- Public Authorities - International Organisations	50	EU
2nd QNano Integrating Conference	02.2013	Prague (Czech Republic)	HWU	- Industry - Public Authorities - International Organisations	200+	International
Pittcon 2013	03.2013	Philadelphia (USA)	YED	- Industry - International Organisations	20,000	International
Health and Environmental Impact of nano-enabled Products along the Life Cycle	05.2013	Barcelona (Spain)	ITENE	- Industry - Public Authorities - International Organisations	50	EU
9th Nanoscience and Nanotechnology Conference (Nano TR-9)	06.2013	Erzurum (Turkey)	YED	- Industry - Public Authorities - Industry Associations	1,000+	International
Euronanoforum 2013	06.2013	Dublin (Ireland)	ITENE	- Industry - Public Authorities - International Organisations - Industry Associations	1,500	International
Nanotechnology and the Coatings Industry	10.2013	Nottingham (UK)	HUB	- Industry - Public Authorities - Industry Associations	50	UK

7 Conclusion To-Date & Plan for the Future

- Current results show how the toxicity can be reduced using adequate surface modifiers, however the properties of the nano-products are also modified, being necessary an adequate viability study.
- The test conducted in relevant cell lines and model organism suggests that the most hazardous NPs in mammalian and environmental systems are ZnO, CdSe QDs and Ag
- The results of the scoping visits suggested that exposure levels during most processes will be very low due to the small scale, the nature of the process and the control measures used. New studies are needed

- The control of exposure via inhalation is a key priority for ENPs. The tested ENPs were able to cross the respirators tested and reach the tracheobronchial area of the Sheffield head, where the real time measurement devices where located
- An improvement on the synthesis methods ins key to ensure the scaling of the project, including reduce the polydispersity and variations in the surface chemistry
- A better understanding of the workplace, consumer and environmental exposure will provide the stakeholders with scientific and consensual data to conduct regulatory actions

All the activities described above will be performed with the support of the industrial partners, which will be in charge of the validation of surface modifiers, as well as the application of the new risk management measures.

The dissemination related activities will continue with the scheduled task, especially with the preparation of a new project brochure and dissemination materials to be employed in next dissemination events.

Finally, a key issue related with the coordination and management of the project will be the preparation of the final report in March 2015.

8 Directory

Table 3 Directory of people involved in this project.

First Name	Last Name	Affiliation	Address	e-mail
Carlos	Fito	Packaging, Transport and logistics research center	Albert Einstein, 1 CP.46.980 Paterna (Valencia) – Spain	cfito@itene.com
Araque	Eva	Packaging, Transport and logistics research center	Albert Einstein, 1 CP.46.980 Paterna (Valencia) – Spain	earaque@itene.com
Enrique	De la Cruz	Packaging, Transport and logistics research center	Albert Einstein, 1 CP.46.980 Paterna (Valencia) – Spain	ecruz@itene.com
Jose	Planelles	Packaging, Transport and logistics research center	Albert Einstein, 1 CP.46.980 Paterna (Valencia) – Spain	jose.planelles@itene.com
Socorro	Vázquez-Campos	LEITAT Technological Centre	C/ de la Innovació 2 08225 Terrassa (Barcelona), Spain	svazquez@leitat.org
Rob	Aitken	Institute of Occupational Medicine	Research Park North / Riccarton UK-EH14 4AP Edinburgh United Kingdom	rob.aitken@iom-world.org
Steve	Hankin	Institute of Occupational Medicine	Research Park North / Riccarton UK-EH14 4AP Edinburgh United Kingdom	steve.hankin@iom-world.org
Martie	Van Tongeren	Institute of Occupational Medicine	Research Park North / Riccarton UK-EH14 4AP Edinburgh United Kingdom	martie.VanTongeren@iom-world.org
Teresa	Fernandes	Heriot-Watt University	Edinburgh, UK EH14 4AS +44 131 451 3460	t.fernandes@hw.ac.uk
Vicky	Stone	Heriot-Watt University	Edinburgh, UK EH14 4AS +44 131 451 3460	v.stone@hw.ac.uk
Mustafa	Culha	Yeditepe University	26 Ağustos Yerleşimi, Kayışdağı Cad. 34755 Kayışdağı-Ataşehir Istanbul Turkey	mculha@yeditepe.edu.tr
Nicola	Pinna	Humboldt-Universität zu Berlin Institut für Chemie	Ziegelstr. 30 10117 Berlin	nicola.pinna@hu-berlin.de
Steffi	Friedrichs	Nanotechnology Industries Association	101 Avenue Louise 1050 Brussels Belgium	steffi.friedrichs@nanotechia.org
Pascal	Pierron	Ardeje	4, rue Georges Auric 26 000 Valence - France	pierron@ardeje.com
Mickaël	Barret	Ardeje		barret@ardeje.com
Christophe	Mercier	Ardeje		mercier@ardeje.com
Alicia	Rodriguez	Pinturas Montó	Carretera de la base Militar. 46163 Marines (Valencia) – Spain	alicia.rodriguez@montopinturas.com

First Name	Last Name	Affiliation	Address	e-mail
Alexei	Antipov	Plasmachem GmbH	Rudower Chaussee 29 D-12489 Berlin Germany	antipov@plasmachem.com
Alexev	Kalachem	Plasmachem GmbH	Rudower Chaussee 29 D-12489 Berlin Germany	plasmachem@t-online.de
Francisco	Sanmiguel	Torrecid, S.A.	Ptda Torreta s/n – Apdo. 18 – 12110 ALCORA (Castellón) España	smiguel@torrecid.com
Carlos	Concepcion	Torrecid , S.A.	Ptda Torreta s/n – Apdo. 18 – 12110 ALCORA (Castellón) España	carlos.concepcion@torrecid.com
Lorenzo	Calabri	Tec Star , Srl	TEC Star Srl	calabri@tec-star.it
Alberto	Borghi	Tec Star , Srl	Viale Europa, 40 41011 Campogalliano (MO) - Italy	borghi@tec-star.it

9 Copyright

NanoMILE

Engineered nanomaterial mechanisms of interactions with living systems and the environment: a universal framework for safe nanotechnology

Contract Agreement: NMP4-2012-Large-310451 Website: http://www.nanomile.eu
Coordinator: Eugenia (Eva) Valsami-Jones, University of Birmingham, Edgbaston, Birmingham B15 2TT, United Kingdom

No.	Beneficiary name	Short name	Country
1	The University of Birmingham	UoB	United Kingdom
2	Karlsruher Institut fuer Technologie	KIT	Germany
3	University College Dublin, National University of Ireland, Dublin	NUID UCD	Ireland
4	Commissariat a l'Energie Atomique et aux Energies Alternatives	CEA	France
5	Joint Research Centre of the European Commission	JRC	Belgium
6	Eidgenössische Materialprüfungs- und Forschungsanstalt	EMPA	Germany
7	Eidgenössische Anstalt für Wasserversorgung, Abwasserreinigung und Gewässerschutz	EAWAG	Switzerland
8	University of Geneva	UoGEN	Switzerland
9	Rijiksinstituut voor Volksgezondheid en Milieu / Institute for Public Health and the Environment	RIVM	Netherlands
10	The University of Exeter	UNEXE	United Kingdom
11	Ludwig-Maximilians Universität, München	LMU	Germany
12	The Regents of the University of California	UCLA	United States
13	Duke University	DU	United States
14	University of Utrecht	UU	Netherlands
15	National Research Centre for the Working Environment	NRCWE	Denmark
16	University of Edinburgh	UEDIN	United Kingdom
17	Institut fur Umweltmedizinische Forschung an der Heinrich-Heine-Universitat Dusseldorf GMBH	IUF	Germany
18	Vitrocell Systems GMBH	VC	Germany
19	Novamechanics Ltd.	NM	Cyprus
20	Nano4imaging GMBH	N4I	Germany
21	University of Ljubljana	UNI-LJ	Slovenia
22	Promethean Particles Ltd.	PROM	United Kingdom
23	Eurofins Agroscience Services GMBH	EF	Germany
24	European Virtual Institute for Integrated Risk Management	EUVRI	Germany
25	BASF SE	BASF	Germany
26	Biomax informatics AG	BIOMAX	Germany
27	Atanna AB	ATTANA	Sweden
28	Nanosight LIMITED	Nanosight	United Kingdom

Contents

1 Summary

Project Duration: 48 months

Project Funding: 10 M€

Nanotechnology is a rapidly evolving enabling technology with the potential to revolutionise modern life. On the nanoscale, common materials can take on entirely new chemical, physical and biological properties. These properties open up new possibilities for exploitation and commercial enterprise. However, an increasing body of scientific evidence would suggest that some materials in their nano-form may induce harmful biological or environmental effects through a variety of potential mechanisms, not all of which are fully understood or quantified as yet. Such questions are addressed by the rapidly expanding field of "nanosafety". Indeed, although significant research efforts have been made to make the risk assessment of nanotechnology possible, we are still lacking a mechanistic and systematic understanding of which physico-chemical parameters, or combination of parameters, govern the toxicity of nano-sized objects. Thus, we remain unable to ensure the protection of health and the sustainable commercialisation of nanotechnology.

NanoMILE intends to revolutionise nanosafety research through its robust and novel approaches to the selection and development of the test nanomaterials, its technically and computationally advanced integration of systems biology, its thoughtfully balanced toxicological / ecotoxicological approaches, its development of novel high throughput platforms for screening and its feedback loops for development of nanomaterials that are safer by design. Together, these approaches will result in ***a robust framework for classification of nanomaterials according to their biological impacts***. The advanced scientific expertise offered by the academic partners has been matched by a complement of fully committed and well integrated industrial partners, capato ble of contributing to or advancing the innovations of NanoMILE to industrial applications.

The NanoMILE project commenced on 1st March 2013 and will run for 48 months.

2 Background

NanoMILE builds on several highly successful previous FP7 projects lead by the coordinator, specifically NanoReTox and ModNanoTox. In particular, NanoReTox developed an approach to normalize data across the concentration ranges utilized for *in vitro* and *in vivo* studies and developed a heat-map approach to categorizing nanomaterials according to their toxicity. A key finding from NanoReTox was that intrinsic nanomaterial composition is the primary driver of toxicity, with copper oxide being the most toxic from the panel of metal oxide and metal particles assessed within that project. Building on that knowledge, NanoMILE has made particle choices that include both known benign materials, that we will attempt to make toxic by altering their properties in a systematic manner, and known toxic nanomaterials that we will attempt to make safer by systematically varying their properties, in order both to isolate (and derive a threshold value) for the various drivers of toxicity, and to develop a set of rules for safer by design nanomaterials. The systematically varied libraries of nanomaterials developed in NanoMILE will also form the basis for high content screening approaches and on the basis of the outcomes from the screening, for detailed assessment of toxicity and ecotoxicity across a range of end-points and species, in order to identify commonalities in terms of mechanisms of action.

From the ModNanoTox project, which was one of the EU-US modeling projects, focused on development of models for assessment of the environmental impact of nanomaterials, NanoMILE builds on the experiences regarding the limitations of modelling approaches, where Lazar modelling approaches were demonstrated to be inapplicable for nanomaterials in an environmental context at present due to significant data gaps that resulted in the training data set being insufficiently offset from the test data set for reliable correlations to be achieved (a public report on the outcomes and challenges is available for the NanoSafety Cluster). Thus, there are also some specific particle requirements for the QSAR modelling and the data integration that have been factored into the development of the nanomaterials libraries for NanoMILE.

3 Scientific and Technical Challenges

Despite being relatively new, nanoscience and nanotechnology have advanced rapidly in terms of generating scientific discoveries along with commercial applications. However, the field of nanosafety, which is the science of assessing hazards and risks from novel nanomaterials, has not kept pace with these developments and relevant to this project are some key areas where the current state of the art requires urgent progression and advancement in understanding. Potentially the greatest concern in the science of nanosafety is the lack of a paradigm for MNM mode of action, as emphasised in the recently published report by OECD Sponsorship Programme for the Testing of Manufactured Nanomaterials, which necessitates that each MNM is considered individually for its toxicity.

Here we highlight some key shortfalls and gaps in knowledge regarding nanosafety and illustrate how the NanoMILE project will address these and ultimately provide a new paradigm in nanosafety, thus substantially advancing the field beyond the current state of the art.

Challenge 1: *A large number of MNMs exist, many already in industrial production. Often behaviour and toxicity of nominally identical MNMs vary, perhaps a result of poor characterisation or understanding of their structure and complexity or perhaps resulting from batch-to-batch differences or poor synthesis control. Studies of the effect of a systematic variation in properties of MNMs on biological reactivity including toxicity are virtually non-existent. A paradigm systematically linking MNM properties with biological effects / toxicity is urgently needed.*

NanoMILE will select, synthesize/procure MNMs suitable for hypothesis-driven development of mechanistic models of nanomaterial interactions with organisms and the environment. To advance the current state of the art, it is essential to include in our study material MNMs designed to display systematic property variations, so that prototypic mechanisms of action of MNMs can be linked directly to specific properties and input into QS(P)AR models. Far from allowing these "designer" MNMs become obsolete at the end of the project, NanoMILE will redesign these MNMs in WP9 to make them safer by design.

A smaller range of MNMs will be purpose-designed for the project to address specific needs, where, for example, systematic property changes need to be tested or where freshly produced particles are required (e.g. respiratory effects of free MNMs versus aggregates/agglomerates, redox sensitive MNMs) or special labels need to be introduced (e.g. stable isotopes). This approach will give NanoMILE powerful tools to advance the current state of the art, held in many cases by project partners. Purpose made MNMs will also allow a systematic investigation of the effect of size within critical relevant size range as well as the role of shape and the presence of inorganic and organic nanomaterial coatings.

Extensive testing of a great number of MNMs is only possible through the high throughput platform of NanoMILE (see below). All materials procured or developed within this work package will be subjected to extensive physicochemical characterization using state-of-the-art methods (imaging, compositional and structural, and following where possible established (e.g. through QualityNano) protocols, thus avoiding problems of unreliable cross referencing of experimental results. The MNM characterization data will be integrated into the database of nanoparticle information being developed by partner Biomax.

Challenge 2: *Many MNMs are likely to undergo significant transformations during their life cycle, following their release and as they move into different biological or environmental compartments. These transformations have received limited attention to date & predictions of MNM behaviour are unsupported by robust data.*

NanoMILE will investigate and quantify the alteration and transformation of MNMs in products and during their use and release into the environment or biota. Exposure to MNMs in occupational, consumer or environmental settings may either be to the original, parent MNMs or to MNMs that have been incorporated into products and subsequently released, either in their original or altered form due to industrial or natural processes.

To date, few studies have tried to establish the changes that MNMs undergo when incorporated into, and released from, products [2] MNMs in textiles, paints, and sunscreens have, to some extent, been studied [3]. It has been shown that MNMs released from these products may be altered considerably and change their physical and chemical properties compared with the original MNM. Furthermore, the transformations that take place may vary considerably between MNMs, with some metals, such as Ag will potentially transform to sulfides, whereas certain metal oxides such as TiO_2 will remain largely unchanged over relevant timescales. A whole range of other behaviours may also take place, for example dissolution, complete or partial for some metal/metal oxide MNMs, or stabilisation by natural organic matter (humics and biomacromolecules) or proteins (see also below). As a result there is major uncertainty as to the state of many MNMs following their release.

WP3 will expose relevant MNMs selected from the libraries of WP2 to different processes, different biophysicochemical conditions, in order to characterize the changes in the MNM, and either deliver altered MNMs or provide detailed protocols on how to induce these alterations, to alternative WPs. These altered MNMs will then be used alongside the parent particles in WP4-9. Predictive models will be developed that describe release of MNMs from products to the environment and qualitatively and quantitatively assess the changes of MNMs properties during these processes. Significant advancement of the current state of the art will be through the generation of libraries of modified (but stable) MNMs for testing in subsequent WPs and by incorporating the effect of ageing as a further descriptor in the project's QS(P)AR models.

Challenge 3: *There are simply too many different MNMs to be tested by any one project or lab. Harmonisation of data across labs is a further challenge. A high throughput platform for hazard ranking is required.*

Cell lines and zebrafish embryos were recently used successfully for hazard ranking of ENM with HT/CS3, in a study first of its kind. Furthermore, using novel high throughput imaging approaches and advanced image analysis software multiple biological endpoints can be investigated, and in some cases in real time, in cell cultures and in zebrafish embryos. The availability, via the European Zebrafish Resource Centre (EZRC)[4], of thousands of mutants and transgenic lines which have specific gene alterations facilitates enormously the identification and confirmation of toxicity pathways.

One of NanoMILE's pioneering approaches is the practical incorporation of a high throughput platform, which will allow screening of a large numbers of MNMs/MNM variants at the start of the project, in order to identify "lead candidates" for subsequent work. High throughput and content screening (HT/CS) *in vitro* (cell culture) and *in vivo* (zebrafish) will therefore be established. The same high-throughput approach will be used again later on for the validation of results and establishment of causality of the discovered biomarkers for subsequent toxicity by using chemical and genetic interference strategies. The large volume of data generated by this work will be instrumental for the quantitative structure (property)-activity relationships (QS(P)ARs), to allow identification of no-observed-adverse-effect levels (NOAELs) and to predict the impacts from physico-chemical characteristics or "initial" corona characteristics. Notably, latter aspects of these innovations will be advanced to demonstration stage by industrial partners.

Challenge 4: *MNMs transform upon contact with biological or environmental media, and it is likely that a layer of biomolecules or geomolecules ("corona") cover their surface. The nature, properties*

and robustness of this layer and interactions between the core and the corona are currently poorly understood; it is also not clear how different environmental or biological compartments will impact on the formation of this corona.

The importance of the protein corona formed around nanoparticles upon contact with biological fluids or living organisms has recently been highlighted1, and it is now understood that it is not the bare nanoparticles that interact with living systems but rather the biological interface conferred by the adsorbed biomolecules that organisms actually "see", with the nanoparticle acting only as a scaffold [5, 6]. This corona is, when sufficiently long-lived, thought to govern the particles' biological fate. However, even this long-lived "hard" corona evolves and re-equilibrates as particles pass from one biological fluid to another, which may be an important feature for long-term fate. It has recently been shown that transfer of nanoparticles from one biological fluid (plasma) into another (cytosolic fluid), used as a simple illustrative model for the uptake of nanoparticles into cells, resulted in significant evolution of the corona in the second biological solution, but the final corona contained a "fingerprint" of its history [7] .

An important hypothesis is that this evolution could be used to map the transport pathways utilized by nanoparticles, and eventually to predict nanoparticle fate and behaviour based on characterisation of the initial corona in a representative biofluid. A similar concept for MNMs exposed via aquatic or terrestrial media containing natural organic matter (NOM, initial corona) taken up into organisms (final corona) has also been shown to exist [4, 5] and needs to be further investigated.

Beyond the current state of the art, NanoMILE will focus on the quantification (which has not been addressed to date) of MNMs interactions with environmental and biological macromolecules (proteins, lipids, sugars, nucleic acids, humics) before and after uptake and localisation, and correlation of nanomaterial-associated biomolecules with nanomaterial fate and behaviour in cells, organisms and animals. An important and novel objective will be to establish the precise nature and transformations of the coronas with time in realistic environmental conditions. Modelling of NP-biomolecule interactions will be included and data will feed into the development of QS(P)ARs. Methods will be optimised to be applicable for identification and quantification of proteins, lipids, sugars, natural organic matter etc., associated with nanomaterials over timescales of relevance for biological interactions (minutes) and each of the tasks will be conducted for a range of different biofluids, representative of the different exposure routes (inhalation, ingestion, intravenous, environmental (e.g. aquatic/terrestrial).

Challenge 5: *Although toxicological studies exist for a number of different species, many such studies produce different results and there is no framework for comparisons across species and in different environmental compartments (terrestrial / marine / freshwater). It is becoming clear that nanoparticles react with a biota in a nanoparticle specific manner where toxicity is one of the outcomes of these interactions. Others may include reduced energy reserves, reduced fitness and ultimately increased vulnerability.*

The current state of the art in this arena has advanced to the point where some patterns of toxicity emerge and there is understanding of internalisation of MNMs in biota. Recent advances also include novel tracers (stable isotope labelled MNMs) and better understanding of alternative sources of uptake (food versus water) by biota6 1. There is however currently no overarching framework for risk assessment.

NanoMILE will carry out investigations into in vivo bioavailability and effects related to nanoparticle exposure across wildlife species from single celled organisms to lower vertebrates (fish) and from subcellular to ecosystem level thus creating one coherent set of parameters for multiple species and MNMs. We will test hypotheses that specific features of MNMs confer toxicity through the use and application of modified MNMs and identify common effects across a wide range of wildlife taxa and establishing the most vulnerable organisms for potential harm. The focus will be on algae, daphnia, aquatic isopods and worms, and fish (zebrafish: adults and embryos), and for terrestrial animals *Caenorhabditis elegans*, earthworms (*Eisenia fetida*), springtail (*Folsomia candida*), and soil mite (*Hypoaspis aculeifer*) and a range of isopods with varying ecological niches. ENP selection will be based on results from the high throughput testing (WP4). This is an extensive set of organisms and MNMs tested under a universal framework and will generate a unique and valuable database.

There are currently no dedicated toxicity tests for MNMs in the soil environment, and NanoMILE will develop a dedicated demonstration study, by an industry partner, adapting findings from this WP.

Challenge 6: *Although a substantial volume of mammalian toxicological studies exist (in vivo and in vitro) a model for human toxicity has not yet emerged.*

Currently there is extensive state of the art on MNM toxicity that is obtained by in vitro studies. Such *in vitro* studies are very useful for identification of toxic potency and mechanistic studies, and can support the outcome of *in vivo* studies. However, the information does not fit well in risk assessment.[8] In addition, the availability of *in vivo* repeated dose toxicity studies is limited.[9] Such *in vivo* data are therefore urgently needed, as are new paradigms based on low doses and closely linking toxicology and biokinetics.

NanoMILE will evaluate distribution (biokinetics) and toxicological endpoints after exposure of cells, isolated organs and organisms. Nanoparticles with defined composition, size distribution, and surface properties from WP2 will be transferred into an aerosol with defined size/morphology, and deposited on lung cells via the air/liquid interface with well defined mass, number, and surface doses. For other cell types, submerged systems will be used. Mechanisms of toxicity (e.g. oxidative stress, inflammation, thrombogenicity) indicative for the induction of clinical adverse effects will be identified and correlated over the various physico-chemical characteristics and test systems in the project. There will be a focus on inhalation toxicity studies using aerosols, as this is one of the most likely exposure routes for humans, but both oral and intravenous application will also be used as relevant routes of exposure. Migration of MNMs, physical stress including frustrated phagocytosis and more complex responses of the immune, cardiovascular or central nervous system might be predicted using novel cell based *in vitro* systems as applied in this project.

The objectives will be realised by using realistic inhalation, oral and intravenous exposure scenarios, mimicking occupational, dietary and medical use of MNMs. Specific attention will be paid to low dose exposure and long term effects and to what extent short-

term toxicity testing plus toxicokinetics can predict the outcomes of long term exposure. In particular the predictive value of this approach for tissue accumulation will be assessed. In addition to assessments on local adverse effects of MNMs at the port of entry via routine pathology and biomarker evaluation, analyses of systemic toxicity, including effects on the immune, cardiovascular and central nervous system will also be determined.

In vitro experiments will be focused on identification of mechanisms involved in the acute toxicity for various endpoints (cell death, cytokine induction, oxidative stress, DNA damage (repair), proliferation, DC maturation etc.) and using models for identification of migration of MNMs across cellular barriers, whereas *in vivo* experiments (acute and repeated dose up to 28 days) will focus both on local effects depending on the exposure route, and systemic effects, especially immunotoxicity, neurotoxicity and cardiovascular effects, including models for diseases (allergy to proteins and low-molecular weight chemicals, atherosclerosis, deficient biological barriers, neurodegenerative disease).

Toxicokinetic experiments will be performed to evaluate MNM translocation and migration as predicted from in vitro models. Different routes of exposure will be explored, as well as the particle-characteristics that determine translocation (e.g. particle size and charge, presence of (protein-) coatings). The results for selected parameters will be evaluated against results obtained in non-mammalian species (zebrafish or *C. elegans*, see above) as proxy to determine possibilities for applying one of the 3Rs for alternative testing of the safety of MNMs.

Challenge 7: *Systems biology has in recent years emerged as a powerful tool for understanding biological mechanisms at the molecular level and using such information to generate predictive and mechanistic approaches in disease. These advances have yet to be applied in the field of nanosafety.*

NanoMILE will seek to discover and compare mechanisms and potencies of the potential harmful effects of different MNMs using an integrated Systems Biology approach, including transcriptomics, metabolomics, lipidomics and computational biology. These consortium participants are highly experienced in the application of 'omics technologies to studying biological responses to toxicants1. The overall aim is to identify prototypic mechanisms of action of MNMs, including both species-specific and evolutionarily conserved responses, with the latter likely to provide extremely powerful biomarkers in relation to assessing MNMs impacts on environmental and human health. This WP is linked tightly with high throughput work (WP4), both in regard to the initial selection of MNMs for detailed analysis and the application of the discovered novel molecular biomarkers in subsequent high throughput screening (HTS).

NanoMILE will employ both static and dynamic modelling to identify subsets of the multi-dimensional, information rich, 'omics datasets that represent adverse outcome pathways (AOPs), i.e. mechanistically based molecular biomarker signatures that can be implemented into diagnostic screening assays to identify and characterise the impacts of nanomaterials. So-called "Reverse Engineering" approaches, which are a branch of Systems Biology, will be used to reconstruct the underlying structure of biological pathways from observational 'omics data. Research by WP leaders [10, 11] has shown that these methodologies can be tremendously effective in biomedical research where they have already contributed to identifying networks predictive of clinical response, drug resistance and novel therapeutic targets. The dynamical models will also enable *in silico* simulations of the toxicity responses to MNMs, which will be tested experimentally.

WP8 will encompass several species/cell type spanning ecotoxicology and human toxicology, including algae (model plant), daphnia (model invertebrate), zebrafish (model vertebrate) and a human cell line. All this work is completely novel and represents advancement of the state of the art, both in scale and detail. At the same time the work is achievable being supported by other WPs (notably WP2 and WP4), and through the data integration and management capabilities of an industrial partner.

Challenge 8: *No platform exists for referencing and comparing the activity, in terms of toxic behaviour, of MNMs; no fundamental concept of safe MNM design has yet been developed.*

Following early work within NanoMILE which will discover systematically the precise mode of action of MNMs properties, key later activities will be carried out towards:

a) practically test such features by designing them in or out (both at bench and pilot scale);

b) develop models of quantitative structure (property) –activity relationships (QS(P)ARs) enabling predictive work to evolve and feed into risk assessment; and

c) provide an integrated platform for risk assessment.

Current understanding of MNM mode of action suggests there may be specific physicochemical features in MNM design that confer or influence toxicity. Such features or descriptors include aspect ratio ("asbestiform" MNMs or HARNs[1]), surface modifications and their stability, (oxidative) reactivity, hydrophilicity/hydrophobicity and size [12, 13]. More novel descriptors such as band gap have also been evoked.[14]

In order to design safer MNMs, the work in NanoMILE will involve a central iterative link between MNM properties and biological/environmental effects, i.e. if certain features of the particles become clear as inducing toxicological effects, then these features will be designed out in WP9 (keeping all other parameters constant as far as possible) and the particles will be re-tested to confirm those features conferred the observed toxicity; the opposite (design in features to create positive controls of certain magnitude) will also be applied. Once these modifications are tested and the principles of safer designs are established for one group of MNMs, similar principles will be transposed to other families of MNMs, to establish whether these apply and whether generic patterns of safer designs may begin to emerge.

One of the ultimate goals will be to test if this approach works across structurally and chemically different MNMs and across a

[1] High aspect ratio nanomaterials is a major focus for project NanoReg, and it has been decided to exclude from NanoMILE to avoid overlaps. However, high throughput work will cover HARNs and thus link up with NanoReg results.

range of sizes. Carbon based materials form a separate class of materials, although similarities in issues related to surface modifications apply across all classes. Designing safer MNMs will then be implemented at demonstration level by industry partners.

QSARs, perhaps more appropriately termed QPARs (as it is physicochemical "properties" rather than "structures" that need to be linked to a specific mode of hazardous activity) will form a fundamental component of NanoMILE. There are two main difficulties related to the development of nano-QSARs: The first is lack of sufficiently numerous and systematic experimental data and the second is the currently limited knowledge on mechanisms of toxic action. The former is being addressed in a number of major EU funded projects, data from which will feed directly into NanoMILE, via common project partners. The latter will be addressed within NanoMILE and knowledge acquired will transfer to this WP. An understanding of the relationship between the physical and chemical properties of the nanostructure and their *in vivo* behavior would provide a basis for assessing toxic response and more importantly could lead to predictive models for sub-classes and OECD recommends1 a procedure for the grouping of chemicals.

The overall objective of nano QPAR models is to relate a set of descriptors characterizing MNMs with their measured biological effects, for example, cell viability, or cellular uptake. Such models can then be applied to newly designed or commercially available MNMs in order to quickly and efficiently assess their potential biological effects.

The integration of technology and risk assessment with life cycle perspectives enables to identify innovation pathways for sustainable and responsible nanomaterials. With an integrated technology assessment NanoMILE aims to identify opportunities of new materials by integrating the results from all other work. We are linking the state of knowledge in research with the innovation processes in industries to facilitate sustainable innovation.

Challenge 9: *A lot of projects operate in isolation both laterally by not interacting with other concurrent research on the same or similar topic and temporally by missing existing background and allowing the generated foreground to lapse after the project ends.*

NanoMILE has a WP & team ensuring interactions with other major funded projects, to ensure recently acquired state of the art flows smoothly into the project, parallel developments from ongoing work are known to the research teams and future developments through NanoMILE flow into other projects and applications, ensuring the maximum possible impact by the project.

4 Project Objectives

The overarching objective of NanoMILE is to formulate an intelligent and powerful paradigm for the mode(s) of interaction between manufactured nanomaterials (MNMs) and organisms or the environment to allow the development of a single framework for classification of nanomaterial based on their potential toxicity and to create a universally applicable framework for nanosafety.

The specific objectives, in chronological order of development, are:

• **Objective 1:** To select and synthesise/procure MNM libraries suitable for hypothesis-driven development of mechanistic models of nanomaterial interactions with organisms and the environment, in harmony with, and linking to existing EU funded platforms, such as the EU funded QNano or the sponsorship programme of the OECD Working Party on Manufactured Nanomaterials (WP2).

• **Objective 2:** To establish an understanding of changes in the nature of MNMs as they undergo transformations within products and biological or environmental compartments across their life cycle and critically to feed this information into subsequent research to ensure that these "aged" and transformed MNMs are tested for their biological/environmental role (WP3).

• **Objective 3:** To establish a screening platform (WP4) based on high throughput techniques at two stages: a) at the start of the project, to screen for the most relevant MNMs and endpoints (using both classical and novel biomarkers) to provide a focus for subsequent WPs (5-8) and later, b) to screen the mechanistic discoveries from WP5-8 and develop test methods of the future.

• **Objective 4:** To qualify and quantify nanomaterial interactions with environmental (humic acids, polysaccharides, clays) and biological molecules (proteins, lipids, sugars, nucleic acids) before and after uptake into biological systems to enable understanding of how these interactions alter MNM fate and behaviour in cells, organisms and animals. To generate a computational-based screening platform for bionano interactions to allow tests on a comprehensive dataset of MNMs (WP5).

• **Objective 5:** To establish in-vitro and in-vivo reactions between MNMs and a carefully selected range of cell-lines/organs/organisms, representative of a wide range of species with increasing biological complexity, from algae to fish, aquatic and terrestrial species (WP6) and humans (WP7).

• **Objective 6:** To complement the above with a carefully selected range of systems biology based studies (WP8) to support the understanding and comparisons of mechanisms of MNMs activity across several species of increasing complexity.

• **Objective 7:** To more intelligently design safer MNMs (WP9), using the previous WPs as a guide, and working towards designing out adverse effect causing features.

• **Objective 8:** To develop appropriate models linking quantitative structure(property)-activity relationships (QS(P)AR), established from the biological effects studies above, to population response models, thus enabling predictive work to evolve from molecular mechanisms (specific toxicity pathways **and classification of MNMs according to their mode of action) to the scale of the ecosystem (WP9).**

• **Objective 9: To interact closely with other EU and US funded projects and the NanoSafety Cluster, to ensure maximum integration of prior state of the art within the project and progression along and beyond paths and platforms thoughtfully designed by these projects (WP10).**

The Workpackages (WPs) listed in the text above are interlinked and in constant communication with feedback-loops where information is iteratively fed into the WPs as shown in Figure 1.

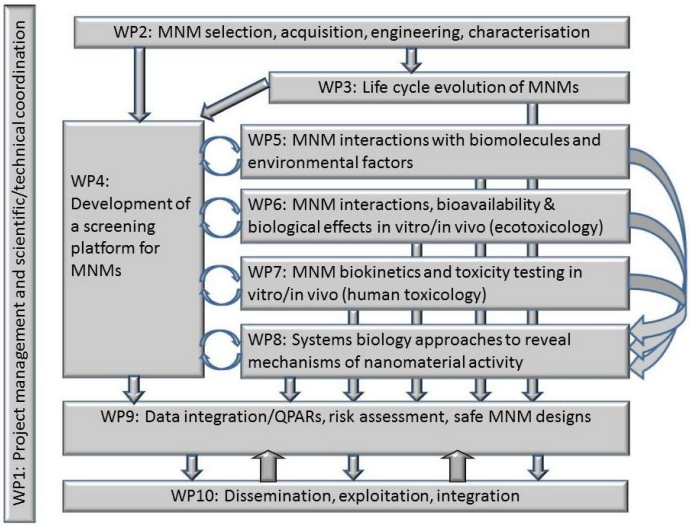


Figure 1. NanoMILE WP flow diagram and interdependencies

The scientific and technical goals of NanoMILE, as outlined in Section 2 above, could not be achieved by an effort at a national level. All the project partners are leaders in their respective fields, and have truly complementary scientific skills. None of the European states involved would individually have access to such a pool of competencies. This also applies to the range of facilities and resources mobilised by NanoMILE.

The NanoMILE consortium comprises 28 partner organisations selected for their ability to play unique and essential roles in the consortium, so as to address the call topic "NMP.2012.1.3-1: Systematic investigations of the mechanisms and effects of engineered nanomaterial interactions with living systems and/or the environment" in its entirety and at the highest technical level.

Of the 28 organisations, 10 are universities, 3 are research facilities, 5 are government bodies, 2 are multinational companies and 8 SMEs (3 technical consultants, 4 materials/instrumentation manufacturers). The two US partners are critically selected and ideally placed to add strength to the consortium by providing expertise at the highest technical level, thus matching and augmenting the capabilities of the European part of the consortium.

5 Progress and Outcomes to date

All workpackages are progressing well, with a range of exciting outputs presented at the 1st Annual meeting held in Antalya, Turkey in conjunction with NanoTox2014. The first technical deliverables, including reports on the Phase 1 particle library, the approaches to ageing of nanomaterials, and the approachces for time-resolved characterization of nanomaterials have been delivered. Summaries of some of the key outcomes to date are reported below.

Selection of Nanomaterials

NanoMILE aims to develop mechanistic models for the interaction of MNMs with biological systems and in the light of this approach the selection of the materials for study will originate from a consideration of physico-chemical properties rather than the more commonly adopted approach of simple chemical compositions, industrial scale or perceived commercial relevance. The final selection of MNMs for mechanistic assessment of their interactions with living systems was based on a consideration of a number of factors, including:

1) The hypothesised mechanism of biological activity.
2) The availability of MNMs via one of the NanoMILE sources.
3) The feasibility of systematically modifying key physicochemical parameters for the basic material type.
4) The availability of existing toxicological data (avoiding duplication).
5) The identification of gaps in existing materials evaluation.
6) Special needs such as for MNM detection and labelling (stable or fluorescence).

To achieve this, the MNM libraries to be developed in the NanoMILE project have been selected following careful review of other relevant projects (see review of current literature included in Deliverable Report D2.1), commercial availability as well as expertise and capabilities available within the NanoMILE project. The NanoMILE project aims to be comprehensive in its coverage of MNMs, and thus a wide range of MNMs will be selected from the major classes of materials - metals, metal oxides, carbon based structures, functionalised structures and core-shell structures.

A tiered MNM procurement/production strategy is being adopted within NanoMILE, as follows:

- A first group of MNMs has been sourced from variety of external sources (industry, commercial, research repositories, MNM libraries from other EU funded programs/projects as well as national/international standards organizations) and will be rapidly available to the partners for establishment of test protocols and harmonisation of approaches within and across WPs.
- A second group of MNMs will be produced by appropriate chemical or physical modification of selected members of the first group of MNMs. Key partners in WP2 will be involved in these modifications
- Finally, a third group of specialised and highly tailored materials will be fully synthesised in-house by selected project partners. Here the focus will be on development of nanoparticle libraries and on systematically designing out key properties linked to toxicity (e.g. dissolution or positive surface charge) and assessment of the effect of such changes on other physico-chemical properties and on biological impact. For example, it is conceivable that changing the dissolution potential of an MNM may introduce other toxicity features such as biopersistence, and these aspects will also be assessed, and recommendations regarding optimal design criteria developed.

Following early work within NanoMILE which will discover systematically the precise mode of action of MNMs properties, key later activities will be carried out towards:

- a) practically testing such features by designing them in or out (both at bench and pilot scale);
- b) developing models of quantitative structure (property) –activity relationships (QS(P)ARs) enabling predictive work to evolve and feed into risk assessment; and
- c) providing an integrated platform for risk assessment.

All materials procured or developed within NanoMILE are being subjected to extensive physicochemical characterization using state-of-the-art methods (imaging, compositional and structural, and following where possible established (e.g. QualityNano, NanValid, NanoReTox) protocols, thus avoiding problems of unreliable cross referencing of experimental results.

By adopting this three-pronged strategy NanoMILE intends to produce a sufficiently large matrix of MNMs in a relatively short time period so as to be able to benefit adequately from the availability of the high throughput test facilities which are a key aspect of this project. The procurement and production activities will be conducted in parallel.

Table 1: Initial selection of MNMs for assessment in NanoMILE and key descriptors that will be systematically assessed / varied.

Nanomaterial	Justification for selection	Key descriptors	1st group (commercial)	2nd group (modified)	3rd group (bespoke)
CeO_2	Low solubility -> low toxicity Redox variations Isotopic label available Commercial value	Redox state Size Shape Solubility	Sigma JRC repository	Yes (PROM, UoB)	To be decided by Month 12 (MS3)
ZnO	High solubility -> high toxicity Isotopic label available High commercial importance (multiple applications)	Size Shape Dissolution rate / coating	JRC repository	Yes (UoB)	To be decided by Month 12 (MS3)
Ag	Variable solubility -> variable toxicity Isotopic label available High commercial value	Size Shape Dissolution rate / coating Surface defects	Sigma Sciventions Ltd.	Yes (JRC)	To be decided by Month 12 (MS3)
FexOy	Likely low solubility -> low toxicity Multiple structures & Magnetic properties Potential for labelling Medical applications	Crystal structure / phase Magnetic properties Coating Size	Sigma	Yes (PROM, N4I)	To be decided by Month 12 (MS3)
Graphene / other carbon-based MNM	High commercial relevance (e.g. Graphene Flagship) Non-spherical -> potential for alternative mechanisms of action	Aspect ratio Shape / structure C/O ratio / surface groups Surface functionalisation	Thomas Swan	Yes (CEA) (also negotiations with Graphene Flagship partners underway)	To be decided by Month 12 (MS3)

SiO_2	Easily fluorescently labelled Multiple synthesis routes Low toxicity generally, though evidence that structural transformations can induce toxicity (e.g. fumed silica)	Size Porosity	JRC repository IRMM standards BAM - Federal Institute for Materials Research and Testing	Yes (JRC)	To be decided by Month 12 (MS3)
TiO_2	Low solubility -> low toxicity Multiple coatings available Different crystal phases Commercial value Photoreactive	Crystal structure / phase Coating (ageing) Size ROS production	JRC repository NIST standards	Yes (PROM)	To be decided by Month 12 (MS3)

Towards a strategy for grouping and classification of Nanomaterials

A novel approach to identify interlinked physicochemical descriptors, and on this basis identify overarching descriptors (axes or principle components) which can be used to correlate with toxicity is proposed. An example of the approach is provided, using three principle components which we suggest can fully describe each NM, these being the *composition*, the *intrinsic* (inherent) properties of the NM, and *extrinsic* properties (interaction with media, molecular coronas etc.).

Within NanoMILE, we developed a hypothesis that NM toxicity can be predicted as the sum of three "quantifiable" parameters (principle components, PCs) that capture the diversity of modes of action of Nanomaterials, namely*:*

- *Composition* which includes inherent molecular toxicity, charge, hydrophobicity and coating (although also linked to both the intrinsic and extrinsic axes).
- *Intrinsic* properties which are inherent to the nano-form of a material, and include e.g. structure and structural strain. A number of NM physicochemical properties map onto the *intrinsic* axis, including shape, porosity, structural configuration and bandgap.
- *Extrinsic* properties which are those corrected to the surface area of the NM, including e.g. surface interactions and transformations of NM surface and biomolecules (e.g. unfolding, receptor activation, membrane damage, fibrillation etc.) as a result of binding.

Clearly, each of these PCs/axes will have multiple contributors, and the relative contribution will vary for different NMs and will need to be teased out as part of the overall quantification of each PC. Key features of this approach are that: (1) it allows separation of modes of action (e.g. dissolution is primarily associated with specific NM *compositions*, but can be facilitated by specific *intrinsic* properties such as high strain conformation associated with, for example, pointed structures such as needles or nano-stars, and by *extrinsic* factors such as strongly binding ligands that unfold and expose cryptic epitopes for example; and (2) identities the major physico-chemical descriptors driving the toxicity from the weight of contribution to each PC/axes can be explicitly determined (i.e. the loadings), as can the main mode of action as this will be the PC with accounting for the highest amount of the variability in the data, as shown schematically in Figure 2. Thus, we envisage utilisation of a set of scales from low to high (toxicity) for each of the three parameters with the overall toxicity being the sum of the three axes. The fact that the same physico-chemical descriptor can contribute to more than one PC is a key feature of this approach, which we expect will enable development of QNARs, facilitate the grouping of NMs on the basis of where they sit in this 3-dimensional space, and support regulatory decision-making. The role of *intrinsic* (structural) and *extrinsic* (surface and interface with media) properties has only recently begun to emerge in the context of nanotoxicity descriptors; the relative significance of these two groups of properties, as well as internal scaling are yet to be established.

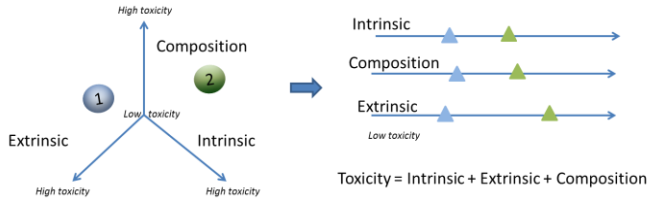


Figure 2. A principle components approach to predicting nanomaterial toxicity, where the experimentally determined physico-chemical parameters are mapped onto 3 axes (principle components), proposed here as and composition, intrinsic properties (i.e. those inherent to the nanostructure that are independent of the surroundings), and extrinsic properties (i.e. those affected by the surroundings). By determining/predicting where a specific nanomaterial sits in terms of each scale it will be possible to predict its toxicity. From : I. Lynch, et al., Nano Today (2014), http://dx.doi.org/10.1016/j.nantod.2014.05.001.

Linking characterisation data to toxicity and ecotoxicity assays

A key feature of the NanoMILE project is the development of integrated datasets regarding mechanisms of NMs toxicity across a range of environmental species and cell models, and thus it is vital that the characterisation work undertaken here and in WP2 is closely integrated with the assays that will be performed in the other workpackages (WPs), including utilising

agreed media and serum (where appropriate). To this end, WP5 worked closely with WP4 and WP6 – WP8 to ensure that the selection of representative media for WPs 6-8 (MS10) was fed into the characterisation studies of WP5 related to characterisation of interactions of MNMs with biomolecules and environmental factors e.g. natural organic matter.

Based on this WP5 undertook time resolved characterisation of MNMs in different media – deionised water, PBS, 10% FBS dispersed in PBS, and Zebrafish embryo media (ZEM). These media were selected to provide a range of representative dispersions and to increase in complexity of composition and thus potential for impact on MNM dispersion quality and stability. Deionised water as a control, FBS (NanoMile centralized serum) used to represent conditions in biological systems, PBS as a standard buffer and zebrafish embryo media (recipe supplied by WP6) representing ecotoxicological media. Subsequent studies in WP5 will also utilise these representative media to ensure cross-comparability of datasets and facilitate data integration.

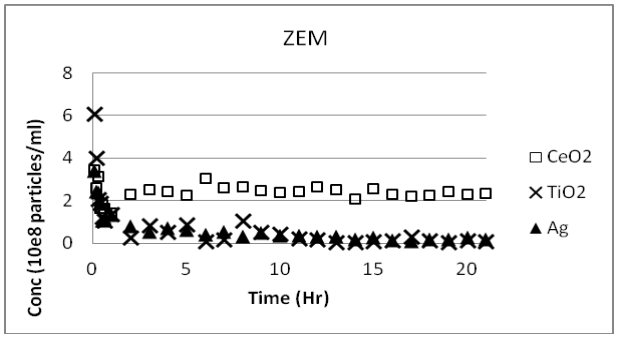


Figure 3. Graphs displaying concentration values for CeO_2, TiO_2 and Ag MNMs dispersed in Zebrafish embryo medium (ZEM) over a 21 hr duration.

6 NanoMILE's Expected Impacts

"Nanotechnology businesses and organizations will restructure toward integration with other technologies, distributed production, continuing education, and forming consortia of complementary activities." [15]

The volume of MNM production has led to significant concerns about the risks to human health and environmental impact as potential pollutants of considerable importance. Sustainable development of ENMs in industry requires the minimisation of these risks. The results of the NanoMILE project will be formulated into a number of tools to assist industry and regulators in identifying where specific safety assessments might be necessary, and as such close links with NanoFutures, and the relevant ETPs will be implemented. A priority will be to support both industry and public acceptance via development of scientific principles as the basis for improved regulation with clear and simple rules. Currently, there appears to be a lack of knowledge in the general public, although there is broad support for nanotechnology where knowledge exists; an improved general knowledge of hazard, risks and benefits is therefore essential.

NanoMILE will contribute significantly to the efforts to reduce the many uncertainties about the potential impact of MNMs on health and the environment, which is urgently needed for the development of a sound regulatory framework. It is crucial to learn what the parameters are that govern the toxicity of nano-sized objects and what the underlying mechanisms are for the sustainable development of MNMs. It is also important to note that regulatory uncertainly leading to delays in commercialisation is more costly to business than clear additional regulatory requirements.[16] A sound regulatory framework has also been requested by the European Parliament which considered it particularly important to address MNMs explicitly within the scope of legislation on chemicals, food, waste, air and water, and worker protections.

From the technical challenges identified above, and the workpackage structure designed to address these challenges, the NanoMILE consortium have identified a number of key outputs that will have significant impact for the various stakeholders involved in the nanosafety and nanocommercialisation question. Table 1 below summarises the key stakeholders for the outputs from the NanoMILE project, with whom targeted dissemination activities will be undertaken. An outline of the sorts of dissemination activities planned to address the needs of each stakeholder group is also given in Table 1.

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7 Directory

Table 2. Directory of people involved in the NanoMILE project

First Name	Last Name	Affiliation	Address	e-mail
Antreas	Afantitis	NovaMechanics Ltd.	John Kennedy Avenue 62-64 Lefkosia 1046, Cyprus	afantitis@novamechanics.com
Teodor	Aastrup	Attana AB	Grev Tureg 14 3 1/2 TR Stockholm 114 46, Sweden	teodor.aastrup@attana.com
Paul	Borm	Nano4Imaging GMBH	Pauwelsstrase 17, Aachen 52024, Germany	pbo@nano4imaging.com
Marie	Carriere	Commissariat a l'Energie Atomique et aux Energies Alternatives	15 rue des Martyrs, GRENOBLE 38054, France	marie.carriere@cea.fr
Flemming	Cassee	Rijiksinstituut voor Volksgezondheid en Milieu	RIVM PO Box 1, 3720 BA Bilthoven The Netherlands	Flemming.cassee@rivm.nl
Kenneth	Dawson	National University of Ireland, Dublin / University College Dublin	Belfield, IE-4 Dublin Ireland	kenneth.a.dawson@cbni.ucd.ie
Damjana	Drobne	University of Ljubljana	Department of biology, Biotechnical faculty, University of Ljubljana, Vecna pot 111 , Ljubljana	damjana.drobne@bf.uni-lj.si
Peter	Gooden	PROMETHEAN PARTICLES LTD	University Boulevard, Nottingham Science Park, Faraday Building 6 Nottingham NG7 2QP, UK	Pete.Gooden@proparticles.co.uk
John Patrick	Hole	Nanosight Ltd.	London Road Minton Park, Wiltshire SP4 7RT, United Kingdom	Patrick.hole@nanosight.com
Tobias	Krebs	Vitrocell Systems GmbH	Fabrik Sonntag 3, Waldkirch 79183, Germany	t.krebs@vitrocell.com
Iseult	Lynch	University of Birmingham	School of Geography, Earth and Environmental Sciences, Edgbaston,Birmingham B15 2TT United Kingdom	i.lynch@bham.ac.uk
Dieter	Maier	BIOMAX INFORMATICS AG	Robert Koch Strasse 2 Planegg 82152, Germany	dieter.maier@biomax.com
Jutta	Muether-Paul	Eurofins Agroscience Services GMBH	Eutinger Strasse 24 Niedern OSchelbronn 75223, Germany	JuttaMuetherPaul@eurofins.com
Mark	Miller	University of Edinburgh	Centre for Cardiovascular Sciences Little France Crescent 47, Edinburgh, EH16 4TJ, United Kingdom	mark.miller@ed.ac.uk
Andre	Nel	The Regents of the Uni9versity of California	UCLA Medicine, 10833 Le Conte Ave 52-175 CHS, Los Angeles 90095-1736, USA	anel@mednet.ucla.edu
Bernd	Nowack	Eidgenössische Materialprüfungs- und Forschungsanstalt	Technology and Society Laboratory, Lerchenfeldstrasse 5, St. Gallen CH-9014, Switzerland	nowack@empa.ch

First Name	Last Name	Affiliation	Address	e-mail
Francois	Rossi	Joint Research Centre of the European Commission	Institute for Health and Consumer Protection – Unit Nanobiosciences Via E. Fermi 2749, Ispra 21027, Italy	francois.rossi@jrc.ec.europa.eu
Joachim	Rädler	Ludwig-Maximilians Universität München	Geschwister-Scholl-Platz 1 Munich 80539, Germany	joachim.raedler@lmu.de
Olivier	Salvi	European Virtual Institute for Integrated Risk Management	19 Haus der WirtschaftWilli-Bleicher-Strasse, Stuttgart 70174, Germany	salvi@eu-vri.eu
Kristin	Schirmer	Eidgenössische Anstalt für Wasserversorgung, Abwasserreinigung und Gewässerschutz	Environmental Toxicology Ueberlandstrasse 133 Duebendorf 8600, Switzerland	kristin.schirmer@eawag.ch
Roel	Schins	Institut fur Umweltmedizinische Forschung an der Heinrich-Heine-Universitat Dusseldorf GMBH	Auf M Hennekamp 50, Dusseldorf 40225, Germany	roel.schins@uni-duesseldorf.de
Serge	Stoll	University of Geneva	Institute Forel, Physical Chemistry Group, route de Suisse, CP 416 10 Versoix 1290, Switzerland	serge.stoll@unige.ch
Gert	Storm	University of Utrecht	Universiteitsweg 99 Utrecht 3584 CG, Netherlands	g.storm@uu.nl
Charles	Tyler	University of Exeter	Hatterley Laboratories, Prince of Wales Road Exeter EX4 4PS, United Kingdom	euresearch@exeter.ac.uk
Eugenia	Valsami-Jones	University of Birmingham	School of Geography, Earth and Environmental Sciences, Edgbaston,Birmingham B15 2TT United Kingdom	e.valsamijones@bham.ac.uk
Håkan	Wallin	National Research Centre for the Working Environment	Lerso Parkalle 105, Copenhagen 2100, Denmark	hwa@nrcwe.dk
Carsten	Weiss	Karlsruher Institut fuer Technologie	Institute of Toxicology and Genetics, Hermann-von-Helmholtz-Platz 1, Eggenstein-Leopoldshafen 76344, Germany	carsten.weiss@kit.edu
Karin	Wiench	BASF SE	Carl Bosch Strasse 38 Town 15 Ludwigshafen 67056, Germany	karin.wiench@basf.com
Mark	Wiesner	Duke University	Department of Civil and Environmental Engineering, 324 Blackwell Street, Suite 920, Durham 27701, USA	wiesner@duke.edu

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NanoPolyTox

Toxicological impact of nanomaterials derived from processing, weathering and recycling of polymer nanocomposites used in various industrial applications

Contract Agreement: NMP-ENV-2009-247899 Website: http://www.nanopolytox.eu
Coordinator: Socorro Vázquez-Campos, LEITAT Technological Centre, Barcelona, Spain

No.	Beneficiary name	Short name	Country
1	LEITAT Technological Centre	LEITAT	Spain
2	Global Nanotechnologies S. A.	GLONATECH	Greece
3	Institut Català de Nanotecnologia	ICN	Spain
4	Polyrise S. A. S.	POLYRISE	France
5	L'Urederra Technological Centre	L'UREDERRA	Spain
6	DHI Water & Environnent	DHI	Denmark
7	Laviosa Chimica Mineraria S.pA.	LAVIOSA	Italy
8	Lati Themoplastic Industries S.p.A.	LATI	Italy

Contents

1 Summary

NanoPolyTox was a small-medium size collaborative project from the FP7 within the topic NMP-2009-1.3-1: Activities towards the development of appropriate solutions for the use, recycling and/or final treatment of nanotechnology-based products (Joint call with Theme 6: Environment including Climate Change).

The main objective of NanoPolyTox project consisted of monitoring the evolution (nanomaterials (NM) properties and toxicity) of three families of nanomaterials (nanotubes, nanoclays, metal oxide nanoparticles) during their life cycle as nanofillers in polymeric hosts. This project included monitoring of the physical and chemical properties of the nanomaterials and their toxicity from the synthesis, during processing, use (accelerated aging) and recycling to their end of life (disposal), quantifying their migration and/or release to the environment during their use (aging). Data generated also contributed to assess the biological and environmental fate of these nanomaterials. Furthermore, the theoretical analysis of the data obtained during the project led to the development of predictive models for the impact of nanomaterials on human health and environment. Conventional methodology used for Life Cycle Assessment (LCA) analysis was modified to be meaningful for the use of these tools for NM-based products impact analyses. Additionally, two recycling strategies were considered in order to propose solutions for the recovery of innocuous nanomaterials. For this purpose, exhaustive evaluations for the selection of adequate dissolving and separation methods to collect the nanomaterials from the polymeric matrix were performed. Finally, disposal strategies of toxic nanomaterials proposed in the project were based on immobilization of these materials in inert matrices.

2 Background

The global industry is moving forward taking advantages of the new opportunities and prospects offered by nanotechnology; therefore it is necessary that these developments take place in a

safe and sustainable manner. The increasing use of nanomaterials in consumer products has raised concerns over their safety to human health and the environment. Currently, there are major gaps regarding to the health and environment risks presented by nanomaterials. During the cycle life of a nanomaterial, workers and consumers are exposed to these materials. While workers are exposed during the process of production and the process of recycling or disposal of the industrial nanoproducts, consumers are exposed during the use of the products. Moreover, sooner or later the nanomaterials are free to enter the environment. Therefore, an exhaustive characterization and toxicity evaluation at different stages of the life cycle of nanomaterials used in industrial production is required. NanoPolyTox project proposed to study the evolution of nanomaterials physico-chemical and toxicological properties during their life cycle to evaluate their global environmental impact. Furthermore, NanoPolyTox studies included the development of strategies for the recycling and disposal of nanomaterials that are included in polymeric matrices.

3 Scientific and technological challenges

Nanopolytox was focused on after-production stages and addressed the following issues for the products considered: Physical and chemical characterization, hazard characterization (human toxicity and ecotoxicity), environmental and biological fate, transformation, and destiny of nanoparticles. Additionally, this project provides, at laboratory scale, technological solutions for recycling and final treatment of nanotechnology-based products.
This project has provided, for the first time, a complete description (physico-chemical and toxicological data) of eighteen nanomaterials (MWCNT, nanoclays and nanoxides) during their life cycle: processing, use, and end of life processes (recycling/ disposal). Furthermore, the migration and consequently release of the different nanomaterials from different polymeric matrices during their use phase was monitored. Specific information about the biological and environmental fate of some of these nanomaterials has been obtained. This project has provided a predictive theoretical model constructed from the collected data on the dependence of nanomaterials size, morphology and chemical composition with the *in vitro* and *in vivo* toxicological data for the nanomaterials along their life cycle. Finally, Nanopolytox proposed and demonstrated technological solutions for recycling of innocuous NM from polymeric nanocomposites and an immobilization method for disposal of toxic nanomaterials.

4 Objectives

The main goal of NanoPolyTox was to improve the understanding of the potential environmental/health impact of nanotechnology-based products over their life cycle. Gathering and generating data on the possible impact on human health and/or the environmental impact derived from the use, re-use, recycling and/or final treatment and disposal of nanotechnology-based products containing engineered nanoparticles.

The specific objectives were the following:

- The preparation of nanomaterials from three different families (carbon nanotubes, nanoclays and metal oxide nanoparticles) including adequate tailoring functionalities for their inclusion in three selected polymeric hosts widely used in several industrial sectors
- Generation of nanocomposite samples by processing in double screw extruders and injection in test specimens
- Weathering of the raw nanomaterials and the nanocomposite test specimens in climatic chambers
- Fully characterization (physical and chemical properties) of all the samples (raw nanomaterials and nanocomposites) during their life cycle
- Collection of toxicological data (*in vitro* and *in vivo* human toxicity and ecotoxicity) for selected samples to evaluate the risks associated with their manufacturing, use, recycling and disposal
- Development of predictive models based on the data obtained for the evolution of the physico-chemical and toxicological properties of the nanomaterials along their life cycle
- Detection and quantification of possible migrations and/or releases of the nanofillers from the polymeric matrices, establishing a relationship between weathering cycles and migration/release of nanomaterials
- Mechanical and chemical recycling for innocuous nanomaterials
- Development of new solutions for the disposal of toxic nanomaterials based on the calcination of polymer and the inclusion of the recovered toxic nanomaterials, into an inert matrix to create ceramic blocks that prevent any leakage of these nanoparticles.
- Evaluation of the global environmental impact of nanomaterials that are highly used in many industrial sectors during their life cycle by LCA analysis specifically complemented with the data obtained during this project and other European projects related to nanosafety.

5 Progress and Outcomes to date

NanoPolyTox is a 3-year project which started in May 2010; the results and conclusions on the project will be described below.

5.1 Synthesis and characterization of NM

The main goal of WP1 was to synthesize and characterize the nanomaterials to be used for the development of the project.

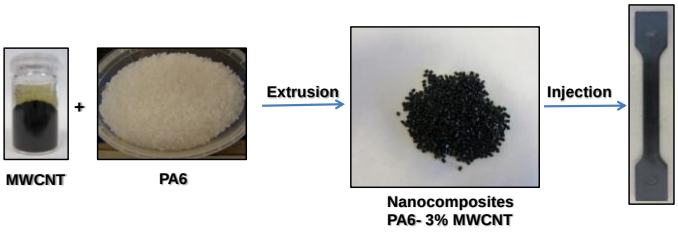
The syntheses of the selected nanomaterials (MWCNT, nanoclays and metal oxide nanoparticles) have been carried out following different methods. MWCNT were synthesized by catalytic chemical vapor deposition (CCVD) processes at high temperatures obtaining CNT with high purities. The synthesis of MWCNT with different surface properties (hydrophobic, amphiphatic and hydrophilic) was performed using wet chemistry procedures. The synthesis of nanoclays (two types of nanoclays with different particle size) was carried out by a two step wet chemistry procedure: Purification of

the natural occurring clays and subsequent ion exchange reaction to modify the nanoclays with three different content or structure of quaternary ammonium salts. Furthermore, metal oxide nanoparticles (SiO_2, TiO_2 and ZnO NP) were synthesized by flame spray pyrolysis which relies on the direct introduction of liquid raw materials into a flame. Metal oxide NP have been functionalized by wet chemistry leading to NP with different surface properties (hydrophobic, amphiphatic and hydrophilic). Full physical-chemical characterization of each NM was carried out and the data collected was included in the technical card which is the ID of the nanomaterials through all their life cycle.

5.2 *Development of polymer nanocomposites*

Polymeric matrices have been selected for the studies of this project; the matrices selected were Polypropylene (PP), Ethyl Vinyl Acetate (EVA) and Polyamide 6 (PA6). Three polymeric matrices with different polarities were chosen to amplify the range of application of the polymeric nanocomposites. The nanomaterials obtained from WP1 were then incorporated into the polymeric matrices by extrusion processes and subsequently injected to obtain the polymeric nanocomposite demonstrators.

The concentration of nanomaterials in the polymeric matrices after each step of processing (extrusion and injection) as well as the compatibilization and dispersibility of nanomaterials in the polymeric matrices were evaluated. Furthermore, physical characterization of the polymer nanocomposite demonstrators was focused on mechanical properties and thermal resistance determination. The results obtained showed that most of the polymer nanocomposites presented improved mechanical properties and/or thermal resistance. These tests also showed that a better dispersion of the nanomaterial in the polymer yielded to nanocomposites with improved properties. Promising results have been obtained with EVA reinforced with nanoclays (big increase in tensile modulus and temperature of oxidation 40 °C higher), PA6 reinforced with MWCNT (higher elastic modulus and noticeable improvement in crystallization temperature, what means easier processing) and with nanoclays (important increment in resistance to impacts). All data was included in the technical cards.



5.4 *Weathering of polymer nanocomposites*

The demonstrators developed in WP2, raw nanomaterials and unmodified polymers were subjected to the selected aging conditions (combination of climate and sunlight radiation during 1000h, under the modified normative ISO 4892-2) and the potential releases of nanomaterials from the polymeric matrices during ageing processes were collected and quantified.

Aged nanomaterials in the powder form were analyzed after being submitted to the whole process of ageing. All the nanomaterials but nanoclays showed alterations compare to the corresponding non-aged nanomaterial. The main difference was the hydration of all the nanomaterials. Furthermore, in some cases, the surface of nanomaterial encountered some modifications in the surface area, pore volume and/or pore diameter. For functionalized nanomaterials, a gain in surface area values indicates a loss of functional groups on the surface of the nanomaterial or a decrease in particle size. In the case of MWCNT, a thermal inestability was observed. ICP-MS analyses showed important metal leaching in the case of ZnO NM, which is reduced in functionalized nanomaterials.

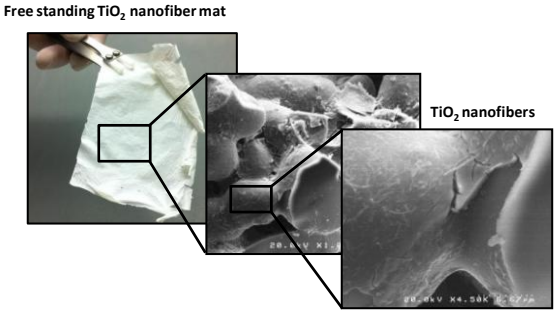
Aged polymeric nanocomposites have also been characterized. As in WP2, mechanical properties and thermal stabilities have been studied. Results showed that in some cases nanomaterials strongly protect the composites from degradation, but in other cases there is no protection or even embrittle the material and do them more thermally unstable.

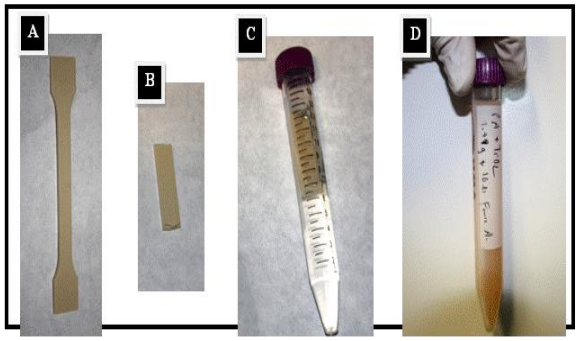
To evaluate the surface degradation and migration of nanomaterial from the polymeric matrices, nanocomposites were analyzed by TEM, SEM and FT-IR and compare with those before ageing. Chemical degradation (visualized by FT-IR) and an increase of the surface roughness, cracks and fissures (observed by SEM and TEM degradation could be observed in all nanocomposites.

These results were complemented with the data obtained for the materials recovered from the simulated rain during aging. The materials released from the polymeric matrices during ageing were composed of a mixture of degraded polymer and nanomaterial surrounded with degraded polymer. Regarding the results obtained, it was concluded that the release of nanofillers from polymeric matrices depends on polymer structure, the compatibility between the nanofillers and the matrices,and the shape of nanomaterials being the fibrous (MWCNT) and platelet (nanoclays) structures those that are less released.

5.4 *Development of non-destructive separation techniques: Proof of concept of NM recycling and disposal techniques*

The main objective of this WP was the development of techniques for the separation of nanofillers from polymeric matrix without inducing degradation of the nanomaterials under the form encountered in the composites. Therefore firstly, research was conducted in the methodologies to dissolve the polymers without affecting the physico-chemical properties of the nanomaterials. From the different methodologies proposed, we selected the mildest conditions. Recovery of nanofillers from polymer nanocomposites was achieved following different strategies (calcination for polymers that were difficult to dissolve under mild conditions, using mild dissolving methods, and mechanical processes). Complete recovery of NM or nanocomposite recycling could be achieved following these strategies.





5.5 *Toxicological and ecotoxicological evaluation of NM at different stages of their life cycle*

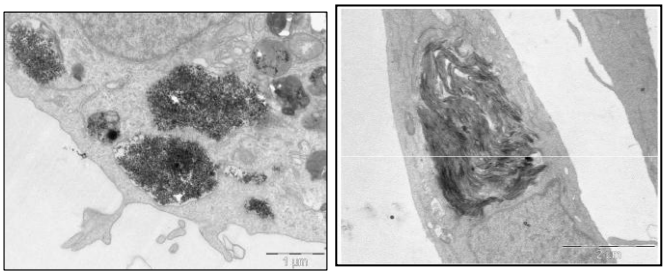
The starting point of the toxicological studies was the dispersion of nanomaterials in aqueous solution. The raw nanomaterials studied in NanoPolyTox project have diverse chemical compositions and surface properties, which make more difficult the selection of the adequate dispersant. The dispersion studies were carried out case by case and the dispersion protocols were described for each nanomaterial. The control over the stability of nanomaterials in the dispersion medium was studied and the best dispersion medium was selected for each nanomaterial.

The (eco)toxicity of raw nanomaterials, both aged and non-aged, and nanomaterials extracted from aged and non-aged nanocomposites were evaluated in a battery of human cell lines and in a fish embryo test. A series of mechanistic assays, such as apoptosis induction, cell proliferation and cell internalization, were performed. The results showed considerable differences in toxic potential and mechanisms of toxicity among the nanomaterials of the project, but relatively good correspondence between the toxic potential in fish embryos and in human cell lines. In both test systems, nanoclays were the most toxic nanomaterials, and their toxicity seemed to be associated to the organic modifiers used in their functionalization.

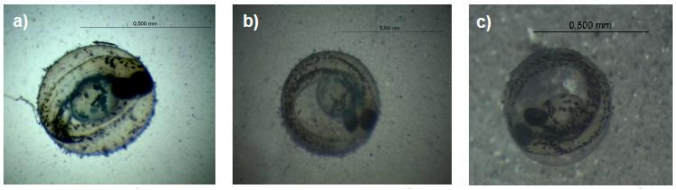
In addition to eco(toxicity), one important factor to understand the possible effects and the biological fate of nanomaterials is the degree of cell internalization and its subcellular location. Nanomaterials were found internalized in cells and most of them were present in cytoplasmatic vesicles. Together with these studies, the bioavailability The last was evaluated by two approaches; Caco-2 cells monolayer *in vitro* model and *in vivo* rat model. The results obtained by *in vitro* and *in vivo* approaches were concordant and they showed that oral absorption of NP is very low and it could be observed a slight statistically significant increase in nanomaterial levels in some places such as small intestine, Peyer's Patches and/or sporadic cells.

Finally, biodistribution, bioaccumulation and environmental fate of nanomaterials were evaluated. A similar biodistribution pattern was observed in which NP are accumulated in tissues of the reticular entothelial system, mainly liver and spleen. In contrast, the elimination pattern did differ depending on the chemical properties of each nanomaterial. On the other hand, bioaccumulation test were carried out in trouts (*Oncorhynchuss mykiss*). Results showed that NP were accumulated in fishes, though were also rapidly excreted again after transition to "clean" feed. These studies were also performed in *Daphnia magna*. In this case, uptake was observed but it was depending on functionalization of NM. Finally, a trophic transfer study was performed with *Danio rerio* feeding on *Daphnia magna* and it was concluded that dietary uptake (*Daphnia magna*) was a major route of exposure by comparing the levels of body burden reached with dietary uptake towards aqueous exposure.

In order to determine the environmental fate of nanomaterials, the adsorption/desorption and leaching of the latter in soil and sediment was studied. It was decided to apply the OECD 312 guideline to investigate the leaching of selected nanomaterials in soil and sediment..The use of higher concentrations of nanomaterial in this test was by far exceeding the expected realistic concentrations in sewage treatment plants and, hence, would not be environmentally relevant. In addition, another important factor have to be taken into account, physicochemical properties of NP could change in contact with water and different conditions of the environment. Therefore, transformation of selected nanomaterials was studied choosing specific conditions of pH, salinity and concentration of dissolved organic carbon. Transferring the results obtained in the lab to the situation in the real environment it may probably expect that nanoparticles in aquatic environment might alternately agglomerate and deagglomerate depending on the conditions such as pH, salinity and concentration of dissolved organic matter. The physical-chemical parameters may also influence the behaviour such as porosity and surface area.



TEM picture of cellular internalization of TiO_2 NP and organoclays



a) SiO_2 (1000 mg/L) b) SiO_2-OH (1000 mg/L) c) ZnO-Octyl (1000 mg/L)

5.6 *Theoretical studies and LCA analysis*

The work in this WP was focused on the establishment of properties-activity relationships, and the second section involves LCA. Although a considerable amount of data has already been generated in the project, the data generated in previous projects have been crucial to obtain reliable conclusions.

LCA studies have been performed in accordance with the ISO standards (ISO 14010:2006 and ISO 14044:2006) in order to analyse the environmental behavior of four selected nanocomposites (MWCNT-PP, nanoclays-EVA, TiO_2 NP-PA, ZnO NP-EVA) over their entire life cycle. A complete methodology has been developed adapting existing databases and impact assessment methods in order to include specific and new data of NM potential impacts.

In the Inventory step, the inputs and outputs of the different processes have been collected including NMs released to the environment. Inventory covered all life stages; synthesis, functionalization, nanocomposite manufacturing, use and waste treatment at end-of-life. For the use stage, data from aging simulations performed during the project have been considered.

In the impact assessment step, environmental impacts have been assessed at midpoint and endpoint level for different impact categories using ReCiPe impact method as a basis. Results allow determining the relative contribution of the different life stages to the overall impact and identify the main impacting parameters. Results on environmental impact regarding the impact distribution among the different life stages of all studied NMs followed a similar scheme. For almost all nanocomposites, nanocomposite manufacturing through extrusion and injection had the highest contribution to overall environmental impacts. In all production and transformation processes, electricity was the most impacting parameter. Therefore, optimization measures in all production and transformation processes are the most effective measure to reduce the overall environmental impact. Due to energy consumption, climate change appeared to be the most relevant impact category at endpoint level, both on Human Health damage (84% - 80%) and Ecosystem Damage (97% - 95%).

Moreover, a model for predicting environmental fate of nanomaterials and their potential ecotoxicity and human health effects have been developed following the principles of the USEtox model (Rousebaum et al, Int. J. Life Cycle Asess. (2008) 13:532). Exposure, fate and (eco)toxicity of NP were assessed in order to derive characterisation factors for Human Toxicity and Freshwater Ecotoxicity categories. In terms of characterization factors obtained, released MWCNTs were the nanoparticles that appeared to have lower potential impacts in human health and ecotoxicity when they are released to air or freshwater. The nanomaterial with higher values in both categories was functionalized TiO_2-OH, followed by bulk TiO_2 and ZnO.

At endpoint level, characterization factors of freshwater ecotoxicity and human toxicity due to released NM were added to final results. In general, results showed that toxicity due to released nanoparticles can be relevant in relation with the overall environmental impact when a worst scenario is considered, especially for human toxicity. In the case of MWCNT, toxicity effect on workers was also assessed for synthesis and nanocomposite stages. Results showed higher values, which corroborated the convenience of perform Risk Assessment in the different stages of the life cycle together to the LCA environment assessment.

5.7 Mechanical and Chemical recycling

Different methodologies to recycle production surpluses or used nanocomposites were proposed and the quality of the recycled products was evaluated. Recycling/disposal strategies were defined in selected representative nanocomposites considering their mechanical and physicochemical properties as well as their toxicological results.

ZnO NP was considered disposable material due to its high toxicity. This nanomaterial was extracted from aged EVA+ZnO nanocomposites by calcination and the recovered nanoparticles were successfully incorporated into a ceramic matrix. The resulting bulk material even at high nanoparticles loading performed well under aging. The waters collected from the aging process were quantitatively and quantitative analyzed in order to determine the possible release of NM. Due to satisfactory results, this method can be suggested as promising for the disposal or nanoparticles recovered from polymer composites.

As representative of recyclable nanocomposites, aged TiO2 NP-PA+, EVA+MMTdell72T and PP+MWCNT were chosen. The first was recycled chemically with the strategy previously defined and used with PA6 (section 5.4), and the recovered material extruded again. The other two composites were recycled mechanically using a grinder. The three nanocomposites were reinjected anew and their mechanical and thermal properties were tested to compare them with the non recycled samples. It was observed that the recycling process led to nanocomposites with slight differences with the new ones. In general, mechanical and thermal properties were maintained.

After it, the recycled nanocomposites were exposed to aging conditions in order to determine if the properties are maintained and if their behaviour is similar to those determined for non recycled aged nanocomposites. The waters collected from artificial rain was qualitatively and quantitative analyzed to determinate the possible released material and/or nanomaterials. In general, it could be determined that nanocomposites maintain their properties regarding the non recycled aged nanocomposites however, the release of the nanomaterial was higher.

6 Expected impact

NanoPolyTox project has achieved significant results and reached a number of important conclusions. The project has addressed the toxicological impacts of nanomaterials that are present in polymeric nanocomposites. The project characterized the effects of these nanomaterials during their life cycle including processing, weathering and recycling of these nanocomposites.

NanoPolytox results will have a significant impact on various industries including the automotive, food packaging, fire retardant insulations and other consumer products manufacturers. Moreover, the project will have significant social impact mainly with respect to the education and information that it will provide the consumers about the safety if the products they use containing nanomaterials. The results of the project will have a significant return and impact on the SMEs and large industries involved in the project that mainly produced the nanomaterials. The identification of the hazards and toxicological effect of nanomaterials in nanocomposites will allow the industries to evaluate their products that are released to the market and how safe they are. This will also motivate the industries to seek new, safe and more effective nanomaterials and nanocomposites. Determining the potential toxicity of its nanomaterials and their long-term consequences on important ecosystems potentially exposed to multiple stressors (e.g. climate change, pollution). This type of characterization is required by the company's customers and collaborators in order for them to feel secure when using and handling nanomaterials and nanocomposites during the various stages of their life cycle. By building trust between manufacturers and end users, the market share of the former will rise through increased market penetration, and this will trigger economic benefits for them. The methodologies and results of NanoPolytox can contribute to the development of testing standards and

guidelines for nanotoxicity characterization and nanomaterials recycling. They can also lead to strategies for improved product design for nanomaterials manufacturers that aim at reducing the release of nanoparticles from polymeric or other matrices during their life cycle, and thus resulting in safer nano-based products. The project has promised and delivered data on the following i) study of physico-chemical properties of the nanomaterial, ii) environmental fate, iii) environmental toxicology, iv) mammalian toxicology, v) emission and exposure thresholds, vi) nanomaterial safety datasheets and safety labeling. The social perception of nanotechnology is still greatly divided. A recent survey across a wide socio-demographic distribution (Eurobarometer 2010) was taken to evaluate the European general public's awareness, opinion and attitude towards nanotechnology. The survey revealed that 46% had heard of nanotechnology and 56% had never heard of it. Following up with perception about the safety of nanotechnology, the survey revealed that one third believed that nanotechnology may cause harm to the environment and is not safe for humans and future generations. One third believed the opposite and one third did not know. The lack of sufficient information about the safety and dangers associated with NMs and products affects the public's trust in such technologies. NanoPolyTox results and dissemination will inform the general public about the nature and safety of the nanomaterials used in consumer goods. The project is also in line with the actions listed in the European Strategy for Nanotechnology regarding the integration of the societal dimension by (i) ensuring public awareness and confidence in nanotechnology, (ii) encouraging a dialogue with EU citizens/consumers to promote informed judgment on nanotechnology, and (iii) committing to ethical principles in order to ensure that R&D in nanotechnology is carried out in a responsible and transparent manner.

7 Directory

Table Directory of people involved in this project.

First Name	Last Name	Affiliation	Address	e-mail
Socorro	Vázquez-Campos	LEITAT Technological Centre	C/ de la Innovació 2 08225 Terrassa (Barcelona), Spain	svazquez@leitat.org
Dirk	Saseta	LEITAT Technological Centre	C/ de la Innovació 2 08225 Terrassa (Barcelona), Spain	dsaseta@leitat.org
Loredana	Mercante	Lati Thermoplastic Industries S.p.A.	Fracesco Baracca 7, Verdano Olona 21040 Italy	lmercante@it.lati.com
Valentina	Ermini	Laviosa Chimica Mineraria S.p.A.	Via Leonardo da Vinci 21, Livorno 57123 Italy	vermini@laviosa.it
Tamara	Oroz	L'Urederra Technological Centre	Area Industrial La Perguita, C/A nº 1, 31210 Los Arcos (Navarra), Spain	tamara.oroz@lurederra.es
Vincent	Gaud	Polyrise SAS	Aveneue Pey Berland 16, Pessac 33607 France	vicent.gaud@polyrise.com
Stephanos	Nitodas	Global Nanotechnologies S.A.	Mesogion Avenue 401 Aghia Paraskevi 15343 Greece	snitodas@glonatech.com
Victor F.	Puntes	Institut Català de Nanotecnologia	Campus UAB, Edifici CM7, 08193 Bellaterra (Barcelona), Spain	victor.puntes.icn@uab.es
Cecilia	López	Institut Català de Nanotecnologia	Campus UAB, Edifici CM7, 08193 Bellaterra (Barcelona), Spain	cecilia.lopez@icn.cat
Margrethe	Winther-Nielsen	DHI Water & Environment	Agern Alle 5, Hoersholm 2970 Denmark	mwn@dhigroup.com

8 Copyright

NanoPUZZLES

"Modelling properties, interactions, toxicity and environmental behaviour of engineered nanoparticles"

Grant Agreement no.: 309837 Website: www.nanopuzzles.eu

Coordinator: Professor Tomasz Puzyn, University of Gdańsk, Poland

Table 1 Consortium List.

No.	Beneficiary name	Short name	Country
1	UNIWERSYTET GDANSKI	UG	Poland
2	INSTITUTO DI RICERCHE FARMACOLOGICHE MARIO NEGRI	IRFMN	Italy
3	ETHNIKO IDRYMA EREVNON	NHRF	Greece
4	LIVERPOOL JOHN MOORES UNIVERSITY	LJMU	United Kingdom
5	INNOBALTICA SP. Z O.O.	IB	Poland

Contents

1 Summary

Project Duration: 1.01.2013 – 31.12.2015 (36 months)

Project Funding: 1'169'800.00 EUR

Nanotechnology is rapidly expanding. However, some types of engineered nanoparticles can be toxic for living organisms and exhibit negative impact on the environment. Thus, the design of new nanomaterials must be supported by a rigorous risk analysis. Following the recommendations by the EU REACH system and regarding ethical aspects, the risk assessment procedures should be performed with possible reduction of living animal use. The main objective of the NanoPUZZLES project is to create new computational methods for comprehensive modelling of the relationships between the structure, properties, molecular interactions and toxicity of engineered nanoparticles. The methods will be based on the Quantitative Structure - Activity Relationship approach, chemical category formation and read-across techniques. Those methods have been widely used in risk assessment of other groups of priority chemicals. But, because of some specific reasons, they cannot be applied directly to nanoparticles. We will be developing novel methods within four complementary areas ("puzzles"), namely: (i) evaluation of physico-chemical and toxicological data available for nanoparticles (NanoDATA), (ii) developing novel descriptors of nanoparticles' structure (NanoDESC), (iii) investigating interactions of nanoparticles with biological systems (NanoINTER), and (iv) quantitative structure – activity relationships modelling (NanoQSAR). Developed methods will be tested and verified for their technical viability by collaborating industry representatives. By implementing the NanoPUZZLES methods, extensive animal testing would be significantly reduced. Moreover, the project will deliver the basis for categorising nanoparticles based on potential exposure, phys-chem, structural and toxicological properties. To maximise its impact, the project is cooperating with other modelling projects within the NanoSafety Cluster (ModNanoTox, NanoTransKinetics, ModEnpTox, MembraneNanoPart, PreNanoTox, Modern) as well as participating in the NanoSafety Cluster Databases and Modelling Working Groups. Discussions are also being held with the US Nanotechnology Working Group and future co-operation is planned with NanoMedicine ETP.

2 Background

The European Community and other countries over the world are witnessing a very important turning point with highly promising prospects for competitiveness and sustainable development across a wide range of industrial sectors such as electronics, magnetic and optoelectronics, biomedical, pharmaceutical, cosmetics, energy, environmental, catalytic, space technology and many others. New approaches for research and development that concern the study of materials having at least one dimension

below 100 nM, called nanosciences and nanotechnologies, lead to the production of novel, useful, and profitable applications. Particles in these size ranges, due to their high surface-to-volume ratio, the quantum size effect, and macro–quantum tunnelling effect, may behave differently from those larger counterparts with the same chemical composition and therefore exhibit remarkable physical and chemical properties. New characteristic properties of nanomaterials including greater hardness, rigidity, high thermal stability, higher yield strength, flexibility and ductility, making them very useful for commercial, technological and therapeutic applications.

Without doubt, nanotechnology is moving out from the laboratory and into the markets. The list of consumer goods developments in the nano-field is long - according to current analysis, about 1015 different products containing nanomaterials were officially on the market in 2009[1] and it is expected that the nano-market will grow exponentially and reach US $10 billion in 2012 and US $2.6 trillion in 2014.

Nanomaterials of all types are poised to register robust growth driven by growing interest from the healthcare and electronics sectors. Oxides and metals are expected to capture a major share of global nanomaterials revenues in the short-term. Emerging nanomaterials such as single-wall nanotubes, and dendrimers are forecast to contribute significantly to market growth. In terms of end-use segments, healthcare and electronics dominate worldwide revenues for nanomaterials. Also, exceptional growth is anticipated from end-use markets such as construction. Commercial usage of nanomaterials is limited to a few applications such as sunscreen lotions, wafer polishing, and treatment of textiles. The United States of America has emerged as the largest regional market with estimated revenues of US $1.12 billion in 2008, as stated by Global Industry Analysts, Inc. Western Europe is the second largest regional market, accounting for over 30% of global revenues. Asia-Pacific is projected to be the fastest growing market, with revenues poised to increase at a compounded annual rate of 38.7% over the analysis period 2002-2015. Worldwide nanomaterial oxide revenues are forecast to reach US $6 billion in 2013. Revenues for nanometals are projected to approach US $3 billion by 2015. Carbon nanotubes are another billion-dollar segment expected to post double-digit growth through 2015. Electronics is the largest end-use market for nanomaterials while healthcare is the most promising[2].

Recent contributions report evident toxicity of selected nanoparticles and highlight the potential risk related to the development of nanoengineering [3] [4] [5] [6] [7]

Nanoparticles, because of their small size, may penetrate cellular membranes such as skin, olfactory mucosa and the blood brain barrier, readily travel within the circulatory system, deposit in target organs and trigger tissue injury. Thus, designing of new nanoparticles should be accompanied by a comprehensive risk assessment. This idea has become an official standpoint of the European Commission, expressed in two communications: Towards a European strategy for nanotechnology[8] and Nanosciences and nanotechnologies: an action plan for Europe 2005 - 2009[9].

Unfortunately, knowledge on the harmful interactions of engineered nanoparticles with biological systems, as well as with the environment, is scarce. In addition, the current understanding of the toxicity of nanoparticles, including possible mutagenic and/or carcinogenic effects, is very limited. Moreover, there are neither theoretical methods nor experimental protocols to be applied for risk assessment of such nanoparticles.

Therefore, novel, fast and inexpensive procedures for risk assessment that can be performed without the need of extensive animal testing are required. This is particularly important in relation to the ethical issues, costs, and the expected increase in number of engineered nanoparticles. Article 13 of the Regulation (EC) No 1907/2006 of the European Parliament and of the Council of 18 December 2006 concerning the Registration, Evaluation, Authorisation and restriction of Chemicals (REACH)[10], says that:

In particular for human toxicity, information shall be generated whenever possible by means other than vertebrate animal tests, through the use of alternative methods, for example, in vitro methods or qualitative or quantitative structure - activity relationship models or from information from structurally related substances (grouping or read-across).

As mentioned in the Article 13, the main groups of computational methods that can be employed for risk assessment are: (i) quantitative structure – activity relationships (QSAR) as well as (ii) chemical category formation and read-across.

The (Q)SAR approach, which was formulated for the first time in 1962 by Prof Corwin Hansch, is based on defining mathematical dependencies between the variance in molecular structures,

[1] W.W.I.C.f. Scholars, Analysis of The Nanotechnology Consumer Inventory of August 25, 2009. Available at: http://www.nanotechproject.org/inventories/consumer/, (2009).

[2] http://www.azonano.com/news.asp?newsID=8393 (updated 29.10.2008)

[3] R. Nirmala, et al. (2011) Bactericidal Activity and In Vitro Cytotoxicity Assessment of Hydroxyapatite Containing Gold Nanoparticles. J. Biomed. Nanotechnol., 7: 342-350.

[4] N. Asare, et al. (2012) Cytotoxic and genotoxic effects of silver nanoparticles in testicular cells. Toxicology, 291: 65–72.

[5] J. Wu, et al. (2011) Neurotoxicity of Silica Nanoparticles: Brain Localization and Dopaminergic Neurons Damage Pathways. ACS Nano, 5: 4476–4489.

[6] T.T. Win-Shwe, et al. (2011) Nanoparticles and Neurotoxicity. Int. J. Mol. Sci., 12: 6267-6280.

[7] M. Kumari, et al. (2011) Cytogenetic and genotoxic effects of zinc oxide nanoparticles on root cells of Allium cepa. J. Hazard. Mater., 190: 613-621.

[8] Towards a European strategy for nanomaterials, COM (2004) 338; European Commission: Brussels, 2004.

[9] Nanosciences and nanotechnologies: an action plan for Europe 2005 - 2009, COM (2005) 243; European Commission: Brussels, 2005.

[10] Regulation (EC) No 1907/2006 of the European Parliament and of the Council of 18 December 2006 concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH), establishing a European Chemicals Agency, and amemding Directive 1999/45/EC and repealing Council Regulation (EEC) No 793/93 and Commission Regulation (EC) No 1488/94 as well as Council Directive 76/769/EEC and Commission Directives 91/155/EEC, 93/67/EEC, 93/105/EC and 2000/21/EC, Official Journal of the European Union, L 396/1; The European Parliament and the Council of the European Union: Brussels, 2006.

encoded by so-called molecular descriptors, and the variance in a given physicochemical (or biological) property in a set of compounds. In practice this means, that if one has experimentally measured substituent constants, other physicochemical properties or has calculated some molecular parameters (called 'molecular descriptors') for a group of similar chemicals and toxicological data are available only for a part of this group, one is able to interpolate the lacking data from the molecular descriptors and a suitable mathematical model.

The use of **the chemical category approach** is already common in a number of regulatory environments outside of the European Union namely in the United States and Canada. In terms of the Organization for Economic Co-operation and Development (OECD), a chemical category has been defined as 'a group of chemicals whose physiochemical and toxicological properties are likely to be similar or follow a regular pattern as a result of structural similarity, these structural similarities may create a predictable pattern in any or all of the following parameters: physicochemical properties, environmental fate and environmental effects, and human health effects'. On a practical level, this process involves treating a closely related (or similar) group of chemicals as a category. Within the category, toxicological data will exist for some, but not all of the chemicals for the endpoints of interest. Thus, data gaps are likely to exist for some of the properties or endpoints for each chemical, with it being likely that differing data gaps will exist for different chemicals within the category. It is for these data gaps that structure-activity relationship methods (such as read across) will have to be utilised to make predictions for the missing toxicological data.

Read-across is a technique in which endpoint information (i.e., toxicity) for one chemical (so-called a "source chemical") is used to make prediction of the endpoint for another chemical (a "target chemical") based on the similarity of both chemicals. The similarity is usually defined by similar chemical structure and/or physicochemical properties. It is assumed that similar compounds should exhibit similar biological activity. Read-across can be either quantitative or qualitative, depending on the data used (numerical or discrete). To make a prediction for a novel chemical, the technique can be performed in two variants: in a one-to-one manner (one analogue is used to make the estimation) and in a many-to-one manner (when two or more analogues are used).

Those methods have been successfully applied for predicting a vast range of properties and toxicity of typical (bulk) chemicals. Chemical grouping methods have been applied successfully for the prediction of skin and respiratory sensitisation, mutagenicity and other human health endpoints[11]. For successful grouping of compounds for the prediction of toxicity, a mechanistic basis is preferred. Once compounds have been grouped together, read-across can be performed. For skin sensitisation it has been shown that calculated indices for electrophilicity are able to predict skin sensitisation potency[12] as well as effects of respiratory sensitisation[13]. In addition, it has been shown that structural similarity can be used to predict complex endpoints such as teratogenicty and reproductive toxicity[14]. These grouping techniques have also been shown to be of use for environmental effects such as acute fish toxicity[15].

Based on the previous experiences of the NanoPUZZLES consortium members and previously published works, it is also possible to apply computational methods for modelling toxicity and behaviour of nanoparticles. The idea and the most significant achievements and further directions related to the application of QSAR methods in the risk assessment of nanoparticles has been described in five recently published contributions. [16] [17] [18] [19] [20]

3 Scientific and technological challenges

There are still many challenges and 'technical' problems to be solved to be able to widely apply computational methods of risk assessment to engineered nanoparticles. The problems include:

- scarce and/or inconsistent experimental data available and lack of conceptual frameworks for grouping nanoparticles according to physicochemical properties and mode of toxic action;
- lack of appropriate descriptors able to express specificity of "nano" structure, to be utilized for grouping based on structural similarities and QSAR modelling of nanoparticles;

[11] Y. K. Koleva, J. C. Madden, M. T. D. Cronin (2008) Formation of categories from structure-activity relationships to allow read-across for risk assessment: toxicity of a,b-unsaturated carbonyl compounds. Chemical Research in Toxicology 21: 2300-2312

[12] S. J. Enoch, M. T. D. Cronin, T. W. Schultz, J. C. Madden (2008) Quantitative and mechanistic read across for predicting the skin sensitization potential of alkenes acting via Michael addition. Chemical Research in Toxicology 21: 513-520.

[13] S. J. Enoch, D. W. Roberts, M. T. D. Cronin (2009) Electrophilic reaction chemistry of low molecular weight respiratory sensitizers. Chemical Research in Toxicology 22: 1447-1453.

[14] S. J. Enoch, M. T. D. Cronin, J. C. Madden, M. Hewitt (2009) Formation of structural categories to allow for read-across for teratogenicity. QSAR and Combinatorial Science 28: 696-708.

[15] S. J. Enoch, M. Hewitt, M. T. D. Cronin, S. Azam, J. C. Madden (2008) Classification of chemicals according to mechanism of aquatic toxicity: an evaluation of the implementation of the Verhaar scheme in Toxtree. Chemosphere 73: 243-248.

[16] A. Poater, A. Gallegos Saliner, R. Carbo-Dorca, J. Poater, M. Sola, L. Cavallo, A. P. Worth (2008) Modelling the structure – property ralationships of nanoneedles: a journey towards nanomedicine. J. Comput. Chem. 30: 275-284.

[17] T. Puzyn, D. Leszczynska, J. Leszczynski (2009) Toward the development of 'Nano-QSAR': Advances and challenges. Small 5: 2494-2509.

[18] T. Puzyn, A. Gajewicz, D. Leszczynska, J. Leszczynski (2009) Nanomaterials – the next great challenge for QSAR modelers. In: T. Puzyn, J. Leszczynski, M. T. D. Cronin (Eds.): Recent advances in QSAR studies: Methods and applications. Springer. pp. 383-409. ISBN: 978-1-4020-9782-9.

[19] T. Puzyn, D. Leszczynska, J. Leszczynski (2009) Quantitative Structure-Activity Relationships (QSARs) in the European REACH System: Could these approaches be applied to nanomaterials? In: J. Leszczynski, M. Shukla (Eds.): Practical Aspects of Computational Chemistry: Methods, Concepts and Applications. Special Issue of Annals - The European Academy of Sciences. Springer. ISBN: 978-90-481-2686-6.

[20] A. Gallegos Saliner, E. Burello, A. Worth (2009) Review of computational approaches for predicting the physicochemical and biological properties of nanoparticles. JRC Scientific and Technical Reports. EUR 23974 EN – 2009

- limited knowledge on the interactions between nanoparticles and biological systems (DNA, proteins, membranes etc.); these interactions lead to the formation of protein coronas, particle wrapping, intercellular uptake and biocatalytic processes that could have biocompatibile or bioadverse effects – thus, the real structure of the active nanoparticle can be different than the structure, for which the descriptors have been calculated;
- lack of rational structure-activity modelling procedures (QSAR) to screen large numbers of nanoparticles for nanotoxicity and hazard assessment; the existing methods and modelling protocols should be specifically profiled for nanoparticles, taking into account size-dependent differences between the bulk and nanostructure.

The NanoPUZZLES project is proposing some novel lines of research which will fill the gaps in knowledge and predictive methodology in a field of nanoscience. We need to ensure that nanotechnology research is carried out with maximum impact and responsibility and that the resulting knowledge is based on an understanding of properties of nanomaterials and on realistic exposure to the materials.

Scientists committed to the NanoPUZZLES project consortium have been involved in studies devoted to the development of robust systems to computationally evaluate the properties as well as the health and environmental impact of engineered nanomaterials for the last three years. In effect, many currently published contributions presenting novel ideas in the area of computational toxicity of nanoparticles are authored or co-authored by them. For example, in 2009 T. Puzyn (UG) and co-workers developed a QSAR model predicting cytotoxicity of 14 nano-sized metal oxides, available on the commercial market, to bacteria E. coli. This was the
first QSAR model predicting toxicity of engineered nanoparticles, recently published in Nature Nanotechnology[21] The same group has written the pioneering paper discussing the possibility of applying QSAR to predict the toxicity of nanoparticles published in 2009 in the Small journal[Error! Bookmark not defined.]. The group of Dr. Papadopulos (NHRF) in 2008 published a 3D QSAR CoMFA/CoMSIA study on fullerene-based HIV-1 PR inhibitors, which was, in fact, the first ever QSAR study with the activity-related endpoint[22].

4 Objectives

The main objective of the NanoPUZZLES project is to develop, within three years, a package of computational algorithms for comprehensive modelling of the relationships between the structure, properties, molecular interactions and toxicity of selected classes of engineered nanoparticles (NPs).

The package (i) will serve as a proof-of-the-concept that the risk related to NPs can be comprehensively assessed with the use of computational techniques and (ii) will define a basis for development of further modelling techniques for a large variety of nanoparticles.

The project is focussing on two groups of compounds:

(i) inorganic engineered nanoparticles (e.g. metal nanooxides) and

(ii) carbon nanoparticles (carbon nanotubes (single-walled and multiwalled), fullerenes and fullerene derivatives).

This choice was dictated by the wide application of these nanoparticles in everyday household products, and by the fact that these compounds are commercially available on the market which eliminates the necessity for their synthesis (leading to a reduction in costs).

Computational algorithms will be developed within four work packages related to the following thematic areas (Fig. 1):

- Quality assessment of physicochemical and toxicological data available for nanomaterials and data exploration (NanoDATA),
- Development of novel descriptors for nanoparticles' structure (NanoDESC),
- Simulating interactions of nanoparticles with biological systems (NanoINTER),
- Quantitative and qualitative structure-activity relationship modelling, grouping and read across (NanoQSAR).

Figure 1. Structure of the NanoPUZZLES project

The specific objectives of the NanoPUZZLES project are defined in four thematic areas ("puzzles") related to the project structure.

The main objective of the first thematic area is to develop a framework for classifying engineered nanoparticles based on the existing data utilising pattern recognition methods. This includes:

- Collecting and evaluating the existing data (physicochemical and toxicity data).
- Developing statistical procedures for data evaluation.
- Exploring the physicochemical and toxicity data with pattern recognition techniques to identify classes of similar properties/toxicity.

[21] T. Puzyn, et al. (2011) Using nano-QSAR to predict the cytotoxicity of metal oxides. Nature Nanotechnol 6: 175-178

[22] S. Durdagi, T. Mavromoustakos, M. G. Papadopulos (2008) 3D QSAR CoMFA/CoMSIA, molecular docking and molecular
dynamics studies of fullerene-based HIV-1 PR inhibitors. Bioorg. Med. Chem. Lett. 18: 6283-6289

- Launching a publicly available database with high quality (evaluated) empirical data or otherwise submitting these data into a suitable, publicly available database.

Along with the work programme requirements, modelling activities incorporated in the NanoPUZZLES project will be based on available physicochemical and toxicity data. The project assumes diversified data sources: NanoBRIDGES project – "Building bridges between specialists in computational and empirical risk assessment of engineered nanomaterials" (FP7-PEOPLE- 2011-IRSES, co-ordinator: Tomasz Puzyn); direct collaboration with Prof. Leszczynski's experimental group (Interdisciplinary Center for Nanotoxicity, Jackson, (MS, US)); scientific literature; existing databases including the NANOhub database (http://www.nanohub.eu) hosted by JRC; research reports from other European experimentally-related projects (NanoSafety Cluster). As mentioned in our reviews [23] [24] , the number of such data is limited.

Thus, extensive and comprehensive experimental data searching is crucial for the project success. Moreover, it is important that, within a given data set, all values are consistent and of high quality. Whenever the variation in data related to the use of different measuring/testing protocols, performed by different laboratories, and/or the errors existing in the data is greater than the real variability in the nanoparticles' properties, application of any classifying and modelling techniques makes no sense. In such a case, one can simply say that poor quality in the input results in poor quality in the output of a model[25]. Therefore, NanoDATA will develop an algorithm for evaluating the experimental data. The evaluation will also include analysis of the meta-data and experimental conditions (in particular the medium composition, the aggregation state etc.), as they might explain the large differences observed between laboratories. The collected and evaluated data will be published on-line as part of a publicly available database: this database will either be developed within the project or the data will be submitted to a pre-existing database. It is worth mentioning that this databases could be utilised by other consortia competing in this call. Finally, we will use these data to deliver a categorisation scheme regarding physicochemical and toxicological properties of the engineered nanoparticles as well as their potential for exposure related to the properties responsible for environmental transport.

The main objective of the second thematic area is to develop a framework for the optimal characterisation of the structure of engineered nanoparticles with use of appropriate descriptors and by categorising them according to structural similarities. This includes:

- Evaluation of the existing systems currently available for structural characterisation of NPs;
- Development of simplified molecular models sufficient to characterise the whole structure;
- Development of descriptors for the nanostructure ("nano-descriptors") of four types:

(i) topological descriptors, which are calculated with molecular graphs, SMILES, InChI, SMART notations, and descriptors based on the technological and physicochemical parameters

(ii) descriptors derived from quantum-mechanical calculations,

(iii)descriptors derived from computational processing of microscopic (SEM/TEM/AFM) images of the particles,

(iv) descriptors based on the anisotropy dimensions (as proposed by Glotzer and Solomon);

- Development of databases of the physicochemical and biochemical properties of the nanomaterials which will be made available via the internet;
- Development of freeware for calculating nanodescriptors.

Development of the descriptors derived from microscopic (SEM/TEM/AFM) images will be realized in collaboration with Prof. Leszczynski's group (Interdisciplinary Center for Nanotoxicity, Jackson, (MS, US).

The objective of the third thematic area is to develop methods to predict and explain interactions of engineered nanoparticles withbiological systems and small molecules. This includes the following:

- Development of a protocol which will provide the guidelines for developing or implementing a model for the study of large interacting systems.
- Development of a hierarchy of computational models for the study of interacting systems involving NPs and biological molecules of varying size.
- Development of techniques for the study of the environment (e.g. solvent) on the interacting system.
- Study of the effect of the computational model (e.g. level of quantum-mechanical theory) on the results.
- Implementation of techniques for the resolution of the interaction energy into various contributions (e.g. those due to electrostatic forces, dispersion etc).
- Design/recognition of functional groups which seriously reduce the genotoxicity and increase the solubility of the considered NPs.
- Study of factors affecting the interaction of the selected systems: nanoparticles (NPs) and{biological molecules. There are several important factors related to the NP. Among those we note:
  - (i) the chemical composition of the NP (e.g. fullerene, CNT, etc.);
  - (ii) the size and shape of the NP;
  - (iii) the particle aggregation;
  - (iv) the surface charge of the NPs, which is known to affect their cellular uptake;
  - (v) contamination. NPs (e.g. CNTs) may involve one or more toxic metals (e.g. Fe, Co, Ni) which may be considered as contaminants;
  - (vi) functionalisation. It is understood that functionalisation may affect the toxicity of the NP as well as its solubility. We shall look for

[23] T. Puzyn, D. Leszczynska, J. Leszczynski (2009) Toward the development of 'Nano-QSAR': Advances and challenges.
Small 5: 2494-2509

[24] T. Puzyn, A. Gajewicz, D. Leszczynska, J. Leszczynski (2009) Nanomaterials – the next great challenge for QSAR modelers. In: T. Puzyn, J. Leszczynski, M. T. D. Cronin (Eds.): Recent advances in QSAR studies: Methods and applications. Springer. pp. 383-409. ISBN: 978-1-4020-9782-9.

[25] J. C. Dearden, M. T. D. Cronin, K. L. E. Kaiser (2009) How not to develop a quantitative structure-activity or structureproperty relationship (QSAR/QSPR). SAR QSAR Environ. Res. 20: 241-266.

functional groups which seriously reduce the genotoxicity and increase the solubility of the considered NPs. Thus we propose to consider how the above factors affect the interaction of the selected systems: nanoparticles (NPs) and{biological molecules.

Moreover, engineered nanoparticles exposed to the environment participate in reactions with other environmental pollutants (oxidation reactions etc.) and can change the reaction rates of degradation (e.g. oxidation) processes of those pollutants, as well as changing reaction pathways and produce new metabolites.

The objective of the fourth thematic area is to develop scientifically justified and technically viable methods for quantitatively modelling relationships between chemical structure and toxicological targets which will extend understanding of toxicity and behaviour of emerging nanoparticles by establishing relations between experimental (based on available, validated data) and computational properties. This includes the following:

- Investigating the impact of size on the physicochemical properties of NPs at the appropriate level of quantum-mechanical theory.
- Developing NanoQSAR models of toxicity and environmentally relevant physicochemical endpoints, based on reliable experimental data and appropriate nano-descriptors.
- Comparing the efficiency of CoMFA/CoMSIA and Hansch Analysis modelling schemes in NanoQSAR.
- Investigating the minimum requirements sufficient for successful validation of NanoQSAR models (minimal number of data, evaluation of the applicability domain etc.) in the light of the OECD Principles for the Validation of (Q)SARs.
- Development of procedures for validating QSPR/QSAR models using probabilistic principles: balance of correlations, balance of correlations with ideal slopes, and filtration of the rare attributes, which can lead to overtraining.
- Estimating the environmental behaviour of NPs based on the physicochemical data predicted with NanoQSAR.
- Evaluation and publication of the NanoQSAR models and the results in scientific journals and with use of QSAR reporting formats (QMRFs) and QSAR prediction reporting formats (QPRFs).
- Updating the database referred to above to include the predicted results.
- Development of the conceptual framework for further grouping NPs based on chemical structure, physicochemical properties, interactions and toxicity.

This part of the NanoPUZZLES project brings together all findings and summarises the results of the project. High quality experimental physicochemical and toxicological data (from Puzzle 1:NanoDATA) and novel descriptors of nanostructure (developed in Puzzle 2: NanoDESC) will be utilised to develop mathematical models describing relationships between the structure and properties/activity. Information on the significance of structural factors responsible for the observed activity will be delivered by Puzzle 3: NanoINTER. The information about the character of interaction mechanisms will be important for an appropriate selection of nanodescriptors representing structural features of the studied nanoparticles.

5 Progress and outcomes to date

WP1: NanoDATA

During the first year of the NanoPUZZLES project, the efforts of WP1 were focussed on three essential tasks: identifying suitable sources of data, preparing an initial data collection and evaluating standards for organising nanomaterial data. These tasks were an essential pre-requisite for achieving the ultimate aim of developing a searchable inventory of knowledge within a publicly available database as well as the more immediate aim of developing datasets which could be used to develop read-across and NanoQSAR models.

Initial datasets were prepared based upon data extracted from approximately 60 publications. The data extracted included data obtained from genotoxicity and cytotoxicity tests as well as indications of inhalation toxicity. Corresponding physicochemical/structural data extracted included size characterisation measurements from multiple technique such as transmission electron microscopy (TEM) and dynamic light scattering (DLS). The data extracted were obtained for fullerenes, carbon nanotubes, metals, metal oxides and silica nanoparticles. Additional data for both carbon based and inorganic nanomaterials were provided by researchers working on the NanoBRIDGES project, yielding a data collection with data from approximately 100 publications.

Originally, all initial datasets were prepared as non-standardised Excel workbooks. Whilst the initial data collection activities were valuable for identifying sources of relevant data and for carrying out some preliminary QSAR modelling, the lack of a suitable, standardised data format was identified as a challenge affecting, *inter alia*, the ability to merge the datasets, readily use them for modelling and to achieving the ultimate aim of uploading the data into a searchable, publicly available database.

Following discussions with the PreNanoTox, ModNanoTox, NanoTransKinetics, ModEnpTox, MembraneNanoPart, and Modern projects, it was decided to adopt the ISA-TAB-Nano data collection/exchange specification which was proposed as a global standard for exchanging nanomaterial data.[26] A thorough evaluation of this proposed standard was carried out and discussions regarding how best to make use of the specification for the NanoPUZZLES project were held with members of other projects within the NanoSafety Cluster as well as the developers of the standard in the US Nanotechnology Working Group.

More recently, data collection templates based upon the ISA-TAB-Nano specification have been designed for organising all of the data within the final NanoPUZZLES data collection. A proposal for assigning quality scores for the structural/physicochemical information, along with associated experimental conditions, corresponding to nanomaterial records in this final data collection has also been developed.

[26] D. G. Thomas, S. Gaheen, S. L. Harper, M. Fritts, F. Klaessig, E. Hahn-Dantona, D. Paik, S. Pan, G. A. Stafford, E. T. Freund, J. D. Klemm, and N. A. Baker, "ISA-TAB-Nano: A Specification for Sharing Nanomaterial Research Data in Spreadsheet-based Format," BMC Biotechnol., vol. 13, no. 1, p. 2, 2013.

Currently, additional literature references are being identified and prioritised for ongoing data collection activities. The NanoPUZZLES data collection is expected to grow until the end of the project.

WP2: NanoDESC

The activities within this WP have been mainly dedicated initially to a survey of the reported chemical descriptors used to model nanoparticles, and then proceeding towards some studies on descriptors which can be used within the project.

The set of classical descriptors, used for classical QSAR, is a useful starting point, since these descriptors have been adopted in several cases. However, there are specific parameters which escape the traditional approach for QSAR, and may be profitably used as descriptors for nanomaterials. The evaluation on the descriptors used in the past has to be done considering the specific nanoparticles and endpoints they refer to. Indeed, different solutions have been applied for the different situations. From the cases we have reviewed, we can see that some nanoparticles have been quite studied, like fullerenes, and nanomaterials based on metal oxides. There are also examples of carbon nanotubes, while studies on other nanomaterials, like carbon black, graphene flakes, dendrimers, quantum dots, nanoclays, are practically absent. There are studies which are quite close to the QSAR models, since they used classical descriptors adopted in the past for traditional QSAR models. There are examples of these "conventional" studies for fullerenes, and nano-metal oxides. In addition, several other models introduced other descriptors, not common in classical QSAR models. The traditional descriptors are anyhow used in many models, alone, or combined to new ones.

In case of "new" descriptors, typically they used certain parameters related to the nanoparticle, like the size, etc. A peculiarity of these studies is that these descriptors are experimental ones, and thus they have to be obtained on a case-by-case basis. Furthermore, another related issue is that these "new" parameters largely depend on the procedure used to measure this parameter, and thus their possible extrapolation on an additional case may be questionable, and anyhow should be carefully verified if the procedure for the new circumstance is homogeneous to the original one which generated the parameter used in the past.

We then proceeded with a consolidation of these cases, applying these principles to some cases. This produced two papers which have been published in 2013[27][28]

We considered several format to introduce the chemical format, such as SMILES, orbital graphs and InChI. So far SMILES results to be more popular, compared to the other two formats. We evaluated the optimal descriptors as a tool for predictive modelling of endpoints related to nanomaterials according to paradigm "Endpoint = F(Eclectic data)". This approach is quite flexible and convenient, since allow the inclusion of the heterogeneous parameters typical of each situation, as we discussed above. Further, more sophisticated an innovative descriptors will be evaluated in the future.

WP3: NanoINTER

A computational protocol for the reliable calculation of the properties (e.g. the structure) of large interacting systems was developed. To have it done, the computational experimentation has taken place which involved:

(a) Ab initio techniques for properly selected systems. We have used a large variety of such techniques, e.g. Hartree-Fock, Møller-Plesset perturbation theory, DFT to compute a series of properties (e.g. ionization potentials, polarizabilities). Basis sets of different size have been employed (e.g. 6-31G, 6-31G*). Semiempirical methods (e.g. AM1) have also been tried.

(b) Molecular dynamics (MD) techniques for the actual systems of interest. The geometries of the interacting systems have been computed by employiong MD methods. The interaction energies of properly selected pilot systems have been computed by using several ab initio approaches, for example those developed by Su and Li, Kitaura and Morokuma, the effective fragment potential approach, the variational perturbation theory. The energy of the hydrogen bond has been computed by employing the atoms-in molecules approach. The interaction energy of large interacting systems systems has been computed by using the Molecular Mechanics Poisson Boltzmann Surface Area (MM-PBSA) method. Thermodynamic integration has also been used for some selected systems.

WP4: NanoQSAR

The quantitative structure-activity relationships (QSARs) techniques were applied to develop the predictive models for: i) membrane damage of by TiO2 nanoparticles [29], ii) cellular uptake of nanoparticles in PACa2 cancer cells[30], and iii) bacterial reverse mutation test TA100 for fullerenes[31]. All of this models utilized SMILES-based optimal descriptors.

Additional endpoints were defined to be modelled by means of QSAR techniques. The efforts have been taken to describe selected nanomaterials by means of topological (DRAGON) and quantum-mechanical descriptors. These descriptors will be applied in nanoQSAR modelling.

Parallel, the researches were focus on investigating the impact of size on the physical/chemical properties of NPs. TiO2 and ZnO oxides were selected to defined mode of size-depending changes of its electronic properties. The efforts have been taken to develop computational protocol for performing calculations of the properties. The selection of oxides differ In toxicity will be helpful in understanding its mode of biological action.

[27] A.A. Toropov, A.P. Toropova, T. Puzyn, E. Benfenati, G. Gini, D. Leszczynska, J. Leszczynksy (2013) QSAR as a random event: Models for nanoparticles uptake in PaCa2 cancer cells. Chemosphere 92 : 31–37

[28] A.P. Toropova, A.A. Toropov, T. Puzyn, E. Benfenati, D. Leszczynska, J. Leszczynski, (2013) Optimal descriptor as a translator of eclectic information into the prediction of thermal conductivity of Micro-Electro-Mechanical Systems J. Math. Chem. 51: 2230-2237

[29] A.P. Toropova, A.A. Toropov (2013) Optimal descriptor as a translator of eclectic information into the prediction of membrane damage by means of various TiO2 nanoparticles. Chemosphere, 93, 2650-2655

[30] A.A. toropov, A.P/Toropova, T.Puzyn, E.Benfenati, G. Gini, D.Leszczynska, J. Leszczynski (2013) QSAR as a random event: Modeling of nanoparticles uptake in PaCa2 cancer cells. Chemosphere, 92, 31-37

[31] A.A. Toropov, A.P. Toropova (2013) Optimal descriptor as a translator of eclectic data into endpoint prediction: Mutagenicity of fullerene as a mathematical function of conditions, Chemosphere, 104, 262-264

6 Expected Impact

The expected outcome of the NanoPUZZLES project is a package of algorithms enabling one to model relationships between the structure, properties, molecular interactions and toxicity of engineered nanoparticles, that can be simply applied within industry to design safe and environmentally friendly nanomaterials. This includes: the following

- tools for evaluating the quality of the experimental data (phys/chem properties and toxicity),
- a dataset, available via a publicly available database, containing high quality data for further modelling,
- tools for calculating descriptors of nanoparticles' structures,
- computational protocols for studying the interactions of NPs with biological systems,
- new grouping schemes for nanoparticles, based on their structure, phys/chem properties, and toxicity,
- a basis for quantitatively modelling the relationships between the structure and phys/chem properties and toxicity of NPs as well as preliminary Nano-QSAR models for selected endpoints.

Application of these methods will provide information on the intrinsic properties of the NPs that will lead to an understanding of their behaviour in physical processes in the environment and to the development of relationships between physicochemical properties and biological activity/toxicity. Detailed physicochemical and toxicological approaches to understand different nanomaterials will ultimately have numerous applications in industry and environmental hazard assessment to guide reliable risk and safety evaluations for these materials to ensure their safety for human health and the environment. Thus, in the long term, the project will contribute to the design of nanomaterials that are safe. This is especially important for further development of nanomedicine. Therefore, the NanoPUZZLES consortium will be closely collaborating with the NanoMedicine ETP.

The studies on human health and the environmental impact of engineered nanomaterials will then be possible without the necessity of performing extensive animal testing. Moreover, employing computational techniques developed within the NanoPUZZLES project should significantly reduce the time and cost of risk assessment for novel engineered nanoparticles. The reduction of costs related to the risk assessment would increase the probability of success of the REACH system and other regulations that implement the European policy of the 3Rs (Replacement, Reduction and Refinement) of animal use (e.g. the Cosmetic Directive). That is the significant argument for the project to be realised at the European (not national or local) level.

Actions undertaken in each work package will have different influences on scientists, industries and citizens (consumers of products containing nanomaterials). The first two work packages will have direct effects on scientists as they enhance the general understanding of nanoparticles' structure and properties, whereas interactions of NPs with human health and the environment is the most important for end users of products containing NPs – citizens. Industry (mostly pharmaceutical and biomedical, but also food, telecommunications and electronics) will benefit the most from practical outputs of the project which are contained in WP4.

The outputs of the project – reports, publications, articles and conferences – will raise the level of knowledge within industry as well as amongst scientists more generally and decision makers. Also, on-line materials (including public deliverables uploaded on the project website) will increase end-users and consumers' knowledge and awareness.

In the conferences planned under WP5 (Dissemination), efforts will be made to get active feedback from scientists, decision makers and industry representatives. In addition, participation in international conferences and trade fairs will be used as a way of getting external feedback and to build up networks with relevant researchers and industry representatives.

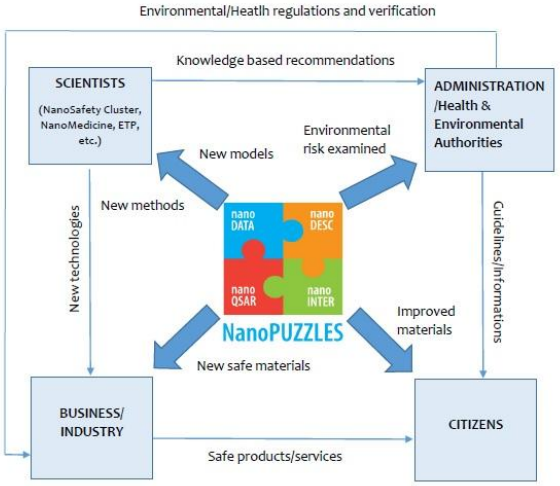


Fig. 2 Impacts of the NanoPUZZLES project on particular groups and interdependencies between them.

The outputs of the NanoPUZZLES project will have a significant impact on various groups in society, including scientists, administration, industry and citizens (Fig. 2, above). The project will directly influence the scientific community by providing new models and methods for testing of the safety of nanomaterials. It will also affect the authorities by providing knowledge driven recommendations. For instance, the REACH regulation in article 13 says that "information shall be generated whenever possible by means other than vertebrate animal tests, through the use of alternative methods, for example, in vitro methods or qualitative or quantitative structure-activity relationship models or from information from structurally related substances (grouping or read-across)". Unfortunately, such computational methods need to be specially adapted to be employed for nanomaterials. Based on a thorough understanding of the behaviour of NPs in the environment and to humans, regulations that must be complied with when introducing new nanomaterials to the market will be implemented. These regulations will force industry (mostly pharmaceutical and biomedical, but also food, telecommunications and electronics) to manufacture nanomaterials in accordance with health and environmental restrictions. Moreover, we believe that, in future, the fact that a particular product is designed in an environmentally friendly way would additionally increase its competitiveness on the market (according to the idea of "green marketing"). Specific needs and expectations of industry will be taken into account during the development of new methods. That information will be gathered from industrial actors during a stakeholder event. During the final conference, industry representatives will be presented with the results of the project and will give us feedback on which aspects of the tools developed should be improved.

NanoPUZZLES scientists will also directly influence industry by providing new technologies for the design of safe nanomaterials. It is expected that the largest impact will be observed for citizens as potential consumers of engineered nanomaterials. They will be provided with new, safe products and informed by authorities about their safe use, application and contraindications. The contribution of this project to particular target groups requires a European approach, especially in the light of implementation of EU regulations towards the REACH Programme. Moreover, development of NanoQSAR techniques follows the 10-year strategy worked out by COST (European Cooperation in Science and Technology) during the latest COST Exploratory Workshop on Quantitative Nanostructure-Toxicity Relationships held in Maastricht (3-6 April 2011). The strategy has been already contributed to by the ideas of members of the NanoPUZZLES consortium: Dr. Puzyn was one of the invited speakers at that meeting. Currently, he is a member of the Management Committee of the Modena COST Action.

The results will also be published, where possible, using QSAR Model Reporting Formats (QMRFs), QSAR Prediction Reporting Formats (QPRFs) and Category Reporting Formats (CRFs) prepared by the European Commission's Joint Research Centre. The new published methods and models will significantly influence the implementation of REACH and other regulations showing necessity of specific treatments of nanomaterials in risk assessment.

Only a few projects specifically devoted to development of QSAR, read across and in silico modelling methodology for nanomaterials have been initiated at the European level within NanoSafety Cluster. Some of the most important ones are ModNanoTox and NanoTransKinetics (NMP.2010.1.3-2 EU-US coordinated call). To maximise the impact of NanoPUZZLES, the coordinators of ModNanoTox and NanoTransKinetic,s as well as representatives of the NanoSafety Cluster and NanoMedicine ETP, will be invited to join the NanoPUZZLES advisory board. This would help to efficiently integrate the project within the existing EU research infrastructure and to facilitate research cohesion with the existing projects. As mentioned, individual "puzzles" (work packages) will be integrated within the structure of the NanoSafety Cluster NanoDATA is collaborating with the NanoSafety Cluster's Database Working Group, whereas NanoDESC, NanoINTER, and NanoQSAR will be collaborating with the NanoSafety Cluster's Modelling Working Group. Indeed, a NanoPUZZLES researcher (Richard Marchese Robinson) is currently acting as co-chair of the Modelling Working Group. We also have offered our contribution to the NPs' risk assessment research strategy for 2015-2020.

Therefore, the NanoPUZZLES project will become a significant part of the European Research Area and the results will deliver to industry new, scientifically justified and technically viable tools for engineering nanomaterials that are safe by design.

7 Directory

Table 1 Directory of people involved in this project.

First Name	Last Name	Affiliation	Address	e-mail
Tomasz	Puzyn	University of Gdańsk (UG)	63 Wita Stwosza Street, 80-308 Gdańsk, Poland	t.puzyn@qsar.eu.org
Agnieszka	Gajewicz	University of Gdańsk (UG)	63 Wita Stwosza Street, 80-308 Gdańsk, Poland	a.gajewicz@qsar.eu.org
Karolina	Jagiello	University of Gdańsk (UG)	63 Wita Stwosza Street, 80-308 Gdańsk, Poland	jagiello@qsar.eu.org
Emilio	Benfenati	Istituto di Ricerche Farmacologiche "Mario Negri" (IRFMN)	Via La Masa, 19, 20156 Milano, Italy	emilio.benfenati@marionegri.it
Manthos	Papadopoulos	National Hellenic Research Foundation (NHRF)	48 Vassileos Constantinou Ave. 11635 Athens, Greece	mpapad@eie.gr
Mark	Cronin	Liverpool John Moores University (LJMU)	James Parsons Building Byrom Street Liverpool L3 3AF Great Britain	M.T.Cronin@ljmu.ac.uk
Katarzyna	Dembowska	InnoBaltica Sp. z o. o. (IB)	Gdańsk Science-Technology Park, building A, 4th Floor 3 Trzy Lipy Street 80-172 Gdańsk, Poland	k.dembowska@innobaltica.eu

First Name	Last Name	Affiliation	Address	e-mail
Richard	Marchese Robinson	Liverpool John Moores University (LJMU)	James Parsons Building Byrom Street Liverpool L3 3AF Great Britain	R.L.MarcheseRobinson@ljmu.ac.uk
Andrea	Richarz	Liverpool John Moores University (LJMU)	James Parsons Building Byrom Street Liverpool L3 3AF Great Britain	A.Richarz@ljmu.ac.uk

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NANoREG

A COMMON European approach to the regulatory testing of nanomaterials

Contract Agreement: 310584 Website: www.nanoreg.eu
Coordinator: Ministry of Infrastructure and the Environment; the Netherlands
Project Coordinator: Tom van Teunenbroek Project Manager: Aart Dijkzeul

Table 1 Consortium List.

No.	Beneficiary name	Short name	Country
1	MINISTERIE VAN INFRASTRUCTUUR EN MILIEU	Min I&M	Netherlands
2	JRC -JOINT RESEARCH CENTRE- EUROPEAN COMMISSION	JRC	Belgium
3	BUNDESANSTALT FUER ARBEITSSCHUTZ UND ARBEITSMEDIZIN	BAuA	Germany
4	DET NATIONALE FORSKNINGSCENTER FORARBEJDSMILJO	NRCWE	Denmark
5	RIJKSINSTITUUT VOOR VOLKSGEZONDHEIDEN MILIEU - NATIONAL INSTITUTEFOR PUBLIC HEALTH AND THE ENVIRONMENT	RIVM	Netherlands
6	BUNDESINSTITUT FUER RISIKOBEWERTUNG	BFR	Germany
7	CENTRE NATIONAL DE LA RECHERCHE SCIENTIFIQUE	CNRS CEREGE	France
8	AIT Austrian Institute of Technology GmbH	AIT	Austria
9	INSTITUTE OF OCCUPATIONAL MEDICINE	IOM	United Kingdom
10	TEMAS AG Technology and Management Services	TEMAS	Switzerland
11	FUNDACION GAIKER	GAIKER	Spain
12	NANOTECHNOLOGY INDUSTRIES ASSOCIATION AISBL	NIA	Belgium
13	BASF SE	BASF	Germany
14	THE PROVOST, FELLOWS, FOUNDATION SCHOLARS & THE OTHER MEMBERS OF BOARD OF THE COLLEGE OF THE HOLY & UNDIVIDED TRINITY OF QUEEN ELIZABETH NEAR DUBLIN	TCD	Ireland
15	KAROLINSKA INSTITUTET	KI	Sweden
16	NORSK INSTITUTT FOR LUFTFORSKNING	NILU	Norway
17	ISTITUTO SUPERIORE DI SANITA	ISS	Italy
18	AGENZIA NAZIONALE PER LE NUOVE TECNOLOGIE,L'ENERGIA E LO SVILUPPO ECONOMICO SOSTENIBILE	ENEA	Italy
19	STATENS ARBEIDSMILJOINSTITUTT	STAMI	Norway
20	ACONDICIONAMIENTO TARRASENSE ASSOCIACION	LEITAT	Spain

21	INSTITUT NATIONAL DE RECHERCHE ET DE SECURITE	INRS	France
22	FACULTES UNIVERSITAIRES NOTRE-DAME DE LA PAIX DE NAMUR	UNamur	Belgium
23	COMMISSARIAT A L ENERGIE ATOMIQUE ET AUX ENERGIES ALTERNATIVES	CEA	France
24	GEOCHEM RESEARCH BV	GeoChem	Netherlands
25	DEPARTMENT OF HEALTH; PUBLIC HEALTH ENGLAND	PHE	United Kingdom
26	CENTRUM VOOR ONDERZOEK IN DIERGENEESKUNDE EN AGROCHEMIE - CODA	CODA-CERVA	Belgium
27	UNIVERSITAT AUTONOMA DE BARCELONA	UAB	Spain
28	FONDAZIONE ISTITUTO ITALIANO DI TECNOLOGIA	IIT	Italy
29	ASOCIACION DE INVESTIGACION DE LAS INDUSTRIAS DE LA CONSTRUCCION	AIDICO	Spain
30	STICHTING DIENST LANDBOUWKUNDIG ONDERZOEK	DLO-RIKILT	Netherlands
31	CONSIGLIO NAZIONALE DELLE RICERCHE	CNR	Italy
32	ASSOCIATION SAINT YVES	UCO	France
33	NEDERLANDSE ORGANISATIE VOOR TOEGEPAST NATUURWETENSCHAPPELIJK ONDERZOEK - TNO	TNO	Netherlands
34	INSTITUT NATIONAL DE L ENVIRONNEMENT ET DES RISQUES INERIS	INERIS	France
35	AGENCE NATIONALE DE SECURITE SANITAIRE DE L'ALIMENTATION, DE L'ENVIRONNEMENT ET DU TRAVAIL	ANSES	France
36	BIONANONET FORSCHUNGSGESELLSCHAFT MBH	BioNanoNet	Austria
37	LUNDS UNIVERSITET	LTH	Sweden
38	GENOK - SENTER FOR BIOSIKKERHET	GenØk	Norway
39	UNIVERSITETET I BERGEN	UIB	Norway
40	EIDGENOESSISCHES DEPARTEMENT DES INNERN	FOPH	Switzerland
41	VENETO NANOTECH SCPA	VN	Italy
42	INSTITUTO TECNOLOGICO DEL EMBALAJE, TRANSPORTE Y LOGISTICA	ITENE	Spain
43	UNIVERSIDAD DE LLEIDA	UdL	Spain
44	STIFTELSEN SINTEF	SINTEF	Norway
45	UNIVERSITAET LEIPZIG	ULEI	Germany
46	NPL MANAGEMENT LIMITED	NPL	United Kingdom
47	LABORATOIRE NATIONAL DE METROLOGIE ET D'ESSAIS	LNE	France
48	LABORATORIO IBERICO INTERNACIONAL DE NANOTECNOLOGIA	INL	Portugal
49	TYOETERVEYSLAITOS	FIOH	Finland

50	UNIVERSITETET FOR MILJO OG BIOVITENSKAP	UMB	Norway
51	FUNDACION TEKNIKER	TEKNIKER	Spain
52	CHALMERS TEKNISKA HOEGSKOLA AB	Chalmers	Sweden
53	INSTITUTO DE SOLDADURA E QUALIDADE	ISQ	Portugal
54	TURVALLISUUS JA KEMIKAALIVIRASTO	TUKES	Finland
56	ARKEMA FRANCE SA	ARKEMA	France
57	STORA ENSO OYJ	Stora Enso	Finland
58	UPM-KYMMENE OYJ	UPM	Finland
59	SP SVERIGES TEKNISKA FORSKNINGSINSTITUT AB	SP	Sweden
60	UNIVERSITY OF LEEDS	Unileeds	United Kingdom
61	ENVICAT CONSULTING	Envicat	België
62	COMET BIOTECH	CBT	Norway
63	INSTITUT DE PASTEUR DE LILLE	IPL	France
64	HERRIOT WATT UNIVERSITY	HWU	United Kingdom

Contents

1 Summary

Project Duration: 42 month

Project Funding: 50 M€

In the NANoREG project, over 60 partners, together with government representatives of participating countries, cooperate to reduce or eliminate the existing uncertainties regarding the way the EHS effects and risks of Nanomaterials will have to be assessed. The project will identify the Environmental, Health and Safety (EHS) aspects that are most relevant from a regulatory point of view. It will provide tools for testing the EHS aspects and the assessment and management of the risks to the regulators and other stakeholders. To assure that the final results of the project can be implemented in an efficient and effective way, Industry and Regulators are strongly involved in the project.

2 Background

Nanotechnology is one of the six "Key Enabling Technologies" (KET's), the European Commission identified in its 2012 communication on this topic. Technologies that are of paramount importance for the transition to a knowledge-based and low carbon resource-efficient economy and for the competitiveness of European industries in the knowledge economy. A serious threat to the capitalization of this potential is the limited understanding of the Environmental, Health and Safety (EHS) aspects of NanoMaterials (NMs). This limited understanding leads to uncertainty on how to judge the EHS aspects of these materials in a regulatory context. This has a negative impact on the investment climate and on societal appreciation of products containing NMs.

To address this thread, it is necessary to develop a common understanding on how to assess the EHS effects and risks of NMs during their complete lifecycle. Questions like "what tests are suitable and reliable for identifying the EHS effects", "how to interpret the results of these test", "how to assess the risks related to the identified effects", "how to predict effects of new materials on the basis of existing data" need an answer. An answer that fits in the

regulatory context and is accepted as "the right answer" by the involved parties.

This is the playground for the NANoREG project: finding answers to the regulatory needs and questions by "testing the tests" and by translating the results in methods for testing, assessing and predicting the EHS aspect of nanomaterials. Methods that fit in the regulatory context for this kind of materials.

3 Scientific and technological challenges

The NANoREG project is demand driven. The questions and needs of the regulatory community and industry are leading. Three themes are designed to address these needs and questions:

Credibility of the regulatory context (short term)
At this moment already hundreds of products containing NMs have entered the market while human or environmental safety cannot be guaranteed. On the other hand evidence-based policy and subsequent regulatory requirements for industry cannot be given. Although this situation highlights the need for quick solutions, all stakeholders will have to deal with the present regulatory framework and its less than optimal fit for emerging technologies like nanotechnology. Dealing with the most urgent issues in this context will be addressed under this theme

Accelerating the regulatory process (mid-term)
Up till now risk assessment of manufactured NMs (MNMs) was approached on a case-by-case basis. Given the huge variety of forms and sizes of MNMs coming or at market, one can imagine that this approach will not hold for long. Moreover, it is a time and money consuming, and inefficient approach for all stakeholders.
Activities within this theme focus on how to improve the pace of getting insight into the risks of MNMs.

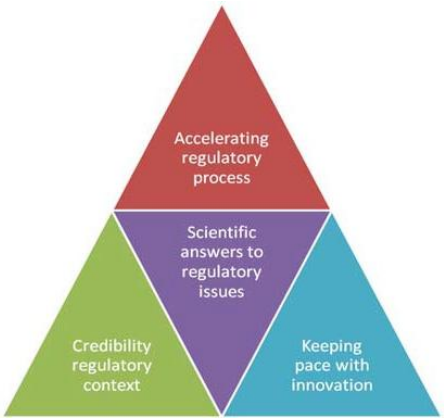

Figure 1: short term, midterm and long term approaches within NANoREG

Keeping pace with innovation (long term)
It is recognized that regulatory risk analysis is lagging far behind the emerging market of MNMs and its products. If the present regulatory approach for risk research is not adapted, the gap between innovation and safety will become larger and larger, hampering innovation and leading to even more uncertainties about health risks. Within this theme tools for linking innovation to risk formulation and risk analysis will be addressed.

4 Objectives

The wider scientific and technical objectives of this project are:

1. To provide legislators with a set of tools for risk assessment and decision making instruments ("NANoREG toolbox") for the short to medium term, by gathering data and performing pilot risk assessment, including exposure monitoring and control, for a selected number of NMs used in products.
2. To develop for the long term, new testing strategies adapted to a large? number of NMs where many factors can affect their environmental and health impact.
3. To establish a close collaboration among authorities and industry with regard to the knowledge required for appropriate risk management, and create the basis for common approaches, mutually acceptable datasets and risk management practices.

5 Organization

The hard core scientific work is organised in five R&D work packages (WP2-6). WP7 is responsible for organising the involvement and input from industry and the regulatory community. WP1 integrates the results from the other work packages and translates those results in "answers to the regulatory questions and needs".
To achieve the previous mentioned objectives, there is a strong need for cooperation between the work packages and other involved parties like regulators, risk assessors and industry.

Figure 2 gives an impression of the workflow of the NANoREG project and the interlinkage between the different WPs. The more detailed objectives and tasks of the WPs will be described below.

Synthesis, supplying and Characterisation (WP2)
Work package 2 will provide the project with a suite of high-quality commercial and tailored test NMs. It will establish (or give recommendations on) SOPs for size-distribution and some specific surface area (VSSA) of MNM in powders and complex viscous matrices, validated generic or "test-specific" MNM dispersion protocols and validated methods for quantitative characterization of the exposure and dose rate for MNM in liquid dispersions. Developing recommendations for a MNM analysis and categorization procedures considering the wide range of different MNM and chemical derivatives (of: derivates?) is also one of the tasks of this work package.

Exposure through life cycle analysis (WP3)
The work of WP3 is aimed at characterisation of real exposures (intensity and frequency) to humans (workers and consumers) and the environment through NM life cycle. The WP will provide tools for companies and legislators for risk assessment and decision making for the short to medium-term for a selected number of MNMs used in products. For the long-term, new testing strategies will be developed adapted to a high number of MNMs. A further objective is to bring together the activities of national authorities responsible for worker protection, public health and environment and create the basis for common approaches, mutually acceptable datasets and risk management practices.

Biokinetics and toxicity testing in vivo (WP4)
For the purpose of risk assessment, work package 4 will obtain information on the mode of toxic action including long-term expo-

sure and the relevant lower dose range, of selected nanomaterials (GBP, HARN). To study putatively relevant effects of selected nanomaterials various standard in vivo studies will be carried out. The work package will assess the systemic distribution of selected nanomaterials after different exposure patterns including the relevant lower dose range, the internal exposure of selected organs and putative systemic toxicity including long-term exposure and the relevant lower dose range. Toxicity and grouping approaches for relevant NM in aquatic systems will be verified.

Advancement of Regulatory Risk Assessment and Testing (WP5)
Work package 5 will develop a proposal for grouping of nanomaterials in categories with similar biological, ecological and/or toxicological effects. Furthermore a strategy for solubility testing and a methodology for alternative predictive screening will be developed. WP5 will combine the results of WP4 and own data to identify the most suitable in vitro model to assess inhalation toxicity. In vitro toxicity assays connected to regulatory questions will be designed. Other tasks are the development of a rapid high throughput screening methodology and a decision tree for risk assessment.

Keeping pace with innovation (WP6)
The work carried out in work package 6 is aimed at making a more effective foresight of the impact of new MNMs applications on human and environmental health possible. An integrated research strategy which addresses product/material design and the safety aspects for humans and the environment will be developed.

Liaisons, Dissemination, Exploitation and Communication
Regulations with respect to EHS are mostly effective on an international scale. The objective of WP7 is to ensure interactions (dissemination, and communication) with the stakeholders on a national and international scale. Liaisons will be established with selected Global Standardisation and Regulation Institutions and National Authorities for EHS regulation and legislation as well as with the industry and the public (National stakeholders). NANoREG results will be actively communicated.

Scientific answers to regulatory questions
The results of the R&D work done in the WPs 2-6, will be brought together in WP2 and used to formulate the answers to selected needs and questions of the regulatory authorities and the industry. It will result in an overall framework, in principle applicable for all/most types of legislations on how to address safety of NMs and a set of tools for risk assessment and decision making instruments ("NANoREG toolbox").

6 Progress and outcome to date

One year after the start of the NANoREG project, the project makes the transition from the initial and preparatory phase to the R&D phase. In the initial and preparatory stage, the foundation has been laid for the R&D work. Regulators articulated their "questions and needs"; gaps in our knowledge were identified.

A suite of cross cutting materials and Standard Operating Procedures (SOPs) were established that all the NANoREG partners will use. Both selections form a prerequisite for the R&D work. They make it possible to link the results of the several projects within NANoREG because partners apply the same MNMs and SOPs.

Type of MNM	MNM Identification codes used by NANoREG
Titanium Dioxide	NM101, NM102, NM103
Silicon Dioxide	NM200, NM203
Zinc Oxide	NM110, NM111
Cerium Dioxide	NM212

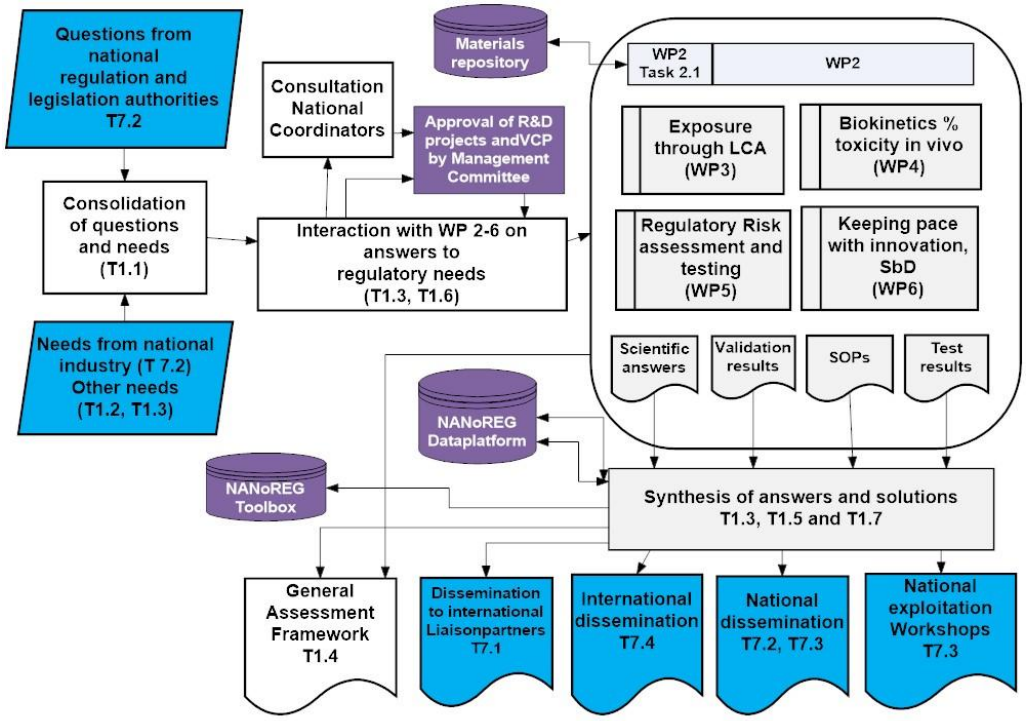

Figure 2: The NANoREG workflow and the interlinkage between the different WPs.

Barium Sulphate	NM220
Silver	NM300K, NM302
Carbon Nanotubes (single and multi-walled)	NM400, NM401, NM410
Nanofibrillar cellulose	NFC Fine, NFC Medium-coarse, UPM Biofibrils AS, UPM Biofibrils NS, UPM Bleached Birch Pulp

Figure 2: Core selection of Nanomaterials

Type of test	Protocol
In vitro studies	NANOGENOTOX
In vivo studies	NANOGENOTOX and ENPRA
Eco toxicity studies	PROSPECT as the basis and a NOM-water protocol for CNT

Figure 3: selection of dispersion protocols

The discussion on minimum requirements for "test design and performance" and data logging will, in short term, result in a first Guidance Document for NANoREG partners. As the development of these minimum requirements is an iterative process, the Guidance Document will be modified and extended during the project.

Apart from the NANoREG overarching activities, preparatory work was done for specific R&D tasks. Protocols and their applicability were tested, exposure scenarios developed, experimental set-ups were designed and a number of experiments, like inhalation tests, already started.

The first ideas for outlines of a "safe-by-design decision tree" were developed. The cooperation with National Coordinators was given a start. As "NANoREG ambassadors" in their country, the National Coordinators played an important role in formulating the questions and needs of regulators. Later on in the project, they will check the results against these questions and needs.

7 Directory

Table 1 Directory of Members of the NANoREG Management Committee

First Name	Last Name	Role in project	e-mail
Juan	Riego Sintes	Workpackage leader WP1	Juan.riego-sintes@ec.europa.eu
Hugues	Crutzen	Co-leader WP1	Hugues.crutzen@ec.europa.eu
Hakan	Wallin	Co-leader WP2	hwa@nrcwe.dk
Keld	Jensen	Workpackage leader WP2	kaj@nrcwe.dk
Marina	Moser	Co-leader WP2	mgm@nrcwe.dk
Armand	Masion	Workpackage leader WP3	masion@cerege.fr
Martie	van Tongeren	Co-leader WP3	Martie.VanTongeren@iom-world.org
Tom	Gebel	Workpackage leader WP4	gebel.thomas@baua.bund.de
Peter	Laux	Co-leader WP4	Peter.Laux@bfr.bund.de
Steffi	Friedrichs	Workpackage leader WP5	steffi.friedrichs@nanotechia.org
Susan	Dekkers	Co-leader WP5	Susan.dekker@rivm.nl
Blanca	Suarez	Co-leader WP5	suarezb@gaiker.es
Adrienne	Sips	Workpackage leader WP6	adrienne.sips@rivm.nl
Susan	Dekker	Co-leader WP6	Susan.dekker@rivm.nl

First Name	Last Name	Role in project	e-mail
Karl	Höhener	Workpackage leader WP7 IPR manager	karl.hoehener@temas.ch
Annemarie	Gemperli	Co-leader WP7	annemarie.gemperli@temas.ch
Tom	van Teunenbroek	NANoREG Coordinator	Tom.van.teunenbroek@minienm.nl
Aart	Dijkzeul	Workpackage leader WP8 Project Manager	aart.dijkzeul@minienm.nl
Joke	Vroom	Project Officer	joke.vroom@nanoreg.eu

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NanoRISK

Best practices effectiveness, prevention and protection measures for control of risk posed by engineered nanomaterials

Contract Agreement: LIFE12 ENV/ES/178 Website: http://www.lifenanorisk.eu
Coordinator: Carlos Fito, Packaging, Transport and Logistics Research Center, Valencia, Spain

No.	Beneficiary name	Short name	Country
1	PACKAGING, TRANSPORT AND LOGISTICS RESEARCH CENTER	ITENE	Spain
2	VITO NV- VLAAMSE INSTELLING VOOR TECHNOLOGISCH ONDERZOEK N.V.	VITO	Belgium
3	AVANZARE INNOVACION TECNOLOGICA S.L.	AIT	Spain
4	CENTRO RICERCHE PLAST-OPTICA S.P.A	CRP	Italy

Contents

1 Summary

Project Duration: 1 October 2013 – 30 June 2016
Project Budget: 1,165,973EUR

The nanotechnology came out as a key enabling technology (KET) to contribute to a sustainable development of "High-Tech" applications under several industrial sectors such as packaging, building, cosmetics, health care, textiles, waste management, household, detergents, electronics, ceramics or painting.

Along with the benefits, **there is an on-going debate about their potential effects on human health or the environment**, considering as a key issue the potential adverse effects of ENPs on workers upon inhalation.

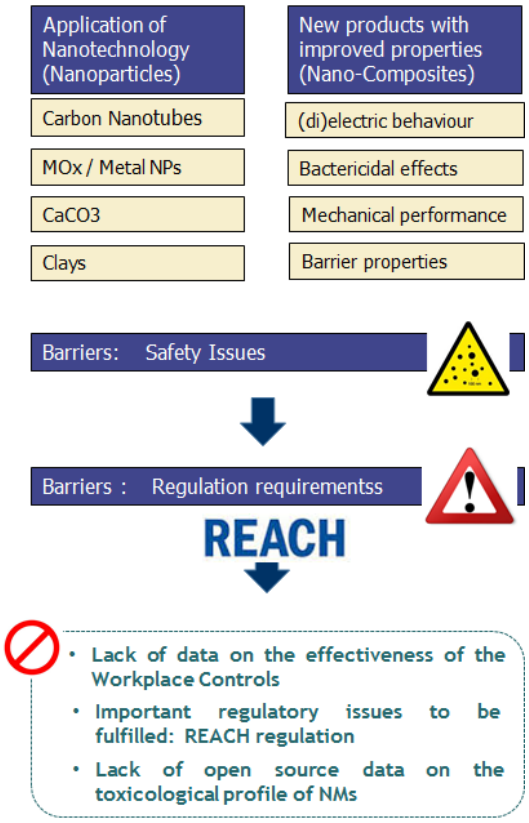
Moreover, recent publications have demonstrated that ENPs can be released to the environment during production, further processing, use and disposal. Recent reports from EU research project as well as other peer reviewed publications have demonstrated the release of nanomaterials to the environment, showing concentrations up to micrograms in rivers, which involves adverse effects in sensitive species. Similarly, studies focused on the release of ENMs from the production sites have found concentrations up to 4.6x 106 pt/cm3, which depending of the type NMs could exceed the current Occupational Exposure Limits (OELs).

In order to address these major concerns , the **main objective of the project is to define proven Risk Management Measures** (RMMs) to prevent or minimize exposure to engineered nanomaterials (ENMs) during the specific workplace situations of the polymer nanocomposite industry, as well as to **support standardization activities concerning the certification of the adequacy of Personal Protective Equipment** (PPE) and **Engineering Controls** (ECs) to protect workers from the risk posed by use of ENMs.

Within this context, the **concept of the project in to ensure a high level of protection of human health and the environment** from the risks that can be posed by the use engineering nanomaterials.

On the other hand, considering the LIFE + programme priorities, the implementation of chemical legislation, and in particular REACH regulation, **plays a central role to ensure the protection of environment and health** from risks posed by chemicals by 2020, meaning that an investigation of proven strategies for controlling existing worker exposures and reduce release in the workplace is therefore not just interesting, but also necessary to provide the political decision-makers and stakeholders with solutions to ensure the sustainable development of the nanotechnology.

In view of the above, and considering the growing production of nanoparticles to develop high-tech applications, the project and its action stems from the need of supporting the mitigation and control of the emerging risk posed by the use of engineering nanomaterials.



2 Background

The use of nanomaterials is steadily increasing daily due to the new properties addressed by the nanotechnology based products. The data published show a significant increase in the production rates of the most representative nanomaterials with growth expected to achieve 2 billion jobs by 2015, being the European Union responsible for 30 % of nanomaterials manufacturing and use.

In this respect, the main materials and substances at the nanometer scale currently produced in the Europe include nanopowders (metals, metal oxides, alloys), magnetic nanomaterials, carbon nanotubes (single, multi-walled), nanoceramics, nano-silica (fumed, colloidal), quantum dots (metal and semi-conducting nanocrystals) and polymer composites containing nanoreinforcements.

Such rapid proliferation results in a key environmental problem due to the fragmentary scientific knowledge of their health and environmental impacts and subsequent effects on ecosystem health.

Taking into account the current situation, the rising production and use of ENMs is generating both environmental and human health impacts. To overcome the adverse effects of the nanotechnology development in the environment, REACH regulation will play a central role to the extent that the responsible of the commercialization must evaluate the environmental exposure across the product life-cycle, reporting to the European Chemicals Agency (ECHA), the necessary measures to achieve an acceptable level of exposure on the basis of the risk characterization process.

Taking into account the current situation, the **project deals with the characterization of highly-efficient work place controls to reduce and control the risk posed by the use of ENMs.**

The project is focused on the polymer nanocomposites industry, where several studies show a substantial release of engineering nanomaterials during the production process. In addition, recent estimations of the levels of release performed by within the FP7 project NanoSafePack show that the current production of nanocomposites will result in a release of 4,895 metric tons of engineering nanomaterials to the environment, where the release via air is the main source of environmental pollutions (71 %), followed by water and soil.

Regarding the levels of exposure, data retrieved from the literature shows levels of exposure up to 4,6x105 pt/cm3 and mass concentrations up to 53 µg/m3. In the specific case of the nanocomposite industry, recent studies demonstrate that the weighing and sanding processes of 600 mg of NMs is able to generate a respirable mass concentration up to 2.68 µg/m3. In addition, measurements in the breathing zone results in mass concentrations up to 31.5 µg/m3. In view of such data, the production of 1 tonne of nanomaterial can generates a mass concentration of airborne NMs up to 4.5 mg/cm3, which depending of the type NMs could cause adverse effects upon exposure via inhalation or dermal exposure.

In view of such data there is an urgent need to provide the industry with proven, technically feasible and economically viable organizational measures, Personal Protective Equipment (PPE) and engineering techniques to control and reduce the risk of exposure to engineering nanomaterials.

Scientists agree that if engineering controls are well designed they will be effective in limiting nanomaterials exposure. However engineering controls need to be supplemented by good work practices and the use of appropriate PPE (Tsai & Hallock 2007), which are especially relevant where other approaches such as elimination, substitution or modification of nanomaterials is not possible.

3 Concept and Objectives

3.1 Project Concept

The **NanoRISK project deals** with the characterization of highly-efficient work place controls to reduce and control the risk posed by the use of ENMs in the nanocomposite industry, as well as with the development of standardized approaches to support the testing and demonstration activities.

The overall aim of NanoRISK project is to improve the protection of environment and health from risk posed by chemicals by supporting the implementation of the REACH regulation with regard to nanomaterials, whose use raise many questions and generate concerns due to their potential health and environmental risks. On the basis of this concept, the following activities will be conducted:

a- Generation of practical information to be used in the context of REACH, including the selection of representative nanoscale materials , the identification of reliable information to evaluate hazard and exposure, as well as the identification of information sources regarding the effectiveness of common Risk Management Measures against nanomaterials , all of them key aspects fort risk assessment purposes on a regulatory basis.

b- Characterization of standard protocols to support the quantitative evaluation of the effectiveness of workplace controls

c- Design and development of an aerosol testing chamber prototype for the for the standardized evaluation of the effectiveness of the working procedures, prevention and protection measures to control the risk posed by engineered nanomaterials.

d- Generation of reliable data on the airborne behaviour and release ratios of relevant engineered nanomaterials, including new data on their aggregation/agglomeration patterns and deposition factors under the specific operative and environmental conditions of use presented in the nanocomposites production facilities

e- Promotion of REACH fulfilment by implementing a RMM library containing reliable information on the protection factors of common risk controls against nanomaterials.

3.2 *Project Objectives*

The main objective of the project is to **define proven Risk Management Measures (RMMs) to prevent or minimize exposure to engineered nanomaterials (ENMs) during the specific workplace situations of the polymer nanocomposite industry**, as well as to support standardization activities concerning the certification of the adequacy of Personal Protective Equipment (PPE) and Engineering Controls (ECs) to protect workers from the risk posed by use of ENMs. In detail, and considering the role of REACH regulation and the LIFE+ priorities, the specific objectives of the project are:

- To support the Library on RMM (RMM library) developed within the REACH Implementation Projects with quantified data on the effectiveness of personal protective equipment (PPE), engineering techniques and organizational measures;
- To develop an aerosol testing chamber prototype to evaluate and demonstrate the performance of the RMM at laboratory scale;
- To improve the knowledge base on the parameters that determine the exposure to ENMs at industrial scale;
- To enhance the knowledge base on the potential releases of ENMs to air, soil and water from industrial facilities on a life cycle basis;
- To analyze the adequacy of current international standards (ISO /CEN /ASTM) to evaluate the effectiveness of PPE and collective protection measures;
- To improve the knowledge on the likely Exposure Scenarios in the nanocomposite industry;
- To support the hazard and exposure characterization for ENMs with the aim to support the industry in carrying out their Chemical Safety Assessment (CSA) as stated by REACH;
- To disseminate the project results for a large community of SMEs and potential stakeholders;
- To support the monitoring of REACH compliment and its impact on risk mitigation and prevention of pollution posed by NMs.

4 Overall view of the Workplan

The NanoRISK project is structured in 5 main actions on the basis of the types of eligible actions under the framework of the LIFE+ call.

The scheduled actions and the responsible partner are included in the following table:

Table 1: Scheduled Actions of NanoRISK

WP n°	WP Title	Action Leader
Preparatory Actions		
A.1.	Selection and Description of the types of nanomaterials.	ITENE
A.2	Information gathering on the conditions of use and risk management measures across nanomaterials life cycle	ITENE
A.3	Compilation of data regarding the efficiency of risk management measures for occupational and environmental exposures	VITO
A.4.	Identification of the pilot plant requirements for standardized testing	VITO
Implementation Actions		
B.1.	Compilation and critical evaluation of the published standards for determining the protection efficiency	VITO
B.2	Design and construction of the test chamber prototype for demonstration activities	ITENE
B.3	Development of the testing activities according to the selected approaches	ITENE
B.4.	Development of a Risk Management Measures (RMM) library tool	ITENE
B.5.	Scaling up to industrial case studies	CRP
B.6.	Guidance on the required measures and controls for mitigating and control the risk posed by the target nanomaterials during its entire life cycle	INVASSAT
B.7.	Training activities for end users and stakeholders	INSHT
Monitioring Action		
C.1.	Definition of the starting situation – baseline	ITENE
C.2.	Quantitative Assessment and monitoring of the protection factors achieved under controlled	ITENE

	conditions	
C.3.	Evaluation of the improvements achieved in industrial conditions	ITENE
C.4.	Promotion of REACH fulfilment by implementing the LIFE nanoRISK project	ITENE
C.5.	Assessment of the socio-economic impact of the project actions	ITENE

As can be derived from the table, the work plan has been split into 3 types of activities or actions and based. The overall objectives of each activity are explained below:

1. Preparatory Actions

A set of four preparatory actions will be conducted aiming at clearly define a set of representative NMs in the context of REACH, identify the specific exposure scenarios across their life-cycle, evaluate the feasibility and accuracy of the current approaches for testing and define in detail the technical requirements of the test chamber.

2. Implementation Actions

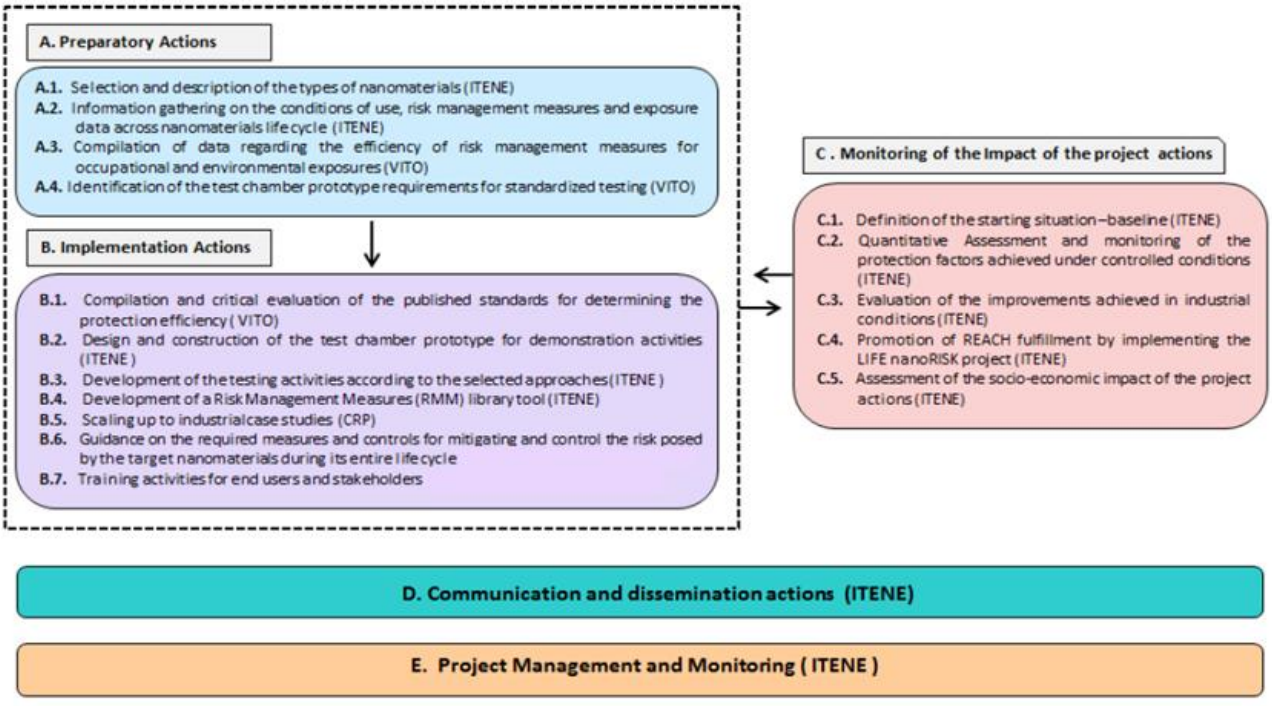
The implementation actions will work on the validation of the workplace controls to prevent or minimise exposure to ENMs including the design of a test chamber prototype for harmonized testing , the development of tools to support the decision making process for selecting control approaches for NMs and Scale up studies.

3. Monitoring Actions

These actions will be focussed on the monitoring of the improvements addressed by means of the project actions, as well as the adequacy of the developed means to address the specific problems and threats.

Besides the above, in order to achieve an optimal management and use of the Project across the EU, management and dissemination actions are also essential to the success of the NanoRISK project.

The scheduled actions and their interdependence are shown schematically below:



5 Added value to EU policies and regulations

The project will explore legal and policy issues, as well as scientific and technical issues, that might arise in the application of the regulatory process related to the use of NMs at the workplace. At this stage, the project results will increase the knowledge about the risk to the human health and the environment, supporting the regulatory activities with scientific data to establish new legal requirements to the use of NMs in the nanocomposite industry in particular and other nanotechnology fields in general.

The project is aligned with the considerations expressed by the European Parliament resolution of 24 April 2009 on regulatory aspects of NMs, which explains that the use of NMs should respond to the real needs of citizens and that their benefits should be realized in a safe and responsible manner, considering the potential EHS problems.

Research activities are ongoing under the Research Framework Programmes and the Joint ResearchCentre, as well as in EU Member States and internationally within the OECD Working Party

on MNMs and the International Organization for Standardisation. According to the Europe 2020 strategy, one of the strategic goals will be ensuring the safe development and application of nanotechnologies by advancing scientific knowledge of the potential impact of nanotechnologies on health or on the environment, and providing tools for risk assessment and management along the entire life cycle. In this sense, the future needs may include identifying and demonstrating the effectiveness of containment technologies for safe handling of NMs through the life cycle, investigating the effectiveness of different work practices for human and environmental exposure mitigation, and strengthening current research on RMM including process enclosure, ventilation and PPE.

Therefore the nanoRISK project is in line with the research areas underpinning risk assessments and management in which new knowledge is more needed, bringing value to the European development of risk management knowledge by the identification of proven measures and controls to reduce exposure to NMs during its entire life cycle.

- A complete description of the current ES across the nanocomposites life cycle, including an in depth description of the existing OC, efficient RMMs and measured exposure levels.
- New information on the release rates to air, surface fresh and marine water, waste water and soil for each relevant stage on the life cycle
- New knowledge on the airborne behaviour of the target NMs, including new data on their aggregation/agglomeration patterns and deposition factors under the specific conditions of use presented in the nanocomposites production facilities.
- A structured compendium of free webinars and workshops to support the training of end users and stakeholders in the use and implementation of the RMM.
- A set of informative material to disseminate the project actions at a Regional, National and European level.
- A network platform to close the knowledge gaps about nanomaterials impact and to develop and implement, in collaboration with scientific committees

6 Expected Results

The main outcomes of the project will be a **library of proven and technically feasible prevention and protection measures** for mitigating and control the environmental, health and safety (EHS) risks posed by nanomaterials during the nanocomposites production, use and release, as well as a set of standardized testing protocols based on the application of a newly designed test chamber to support the quantitative evaluation of the effectiveness of the workplace controls.

In detail, It´s expected to produce the following results:

- A library of proven, technically feasible and economically viable organizational measures, PPE and engineering techniques to control and reduce the risk of exposure to ENMs.
- A functional and newly developed testing chamber prototype for the standardized evaluation of the effectiveness of the working procedures, prevention and protection measures to control the risk
- A compendium of at least 10 well defined and standardized protocols to evaluate the effectiveness of the work place controls against NMs.
- A complete assessment report of the ISO standards for PPE testing

7 Progress to date

The project started officially on October 1st 2012, and had the project had its kick-off meeting at the coordinator facilities on October 23th.

Considering the concept of the project, expected outcomes, and in view of the project timetable and scheduled actions with the project, much of the activities since the beginning of the project have been focused on the definition of the ENMs to be studied within the project, the definition of the nature and extent of processes and activities conducted during the ENMs and nanocomposites (PNCs) production, use and disposal, as well as the determination of the minimum set of information to be generated during the effectiveness testing assays.

On the other hand, several meetings have taken place between partners to define the task under each action, especially between the technical partners ITENE and VITO, both involved in the characterization of harmonized approaches to evaluate the effectiveness of risk management measures against NMs and the design of the aerosol testing chamber prototype.

Finally, ITENE, in charge of the dissemination activities has prepared the first brochure of the project, which will be available in the project web site.

8 Directory

Table 2. Directory of people involved in this project.

First Name	Last Name	Affiliation	Address	e-mail
Carlos	Fito			cfito@itene.com
Enrique	De la Cruz	Packaging, Transport and logistics research center	Albert Einstein, 1 CP.46.980 Paterna (Valencia) – Spain	ecruz@itene.com
Marga	Santamaria			msantamaria@itene.com
George	Boulougouris			George.boulougouris@itene.com

First Name	Last Name	Affiliation	Address	e-mail
Patrick	Berghman	VITO Nv- Vlaamse Instelling Voor Technologisch Onderzoek N.V	Boeretang 200, 2400 Mol, Belgium	patrick.berghmans@vito.be
Evelien	Frinjns			evelien.frijns@vito.be
Sara	Padovani	Centro Ricerche Plast-Optica S.p.A	Via Jacopo Linussio, 1 33020 Amaro (UD), Italy	sara.padovani@magnetimarelli.com
Silvia	Priante			silvia.priante@magnetimarelli.com
Julio	Gómez	AVANZARE Innovacion Tecnologica S.L.	C/Jardines 5. Poligono Industrial Lentiscares 26370 Navarrete (La Rioja) SPAIN	julio@avanzare.com

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NANOSOLUTIONS

Biological Foundation for the Safety Classification of Engineered Nanomaterials (ENM): Systems Biology Approaches to Understand Interactions of ENM with Living Organisms and the Environment

Contract Agreement: Fp7 no: 309329 Website: www.nanosolutionsfp7.com
Coordinator: FIOH

Table 1 Consortium List.

No.	Beneficiary name	Short name	Country
1	TYOETERVEYSLAITOS	FIOH	Finland
2	KAROLINSKA INSTITUTET	KI	Sweden
3	UNIVERSITY COLLEGE DUBLIN, NATIONAL UNIVERSITY OF IRELAND, DUBLIN	NUID UCD	Ireland
4	NEDERLANDSE ORGANISATIE VOOR TOEGEPAST NATUURWETENSCHAPPELIJK ONDERZOEK - TNO	TNO	Netherlands
5	UNIVERSITE BORDEAUX	UB	France
6	UNIVERSITY COLLEGE LONDON	UCL	United Kingdom
7	UNIVERSITY OF PLYMOUTH	UOP	United Kingdom
8	HERIOT-WATT UNIVERSITY	HWU	United Kingdom
9	ASOCIACION CENTRO DE INVESTIGACION COOPERATIVA EN BIOMATERIALES	CIC biomaGUNE	Spain
10	LUDWIG-MAXIMILIANS-UNIVERSITAET MUENCHEN	LMU MUENCHEN	Germany
11	INSTITUTE OF OCCUPATIONAL MEDICINE	IOM	United Kingdom
12	TURUN YLIOPISTO	U. TURKU	Finland
13	TEKNOLOGIAN TUTKIMUSKESKUS VTT	VTT	Finland
14	ACONDICIONAMIENTO TARRASENSE ASSOCIACION	LEITAT	Spain
15	DANMARKS TEKNISKE UNIVERSITET	DTU	Denmark
16	FONDAZIONE TELETHON	FTELE.IGM	Italy
17	UNIVERSITAET LEIPZIG	ULEI	Germany
18	EIDGENOESSISCHE MATERIALPRUEFUNGS- UND FORSCHUNGSANSTALT	EMPA	Switzerland
19	BIOBYTE SOLUTIONS GMBH	BIOBYTE	Germany
20	INSIGHT PUBLISHERS LIMITED	IPL	United Kingdom
21	PLASMACHEM PRODUKTIONS- UND HANDEL GMBH	PLASMACHEM	Germany
22	INKOA SISTEMAS SL	INKOA	Spain
23	BIOTESYS GMBH	BIOTESYS	Germany
24	Zhejiang University	ZJU	China (People's Republic of)
25	Fundação Universidade de Brasilia	UNB	Brazil
26	NATIONAL HEALTH LABORATORY SERVICES	NIOH	South Africa
27	NOORDWES-UNIVERSITEIT	NWU	South Africa
28	NATIONAL INSTITUTE FOR OCCUPATIONAL SAFETY AND HEALTH	NIOSH	United States
29	SAIC - FREDERICK INC CORPORATION SCIENCE APPLICATIONS INTERNATIONAL CORPORATION	NCL	United States
30	NANOCYL SA	NANOCYL	Belgium
31	NANOLOGICA AB	NANOLOGICA	Sweden
32	UNIVERSITA DEGLI STUDI DI SALERNO	NeuRoNe	Italy

33	SOLVAY SA	SOLVAY	Belgium
34	POLYMER FACTORY SWEDEN AB	POLYMERFACTORY	Sweden
35	POLIMEROS Y SISTEMAS DE APLICACION TECNICA SL	POLYSISTEC, S.L.	Spain
36	UNIVERSITY OF MANCHESTER	UNIMANT	UK *

* replaced UCL , partner 6

Contents

1 Summary

Project Duration: 1.4.2013-31.3.2017

Project Funding: Fp7 GA contract no 309329, € 10 000 000

By identifying and elaborating those characteristics of engineered nanomaterials (ENM) that determine their biological hazard potential, NANOSOLUTIONS FP7 will provide a means to develop a safety classification model for ENM based on an understanding of their interactions with living organisms at the molecular, cellular, and organism levels based on their material characteristics.

2 Background

Engineered nanomaterials (ENM) have attracted a great deal of interest over recent years and their potential for economic exploitation has risen exponentially. Some of their properties however have given rise to concern that they may be harmful to humans. Scientists, regulators and industry need an effective test of these properties in order to be sure they are safe to use. While testing of individual applications of ENM is possible, it is expensive and time-consuming and is a barrier to innovation. By identifying those characteristics of ENM that determine their biological hazard potential it will be possible to create a set of biomarkers of their toxicity that will assess and predict their safe use.

3 Scientific and technological challenges

The way ENM interacts with living organisms is complex and their biological effect is largely governed by their surface properties and the way different biomolecules bind to this surface. By understanding the fundamental characteristics of ENM underpinning these biological effects Nanosolutions will provide a sound foundation on which to classify the materials according to their safety. In other words, Nanosolutions will investigate how ENM interact with living organisms at a molecular, cellular and organism level based on their material characteristics. In determining the biological identity of ENM the project will develop a computational model that will predict from the properties of ENM their ability to cause harmful health or environmental hazards. This will give scientists the ability to predict these harmful effects rather than simply describe them once they have occurred.

4 Objectives

The main objective of NANOSOLUTIONS is to identify and elaborate those characteristics of ENM that determine their biological hazard potential. This potential includes the ability of ENM to induce damage at the cellular, tissue, or organism levels by interacting with cellular structures leading to impairment of key cellular functions. These adverse effects may be mediated by ENM-induced alterations in gene expression and translation, but may involve also epigenetic transformation of genetic functions. By determining the biological identity of ENM, the project will create a set of biomarkers of ENM toxicity that are relevant in assessing and predicting the safety and toxicity of ENM across species. This computational, predictive tool will become the global standard for ENM safety classification.

5 Progress and Outcomes to date

In early 2014 the core material synthesis of ENM was finalized and the project completed systematic fuctionalisation. The distribution of materials was completed in April 2014. Distribution protocols (SOP) have been drafted and discussed with the partners concerned.

The realistic simulation process of ENM behaviour in different life-cycle stages of ENM has been defined, and testing has started on a range of commercially relevant, nanotechnology enabled products.

The following preparatory work for the testing phase is currently being carried out:

- The development of reliable testing protocols for qualitative and semi-quantative approaches for protein corona detection

by mass spectrometry, and adapting safety evaluation of ENMs to highthroughput format.
- Planning in vitro test methods for cyto-, immuno- and genotoxicity testing and assays has already been conducted, and decisions have been made on methods. The project will now set up SOP on physico-chemical characterization of ENM for the quality assurance of ENM dispersion.
- The project will continue studies using mammalian (mouse) and environmental species (Daphnia Magna, fish) that aim to explore effects of ENM on cyto-, geno- and immunotoxicity hazard endpoints. Data on the cytotoxicity of large numbers of the chosen ENM is already available, and this shows marked differences in the toxicity between different ENM, core materials and their functionalized modifications.
- Cell culture conditions suitable to modulate the expression of the glycocalyx on cultured human endothelial cells, and to study the effects of nanoparticles with different surface modifications on cultured human endothelial cells with a healthy or a disturbed glycocalyx, have been successful. In addition, it has been demonstrated that the inhalation of certain types of MWCNT (long and rod-like) induces symptoms similar to allergic airway inflammation.
- Ongoing PET in vivo biodistribution studies of 18F-labeled TiO_2 NPs using i.v. administration, and establishing successfully an ex vivo placenta perfusion model.

The information format on nanomaterials and biological specimens has been defined along the ISA-TAB Nano specifications. The basic data repository has been created and tested. A computer algorithm capable of simulating synthetic data, and a novel computational method for feature selection and prioritization based on fuzzy logic and random forests have been developed. In addition, the most advanced GA has been modified for broader and faster data searches, and new methods for inferring the similarity of nanomaterials based on the genomic responses of treated cells have been established. These methods allow working both on genes and on pathways, and have been successfully tested in FDA approved drugs.

Additional activities have been organised, such as the Int. Cong. on Safety of Engineered Nanoparticles and Nanotechnologies (www.ttl.fi/senn2015) on 12-15 April 2015 (Helsinki), and Int. Cong. on Systems Biology of ENM (Stockholm) in November 2015.

6 Expected Impact

The Nanosolutions ENM safety classification model will benefit industry and enable innovation, since being able to effectively assess the safety characteristics of ENM will speed up the innovation cycle and the development of commercially viable products using EN in this format.

7 Directory

Table 1 Directory of people involved in this project.

First Name	Last Name	Affiliation	Address	e-mail
Kai	Savolainen	Dr.		kai.savolainen@ttl.fi
Bengt	Fadeel	Prof.		bengt.fadeel@ki.se
Juha	Kere	Prof.		juha.kere@ki.se
Roman	Zubarev	Prof.		roman.zubarev@ki.se
Kenneth	Dawson	Prof.		kenneth.a.dawson@cbni.ucd.ie
Derk	Brouwer	Dr.		dick.brouwer@tno.nl
Didier	Astruc	Prof.		d.astruc@ism.u-bordeaux1.fr
Kostas	Kostarelos	Prof.		kostas.kostarelos@manchester.ac.uk
Richard	Handy	Prof.		r.handy@plymouth.ac.uk
Teresa F	Fernandes	Prof.		t.fernandes@hw.ac.uk
Sergio E.	Moya	Dr.		smoya@cicbiomagune.es
Fritz	Krombach	Prof. Dr.		krombach@med.uni-muenchen.de
Rob	Aitken	Prof.		rob.aitken@iom-world.org
Riitta	Lahesmaa	Prof.		riitta.lahesmaa@btk.fi
Roland	Grafström	Prof.		roland.grafstrom@vtt.fi
Socorro	Vázquez	Dr.		svazquez@leitat.org
Erik	Huusfeldt Larsen	Dr.		ehlar@food.dtu.dk

First Name	Last Name	Affiliation	Address	e-mail
Paola	Cormio			p.cormio@tigem.it
Irina	Estrela-Lopis	Dr.		irina.estrela-lopis@medizin.uni-leipzig.de
Peter	Wick	Dr.		peter.wick@empa.ch
Ivica	Letunic	Dr.		letunic@biobyte.de
William	Davis	Mr		jbarsby@ipl.eu.com
Alexei	Antipov	Dr.		antipov@plasmachem.com
María	Blázquez			maria@inkoa.com
Jürgen	Bernhardt	Dr.		bts@biotesys.de
Changyou	Gao	Prof.		cygao@zju.edu.cn
Paulo	Cesar De Morais	Prof.		pcmor@unb.br
Mary	Gulumian	Prof.		mary.gulumian@nioh.nhls.ac.za
Victor	Wepener	Prof.		victor.wepener@nwu.ac.za
Scott E.	McNeil	Dr		ncl@mail.nih.gov
Julie	Muller	Dr.		julie.muller@nanocyl.com
Roberto	Hanoi Labrador	Dr.		hanoi@nanologica.com
Roberto	Tagliaferri			rtagliaferri@unisa.it
Jacques-Aurélien	Sergent	Dr.		jacques-aurelien.sergent@solvay.com
Andreas	Nyström			andreas.nystrom@polymerfactory.com
Jordi	Férnandez	Mr.		export@polysistec.com

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nanoSTAIR

Establishing a process and a platform to support standardization for nanotechnologies implementing the STAIR approach

Contract Agreement: NMP4-SA-2012-319092 Website: http://www.nanostair.eu-vri.eu/
Coordinator: Olivier Salvi, European Virtual Institute for Integrated Risk Management, Stuttgart, Germany

No.	Beneficiary name	Short name	Country
1	EU-VRi - European Virtual Institute for Integrated Risk Management	EU-VRi	Germany
2	Institut National de l'Environnement Industriel et des Risques	INERIS	France
3	Finnish Institute of Occupational Health	FIOH	Finland
4	Fundación TECNALIA Research and Innovation	TECNALIA	Spain
5	Nederlandse Organisatie voor Toegepast Natuurwetenschappelijk Onderzoek	TNO	Netherlands
6	Steinbeis Advanced Risk Technologies GmbH	R-Tech	Germany
7	DIN Deutsches Institut für Normung e.V	DIN	Germany

Contents

1 Summary

Project Duration: 18 months

Project Funding: 499.437 EUR

Standardization is one of the most adequate solutions to quickly capitalize and disseminate knowledge in "reference documents", and have it implemented in the industry. It is very important in the field of nanotechnologies since the production of knowledge is very intensive. The overall objective of nanoSTAIR project is to build a sustainable process and platform in the field of nanotechnologies to support the transfer of knowledge gained through research to documentary standards in the context of the STAIR approach promoted by CEN - CENELEC.

The project is organized around several activities that boost the development of new documentary standards. After identifying precisely the barriers that currently limit the transfer from research to standardization and collecting the expression of the needs for standards from various stakeholders, the nanoSTAIR project has set-up a mechanism to identify the opportunities for standardization in the results of research projects (co-funded by the European Commission or by National Research Programmes), based on the semantic analysis of research papers and on expert review. For selected results, nanoSTAIR pools together resources and consortia sharing similar standardization opportunities and provides assistance for selecting the right standardization umbrella (CEN or ISO Technical Committee and Working Group) and for launching new standardization work items. By end of December 2013, 13 research documents have gone through the semantic analysis ("nanoSTAIR check"); two research results have been selected and are being transferred into new standards by the research teams with nanoSTAIR support. One of them is currently (Mid April 2014) submitted to formal TC-voting.

As a result, nanoSTAIR has provided a set of procedures, a tool box and a practical guideline that will be useful to bridge the gap between research and standardization in nanotechnologies. nanoSTAIR structures and eases the development of new documentary standards, and thus enables the European nanotechnology related industry to rapidly operate according to the state of the art and thus increase its competitiveness. Besides, nanoSTAIR has summed up its observations in a report proposing innovative strategies and procedures to translate EU nano-research into standards. Among others, mechanisms are proposed to effectively increase the place of standardization in research calls

and to increase the recognition of performed standardization work in the calls and in the scientific community in general.

The nanoSTAIR team is now proposing, together with the nanoSTAIR platform of experts of nanotechnologies and standardization, its support to funding agencies, existing networks and research initiatives for further exploitation of research results through standardization. A business plan has been designed to make the nanoSTAIR platform sustainable. The service proposed includes:

- A (free) Web based platform, to access/receive targeted information and get support on standardization related to nanomaterials ;
- nanoSTAIR check for of project/results, to identify relevant standards, cluster related initiatives, get in contact with the relevant technical committees, prepare inputs to standardization;
- nanoSTAIR seminar, to get informed about standardization (process, committees, nanoSTAIR support) and to analyse opportunities from project(s).

This support complements the expertise provided by CEN and the National Standardization Bodies.

2 Background

Nanotechnologies are predicted to have giant market potential and by 2020 it is expected that nearly every area of industry will be affected by nanotechnologies. In addition, engineered nanomaterials (ENM) risk issues have been dealt with in a number of recent reports. Growing production and use of ENM leads unavoidably to increased exposure of workers and consumers in terms of numbers exposed and levels of exposure.

New technologies need standards and metrology to support safe and sustainable development and trade. Implementation of this knowledge in the industry will further ensure sustainable competitiveness and operations according to the state of the art.

Standardization appears doubtless to be the key exploitation activity and output of collaborative research, especially of research performed on emerging technologies at European level. Standardization supports the dissemination of research results in an effective, clear and transparent way. All relevant stakeholders, including the research community and the SMEs, can have access to the standardization process.

The new European Standardization Policy 2011 insists on the importance to increase the number of standards and to speed-up the development of standards in a fast changing global landscape. This is particularly true for nanotechnologies that impact a lot of industrial sectors and where safety and social acceptance are important elements. Standards in this field are considered very important because they can facilitate the introduction of new products by bridging the gap between research and marketable products, and also because they contribute to the public acceptance of the innovations.

In order to promote the links between research and standardization, CEN and CENELEC have set-up the STAIR Joint Working Group (STAndardization, Innovation and Research). STAIR has developed the so-called "integrated approach" that is encouraged to be applied to research projects dealing with emerging technologies such as ENM. According to the STAIR approach, standardization does not come as an afterthought but is built into a project proposal right from the start. This introduces significant benefit potential for the project itself and for any actions after the project's life-time.

Several projects and studies have shown the need to bridge the gap between research and standardization. From the results and the experience gained in these projects, it appears that the barriers are related to the four elements: Integration, Awareness, IPR and Resources. On the other hand, it is necessary to develop specific and practical procedures and tools for a sector to bridge this gap. It has to be specific because the features of the barriers are very much industry sector dependant.

3 Scientific and technical challenges

The nanoSTAIR project has been developed to facilitate the emergence of standard based on the results of research projects, taking into account the on-going work of CEN-CENELEC in the context of the mandate M/461, considering any opportunity for standardization but recognizing that the characterization of and exposure from nanomaterials and Health, Safety, and Environment are important drivers.

The project is designed to develop a process supported by a set of tools to practically bridge the gap between research and standardization. It will be achieved by working in synergies with the technical committees (TCs) and working groups (WGs) at CEN, ISO and OECD levels, paying attention not to duplicate the work of the TCs and WGs, but rather offering a liaison with researchers via an effective exchange process and platform.

In addition, running initiatives such as NANOfutures and the NanoSafetyCluster are very important among the research community active at the European level. NanoSafetyCluster is also a natural platform to collaborate with nationally funded projects related to standardization, and with the appropriate key-node activities of the NANOfutures project. nanoSTAIR project also intends to interact with the project that will be funded under the FP7 call on NMP 1.3-3 Regulatory testing for Nanomaterials, because of the obvious synergies between standardization and regulation.

Not everything can be standardized because of resources available, stakeholders' needs, and the priorities have to be defined. Therefore based on these results, the nanoSTAIR project will focus on 1) creating a process to easily launch new documentary standards within projects dealing with nanotechnologies, 2) pooling human resources to reach the critical mass and obtain the relevant expertise and 3) developing a dynamic and speed up the whole preparation of a NWIP.

4 Objectives

The overall objective of nanoSTAIR is to build a sustainable process and platform to support the transfer of knowledge and results gained through research to documentary standards (which could

be EN, TS, TR and CWA)7, in the context of the STAIR approach promoted by CEN-CENELEC.

The aims of the project are definitively but not exclusively:

1. to set-up a mechanism to identify the opportunities for standardization from the results of research projects, co-funded by the European Commission and National Research Programmes using a bottom-up approach;
2. to initiate the process to prepare new standardization work item proposals (NWIP) by pooling resources from consortia, researchers and experts from industry (producers and end-users) to develop the content of the NWIP;
3. to define the type of documentary standard to be drafted and to identify the right host (i.e. the right Technical Committee) to include the NWIP in its work programme and start the NWIP with the support of the European and National Standard Bodies.

The project fosters collaboration between national government ministries/agencies that draft regulations, the National, European and International standardization bodies that draft standards, the research organizations that feed standardization and industry needs and finally the industries that need to securely produce products. When considering the preparation of a new work item proposal, if no CEN TC is identified, the consortium considers the possibility to prepare a CEN Workshop Agreement that will help structuring the European position before further negotiation at international level within ISO or OECD.

5 Progress and outcomes to date

nanoSTAIR ends in February 2014.

The project has set up a collaborative process that enables: 1) flexibility regarding the topics addressed, because the situation on nanotechnologies is evolving very quickly around the world, 2) reactivity to invite new experts according to new technological developments or the evolving policy and public debate and 3) collaboration to support the development of WI with the support of various countries, with the creation of a critical mass.

The project is organized around several activities that boost the development of new documentary standards. After identifying precisely the barriers that currently limit the transfer from research to standardization and collecting the expression of the needs for standards from various stakeholders, the nanoSTAIR project has set-up a mechanism to identify the opportunities for standardization in the results of research projects (co-funded by the European Commission or by National Research Programmes), based on the semantic analysis of research papers and on expert review (Figure 1). For selected results, nanoSTAIR pools together resources and consortia sharing similar standardization opportunities and provides assistance for selecting the right standardization umbrella (CEN or ISO Technical Committee and Working Group) and for launching new standardization work items. By end of December 2013, 13 research documents have gone through the semantic analysis ("nanoSTAIR check"); two research results have been selected and are being transferred into new standards by the research teams with nanoSTAIR support. One of them is currently (Mid April 2014) submitted to formal TC-voting.

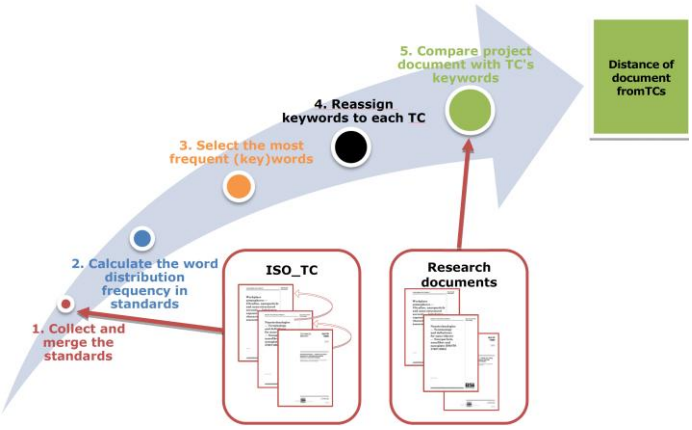

Figure 1: nanoSTAIR's semantic analysis

As a result, nanoSTAIR has provided a set of procedures, a tool box and a practical guideline that will be useful to bridge the gap between research and standardization in nanotechnologies. nanoSTAIR structures and eases the development of new documentary standards, and thus enables the European nanotechnology related industry to rapidly operate according to the state of the art and thus increase its competitiveness.

The most important outcomes of the project are the nanoSTAIR platform that brings together the best experts to launch standardization work item proposals and the nanoSTAIR process to develop new work item proposal.

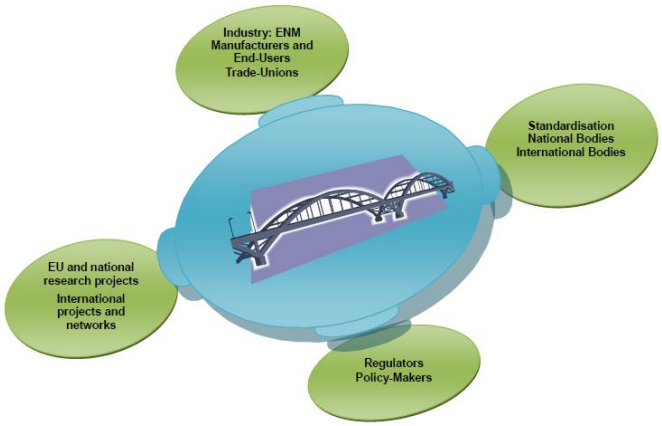

Figure 2: nanoSTAIR platform to bring together the relevant stakeholders and to bridge research and standardization

The nanoSTAIR platform is a place to find information and support, and to find and meet potential partners. Figure 2 illustrates the nanoSTAIR platform which is a virtual table where all stakeholders have the opportunity to share common needs and solutions regarding new documentary standards. In particular, the nanoSTAIR platform bridges the research community and the standardization community. In January 2014, more than 20 nanotechnology and standardization experts have joined the platform.

The nanoSTAIR process will be described in procedures, checklists and communication channels. The Figure 3 illustrates the nanoSTAIR process which can be seen as a turbine that accelerates the preparation of new work item proposals by identifying the potential candidates, by making explicit the needs from the main stakeholders and by pooling the resources and expertise to reach the necessary critical mass.

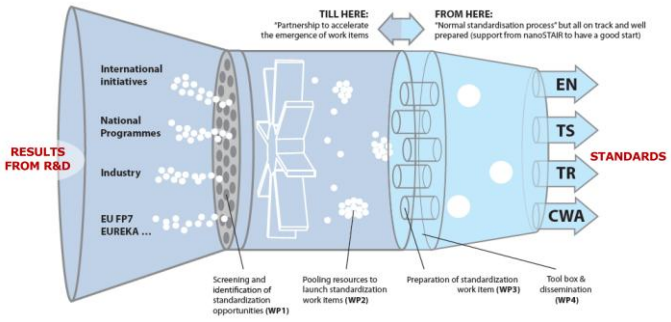

Figure 3: The nanoSTAIR process

Both nanoSTAIR process and platform support the preparation of documentary standards work in the field of nanotechnologies, and help structuring the emergence of new work items that go then to the normal standardization process that is described in the CEN COMPASS. In addition, the nanoSTAIR process and platform speed up the preparation of the proposal and increase the number of proposals.

Knowledge developed during the project has been collated in the form of reports and conclusions of discussion workshops, but also practical tools that will be used and exploited after the end of the project. For instance, nanoSTAIR has summed up its observations in a report proposing innovative strategies and procedures to translate EU nano-research into standards. Among others, mechanisms are proposed to effectively increase the place of standardization in research calls and to increase the recognition of performed standardization work in the calls and in the scientific community in general.

The nanoSTAIR team is now proposing, together with the nanoSTAIR platform, its support to funding agencies, existing networks and research initiatives for further exploitation of research results through standardization. A business plan has been designed to make the nanoSTAIR platform sustainable. The service proposed includes:

- A (free) Web based platform, to access/receive targeted information and get support on standardization related to nanomaterials ;
- nanoSTAIR check for of project/results, to identify relevant standards (Figure 3), cluster related initiatives, get in contact with the relevant technical committees, prepare inputs to standardization;
- nanoSTAIR seminar, to get informed about standardization (process, committees, nanoSTAIR support) and to analyse opportunities from project(s).

This support complements the expertise provided by CEN and the National Standardization Bodies.

All presentations and reports are included on the project website, as well as the tools and the nanoSTAIR practical guideline. To widen and consolidate the potential impact of the project, an International Advisory Board has commented the on-going works and assisted in the dissemination.

Table 1 Workpackages (WP) of nanoSTAIR

WP	Title	Topic
1	*Screening and identification of standardization opportunities*	WP1 aims to define criteria to assess the results of research projects and select candidates for standardization and establish a process to continuously identify possible candidates.
2	*Pooling resources to launch standardization work items*	WP2 aims to identify the needs of various stakeholders for new standards, experts and projects and investigate the possibilities for incorporation of (parts of) the standardization process in running projects.
3	*Verification of the approach with the preparation of standardization work item (Selecting standardization tools and launch of new work item)*	WP3 aims to verify by proof of principle with 2 worked examples that the procedures and tools developed in the project lead to ease the preparation of new standards work items in the field of nanotechnologies.
4	*Tool box and dissemination*	WP4 aims to assemble the outcomes produced in previous WPs to construct tools and guidelines to promote and translate in a practical way European nano-research into documentary standards.
5	*Project management*	WP5 deals with coordinating and managing the project by covering technical, administrative, legal and financial issues and the relation with the EC.

6 Expected impact

The main expected impact of nanoSTAIR is the support to strengthen the position of European nanotechnology industry on the international scene, because nanoSTAIR: 1) Speeds up the emergence of new standards based on research results, 2) Brings together the stakeholders and the content providers able to deliver documentary standards at once 3) Eases the overall process of developing new standards thanks to well established communication channels (with regular meeting with experts of the IAB, the nanoSTAIR platform).

The expected impact as listed in the work programme is related to:

1. Delivery of new standardisation documents (e.g. a CEN new work item); and/or
2. Consolidation of the technical background for standardisation, unification and certification of advanced materials, manufacturing processes and their production environment; and/or
3. A substantial contribution to international standardisation, helping to strengthen the position of European industry; and/or
4. Improved quality control for the entire process chain (from design, over production and certification up to product disposal), increased inter-operability and potentially improved time to market; and
5. Support to EU policies relying on standardisation.

The paragraphs below present more specifically the impacts according to the expectations presented in the work programme and emphasize the benefits of nanoSTAIR.

The European platform to launch new standardization work items in the field of nanotechnologies and consolidate the technical background for standardization

The results of the project will impact standardization work and normative research in nanotechnologies. The outcomes of the project are being widely diffused to all interested groups of public and private researchers. The dissemination of the project findings promotes standardization in this field and thus helps to develop the European and international nanotechnologies market. For European industry and citizens, one of the dissemination routes beside the project website and direct forwarding to the stakeholders will be the European Commission and particularly the DGs concerned such as e.g. Enterprises, Research & Innovation which drafts the European standardization mandates, rules, regulations, directives and FP VII.

Delivery of new standardization documents

During the run of the nanoSTAIR project, two new standard work item proposals have been prepared. These two examples are useful to communicate on the benefits of the nanoSTAIR approach and present concrete outcomes. The consortium and the Standardization Bodies participating in the consortium (CEN and DIN) therefore expect that these examples will path the way for further cases. The nanoSTAIR procedures and platform thus support the preparation of documentary standards in nanotechnologies by capitalizing and exploiting the results of research funded in Europe.

European added value and substantial contribution to international standardization

In the field of nanotechnologies, Europe and in particular the European industry is a key player for research and development. The increased development of standards in the field of nanotechnologies, thanks to the nanoSTAIR approach, will further bring added value for the production of goods using nanomaterials. Another positive impact of nanoSTAIR is the provision of the opportunity to align the European position when a standard is negotiated at an international level, e.g. at ISO or OECD levels. Thanks to nanoSTAIR, the European position in the field of nanotechnologies will be better aligned and therefore stronger on the international scene. As an additional benefit, nanoSTAIR contributes to the transnational cooperation and to the reinforcement of the European Research Area in the field of nanotechnologies.

Contribution to the competitiveness of the European nanotechnology industries

In new technologies, like nanotechnologies, an early start to standardization activities helps to prepare new products for the market. Suppliers and users of new technologies require standards to ensure compatibility before products are placed on the market. nanoSTAIR will have a strategic impact on the EU nanotechnology industry by promoting the collaborative development of standards (since standards better enable to implement results of research). The defragmentation of the standardization in nanotechnologies will therefore give a competitive advantage of this industry in Europe.

Other benefits related to the objectives are: 1)To facilitate industrial development and exploitation of nanotechnologies and to enhance integration and bridge the gaps between research and production fields in nanotechnologies (integration) and 2) To bridge the different aspects of health, safety and environment related to nanotechnologies and to provide the numerous accompanying organizations and structures with information related to the standardization process for nanotechnologies (but not only).

Economical impact

Standardization has a significant impact on the economy. For instance, according to a study contracted by the German national standard body DIN, the economic benefits of standardization for the German national economy were calculated to be 15.9 billion Euro per year. Some other key findings were:

- Economic growth is affected by standards more than by patents and licenses.
- Enterprises participating in the standardization process have advantages in competition and costs.
- Transaction costs are minimized by using European and international standards.
- The research risk and development costs are reduced for all taking part in the standardization process.

The quoted study shows that investments into standardization work creates a significant (exponential) increase in market size

over the next ten years. Investment in standardization in nanotechnologies will have such an effect for all participating sectors. If such study were repeated in all parts of the world the issues and findings would undoubtedly be the same. A comparison of several market studies (from different sources) of the world nanotechnology market shows a leverage effect of nanotechnologies of 100 billion Euros. It has to be noted that the market volume of different real net output ratios were added here.

In an economic context, standards contribute to the development of the free market and the ability of businesses to remain innovative. Common standards permit the free trade of goods and services, cutting out additional modification costs. This approach is followed by the European market, where uniform, harmonized standards apply. In the European market, with some 450 million consumers, and accounting for a quarter of the world´s domestic product and 20% world trade, use of standards creates openings for new, sustainable innovations.

Political and societal impact

In a political context, the incorporation of the results of the work of standards bodies in technical legislation makes the job of legislators easier, and standardization thus effectively contributes to deregulation. A recent study of a large assurance company pointed out, that there is a lack in industrial health and safety standards. Filling this gap will be an important task for standardization. This also has to be investigated in detail by the proposed project. The results will lead to increased safety for labour and environment, and reinforce end-user and consumer confidence due to the fact that nanotechnologies and nano artefacts confronting them at the time being.

It is important to notice that the approach of nanoSTAIR, in terms of process and platform could be applied and repeated to other sectors, and not be limited to the nanotechnology industries. This means that the investment in the nanoSTAIR project may have a wider impact on other industry sectors.

Contribution to EU policy related to standardization

The objectives of nanoSTAIR are totally in line with the new European Standardization Policy proposal 2011 COM(2011) 311 final, that insists on the importance to increase the number of standards and to speed-up the development of standards in a fast changing global landscape. This is particularly true for nanotechnologies that impact a lot of industrial sectors and where safety and social acceptance are important elements. The benefits of nanoSTAIR, such as the increased number of documentary standards in the field of nanotechnologies and the increased rapidity to launch new standards will also facilitate the exploitation of research results by SMEs.

7 Citations

Broekhuizen et al (2011)

8 Directory

Table 2 Directory of people involved in this project.

First Name	Last Name	Affiliation	Address	e-mail
Olivier	Salvi	EU-VRi - European Virtual Institute for Integrated Risk Management	Willi-Bleicher-Strasse 19 70174 Stuttgart, Germany	salvi@eu-vri.eu
Florence	Carré	Institut National de l'Environnement Industriel et des Risques	Parc Technologique Alata-BP2, 60550 Verneuil en Halatte, France	florence.carre@ineris.fr
Arto	Säämänen	Finnish Institute of Occupational Health	P.O. Box 123 00181 Helsinki, Finland	arto.saamanen@ttl.fi
Jesús M.	López de Ipiña	Fundación TECNALIA Research and Innovation	Parque Tecnológico de Alava Leonardo Da Vinci 11 E-01510 Miñano-Alava, Spain	jesus.lopezdeipina@tecnalia.com
Derk	Brouwer	Nederlandse Organisatie voor Toegepast Natuurwetenschappelijk Onderzoek	Utrechtseweg 48, 3704 HE Zeist, PO Box 360 3700 AJ Zeist, Netherlands	dick.brouwer@tno.nl
Daniel	Balos	Steinbeis Advanced Risk Technologies GmbH	Haus der Wirtschaft, Willi-Bleicher Strasse 19 70174 Stuttgart, Germany	balos@risk-technologies.com
Michael	Schmitt	DIN Deutsches Institut für Normung e.V	Burggrafenstraße 6 10787 Berlin	michael.schmitt@din.de
Jean-Marc *	Aublant	Laboratoire National d'essais	1 rue Gaston Boissier 75015 Paris, France	jean-marc.aublant@lne.fr
Micheal *	Stintz	Technische Universitaet Dresden	01062 Dresden, Germany	michael.stintz@tu-dresden.de

*Chair of the International Advisory Board

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NanoSustain

Development of sustainable solutions for nanotechnology-based products based on hazard characterization and LCA

Contract Agreement: NMP4-SL-2009-247989 Website: http://www.nanosustain.eu
Coordinator: Rudolf Reuther, NordMiljö AB, Sunnemo, Sweden

No.	Beneficiary name	Short name	Country
1	NordMiljö AB [Environmental Assessments]	NOMI	Sweden
2	The Institute of Nanotechnology	ION	United Kingdom
3	National Research Centre for the Working Environment	NRCWE	Denmark
4	Technical Research Centre of Finland	VTT	Finland
5	University of Bremen	UniHB	Germany
6	Veneto Nanotech	VN	Italy
7	European Commission Joint Research Centre, Institute for Environment and Sustainability	EC-JRC	Belgium
8	Kaunas University of Technology	KTU	Lithuania
9	National Institute for R&D in Microtechnologies	IMT	Romania
10	Nanologica AB	NLAB	Sweden
11	Nanogate AG	NGAG	Germany
12	UPM Kymmene	UPM	Finland

Contents

1 Summary

Project Duration: 1 May 2010 – 31 May 2013

Project Funding: 2.5 Mio. EUR

After 3 exciting and stimulating years of research, meetings and discussions, the NanoSustain project implemented by 12 partners from 7 different European countries was ending on 31 May 2013. A huge amount of data has been produced and already published that will help to improve our current understanding of the intricate behavior of engineered nanomaterials when released and exposed to man and the environment. The ultimate goal of NanoSustain was to develop new technical solutions for the safe and sustainable handling of engineered nanomaterials, in particular during end-of-life phases, such as reuse/recycling, final treatment and/or disposal. To reach this goal a comprehensive physicochemical (pc) and hazard characterization (toxicology, dose-response, no-effect levels) campaign has been realized, including exposure (human, environment), risk (RA) and life cycle assessment (LCA) of selected ENM (nanocellulose, nano-TiO2, CNTs, nano-ZnO) and associated products, in particular related to their transport, transformation and environmental fate.

NanoSustain succeeded to increase our knowledge on possible risks that may occur along the life-cycle of EN containing products and provided more insight into mechanisms and factors that steer their sustainability. Three EN manufactured world-wide in large quantities have been investigated (TiO2, ZnO and MWCNTs), and

nanocellulose, which is relatively new but derived from sustainable sources and with an enormous potential for future applications. Four life-cycle phases have been studied: production of input EN, production of EN containing products, use of these products and their end-of-life recycling/disposal. Materials used and released at each stage were characterized, toxicity tested, and material + energy flows estimated. Two databases have been developed, a material and a literature database, to make the newly generated data and the knowledge developed by other projects on hazard and exposure of selected EN available to project partners. An online framework was created to systematically collect and document the new pc and biological data, and to allow their statistical treatment, evaluation and correlation. Data generated on the toxicity of pure and lifecycle (LC) relevant materials have been continuously transferred to the material database and Material Data Sheets (MDS) prepared for the 4 selected nanomaterials (NM) (nanocellulose, nano-TiO_2, CNT, nano-ZnO) with safety relevant properties provided by manufacturers and the project. To ensure validation of applied methods and data consistency and comparability across all partner laboratories, standardized protocols have been established for NP characterization by inter-laboratory comparison.

As knowledge is still scarce on how toxicity may change when NM are embedded in a product, test samples have been prepared, including raw, product composites and LCA relevant materials to study toxic effects of after-production NM. Sanding, weathering, abrasion and leaching were used to simulate changes in exposure during handling, transport or reworking, and environmental stress. The source strength of dust emissions was measured from paints (± TiO_2) and epoxy boards (± MWCNT) during sanding, breaking of ZnO containing glass and tearing of nanocellulose containing paper to assess real workplace exposure conditions, and results compared to conventional products. Both pure NP and NP embedded in products, and the collected dust, were characterized and *in vivo* tested in mice for inflammation and DNA damage. There was no increased emission of NP during sanding of NP containing materials compared to reference samples. Dose-response relationships of critical end-points were identified for dust and compared with results obtained from pure or embedded NM. There was no increased toxicity when adding nano-TiO_2 to paint or CNT to epoxy matrix and toxicity seems masked in the product matrix. Only the toxicity of nano-ZnO was conserved when added to coated glass. Also suitability tests of standard bioassays, such as the "Vibrio fischeri" test showed no acute or "nano" specific toxicity for nano-TiO_2 and nanocellulose.

Life-cycle process models were developed for all selected NM and various types of environ-mental impacts assessed by Life Cycle Assessment (LCA) including LCI (Inventory) and LCIA (Impact Assessment). Also an exposure model was completed and generic data on material flow and predicted environmental concentrations (PEC) estimated. Criteria + guiding principles have been established and validated for precautionary design and improved recyclability of NM. LCA results showed a strong dependency of the environmental impact on the type of manufacturing and a high variation of impact factors compared with conventional materials. Also the influence of EN on the environmental impact of new applications was much depending on resource efficiencies but also on the lack of data. None of the selected applications (TiO_2 in paint, ZnO in glass coatings, MWCNT in epoxy material, nanocellulose as paper additive) caused significant exposures to water, air and soil.

As a final goal, the applicability of technical solutions for the sustainable recycling and disposal of EN was tested in the lab based on the newly generated hazard and exposure data, to improve end of life processes. Nanocellulose was used to test the suitability of composting, a CNT containing epoxy composite to assess the feasibility of incineration, and a nano-ZnO coated glass to test glass melting and landfilling as recycling and disposal option. Results showed good degradability and no ecotoxicity of paper containing nanocellulose. Also incineration gave a good combustion of CNT containing boards with no CNT found in bottom or fly ash. Melting of coated glass released NP independent of the type of glass coating, while leaching tests indicated the release of ZnO NP from coated glass.

2 Scientific / regulatory / industry needs and challenges addressed

Since the world-wide production of engineered nanomaterials (EN) is increasing, the amount of products reaching the end of their life cycle may increase, and so exposure to man and the environment. We still do not know how and to what extent certain EN may be released from technical or consumer products and transported, transformed, dispersed or accumulate in man or natural systems, when used or after disposal. For this reason, there is a strong need to improve our knowledge on the impact and fate of products containing nanomaterials along their life cycle and to explore technical solutions for their safe reuse/recycling, final treatment and disposal.

The existing regulatory framework (such as REACH) based on mass (in tons) and concentration metrics may be adequate for areas, where only small amounts of nanomaterials are used (such as research laboratories or small-scale manufacturing shops). However, they may not be applicable for the industrial mass production of nanomaterials, where particle number and/or shape could be more critical for their behavior than for the same bulk chemicals. The applicability of current standardized methods, like those given in the OECD-Guidelines for measuring and testing of hazardous substances, needs to be assessed and where necessary adapted or modified, and validated. In particular the extent to which existing regulatory and risk management strategies and tools can be applied to after-production stages of nanomaterials has to be evaluated, including the suitability of available recycling, final treatment and disposal techniques.

Due to their large surface area, nanoparticles (NP) behave quite differently from bulk materials, why a considerable amount of research is currently undertaken to commercially exploit their unique behavior for a wide range of applications, with a corresponding increase in the number of nanotechnology based products reaching the end of their life-cycle. At the same time, there is a growing understanding that exactly their novel behavior may give rise to unforeseen effects in living systems, including man. Due to their small size (1-100 nm), EN may be more toxic than conventional materials with the same composition, and may cross or adsorb more easily to cell membranes or barriers.

There is increasing concern that the beneficial properties of nanoscale materials and products might also have negative impacts on human and the environmental health. Although much research is now going on world-wide, we still do not know how

exactly nanoparticles (inter)act in the human body or in the environment, to what extent they are released or leach from products, or how they are transported, transformed, or accumulate in living organisms or environmental systems, like soils or waters, in particular after their consumption, reuse/recycling, final treatment and disposal.

Recent toxicological studies show that nanoparticles have implications on human health inducing, e. g., pulmonary and systemic inflammation, and translocation to different parts within the human body, including the brain, after inhalation. However, reliable data on the (eco-) toxicity of nanomaterials is still scarce, although first studies prove that there are toxic effects and potential bioaccumulation in various organisms.

The rapidly increasing amount of nanomaterials produced world-wide causes growing concern about their final fate when used in products and released to the environment, and of possible hazards due to accumulation in animals, plants or the human body. Nanoparticles may be extremely resistant to degradation and accumulate in waters or soils, may aggregate or disperse, which will change their properties compared to single nanoparticles to an extent we still do not know. Also for this reason, existing regulation based on mass metrics alone may not be appropriate to quantify the true exposure to nanoparticles, but needs more accurate data on nano-specific parameters, like surface area, degree of dispersion or aggregation, or particle size concentration.

More reliable scientific data is needed on toxicokinetics, exposure and degradability characteristics of engineered nanoparticles to better understand where, in which form, and to what extent these new materials will end up, to develop more accurate impact, exposure and risk assessment models, and to find efficient ways for product design that in turn favor their sustainable use, reuse and recycling and/or safe disposal. Current chemical characterization and biological test methods are often not appropriate to generate the data we need to reliably assess risk and hazard. As a result, there is an urgent need for preliminary assessment at an early stage of product innovation, and to validate and further develop current characterization and testing methods for these new materials in various matrices and compartments, including reproducible test media to which men and ecosystems are exposed, as well as cell lines, body fluids or tissues.

NanoSustain has addressed most of these questions starting with a comprehensive characterization of the physicochemical (pc) properties of 4 relevant and representative EN and associated products + composites that occur along their life cycle, in relation to possible biological + environmental impacts. One key challenge of the work was to understand to what extent existing RA and LCA approaches can be used, or need to be adapted or modified to take the particular properties and uncertainties of EN fully into account, e.g. by using different dose-specific parameters, such as particle size number instead of mass concentration, or different end-points and their interpretation. For this reason, most important hazard, exposure and risk characteristics have been determined and critical life cycle stages and dose-response relationships as well a no effect levels identified during handling, transport, use, recycling and disposal, including occupational + consumer health risks from direct exposure or indirectly from the environment, or from waste disposal.

The project helped to reduce still existing uncertainties associated with current RA and LCA schemes that are caused by the lack of reliable data, by generating new reliable accurate data on exposure and dose/response that take the specific properties of EN, such as size distribution, aggregation or surface treatment, duly into account, which may have drastic effects on toxicity and exposure and change during a particle's life time. To explore the safety and sustainability of technical solutions for end-of-life phases of nanomaterials, lab experiments have been conducted to simulate composting (organic recycling), melting (glass recycling), incineration (final treatment), and land-filling (final disposal) of EN.

3 Concept, scope and strategy

The project was based on the concept of sustainability meaning that the use of any new materials, such as engineered nanomaterials, must match the needs of future generations and take all risks duly into account that may come up along a products life-cycle, why reusability/recyclability, fate and disposal became crucial questions. This concept was tested and realized by characterizing the properties of representative and relevant nanomaterials and associated products at various stages of their lifecycle in relation to possible impacts on human health and the environment, and by taking their reusability/recyclability and/or ability for safe final treatment and/or disposal, or reintegration into geological cycles into account as requirement for their sustainable development.

NanoSustain selected and investigated four main aspects of the life cycle of the 4 selected nanomaterials and of LCA relevant test materials (such as prototype products, dusts, ashes etc.) generated from these materials, including, (a) their design, (b) manufacture, (c) application, and (d) recycling/disposal. Although most studies still focus on possible toxic effects of purely nanocomponents after exposure for risk assessment, the potential contribution of these materials to all impacts has been examined, when added to products or processes, to better understand the importance of underlying choices involved in the implementation of this new technology.

The project strategy included the following main tasks to assess:

- the hazard of selected nanomaterials based on a comprehensive data survey on their properties (physicochemical characteristics, exposure probabilities, etc.) and the adaptation, evaluation, validation and use of existing analytical, testing and LCA methods;
- the impact of selected products by LCA (in relation to material and energy flows);
- the impact of these materials in relation to toxicology, eco-toxicology, exposure, environmental and biological fate, transport, transformation, and destiny;
- the feasibility and sustainability of new technical solutions for end-of-life processes, such as reuse/recycling, final treatment and/or disposal.

4 Technical approach and work description

NanoSustain has been structured and organized around 4 technical (vertical) and 2 horizontal Work Packages (WPs), each with distinct tasks, deliverables and milestones (see Fig. 1):

Work Package 1 (WP1): Project management and scientific-technical coordination

Work Package 2 (WP2): Data gathering, generation, evaluation and validation

Work Package 3 (WP3): Hazard characterization and impact assessment

Work Package 4 (WP4): Life Cycle Assessment (LCA) and preliminary Assessment

Work Package 5 (WP5): Exploring technical solutions for recycling, treatment and disposal

Work Package 6 (WP6): Dissemination and exploitation of results

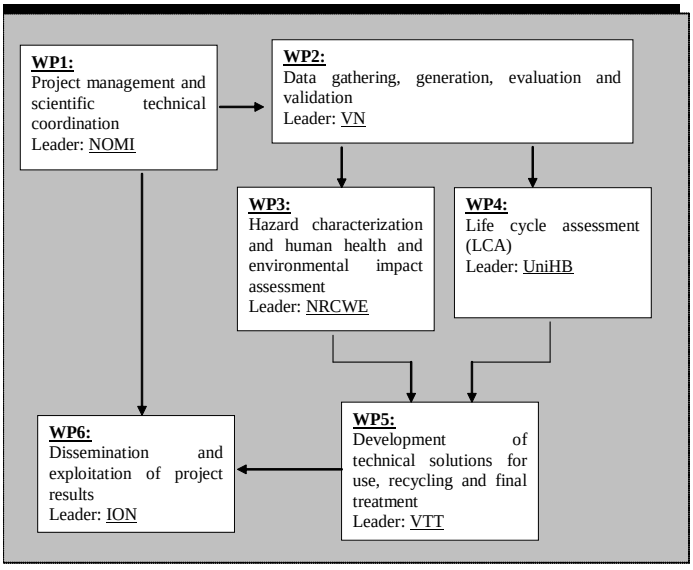

Figure 1: Structure and interdependencies of work packages

5 Main results achieved

NanoSustain has achieved main goals and main results include the following:

(1) Implementation of a comprehensive hazard characterization, exposure analysis and risk assessment of pure nanomaterials and associated composites and products, including dust and other LCA relevant materials derived from experiments that simulate handling and transport of NM as well as relevant after-production life phases that may occur during use or at the end-of-life of selected NM.

(2) Establishing critical dose-response characteristics and no-effect levels to humans and the envi-ronment in particular for materials from after-production and LCA relevant stages (such as use, reuse/recycling, final treatment and disposal), also to evaluate the applicability of current risk and life cycle assessment methods to NM.

(3) Performing a preliminary life cycle assessment (LCA) for selected NM by applying leading edge methodology to identify potential environmental impacts throughout the whole life-cycle (from production, application and use phase to recycling and disposal), and further developing and improving the applicability of existing LCA methods (such as prospective LCA) to NM and their use for precautionary design and risk management.

(4) Assessing human health and environmental impacts of selected NM by producing a set of standardized nanomaterials that represent changing pc properties, LCA phases and processes (e.g. pure NM, NM embedded in a product, dust, ashes, compost, leaching water etc.) by using the inflammatory reaction that follows human exposure to nanomaterials in the lung, the comet assay to determine geno-toxic effects as a measure of the reactive oxygen species (ROS) formation and for possible primary and secondary DNA damages in cells and animals, and by using eco-toxicity tests, such as the kinetic luminescent bacteria "Vibrio fischeri" test, to assess effects on aquatic systems and toxicity mechanisms that may arise directly during transport, after manufacturing, during use phase and/or recycling or disposal, or indirectly from the environment, e.g. by accidental release or leakages.

(5) Exploring performance of technical disposal solutions by lab-scale experiments to elucidate the behavior and fate of nanomaterials during end-of-life phases, such as recycling (composting and glass melting), final treatment (incineration) or disposal (land-filling), to get the data and knowledge needed to better design, engineer and fabricate more safer and sustainable NM. This specific goal was achieved by 1) testing and improving the biodegradability and suitability of nanocellulose based materials for organic recycling and the safety of resulting compost materials, (2) by measuring NP emissions during glass melting to recycle nano-ZnO-containing glass, (3) by exploring the suitability of incineration of CNT containing epoxy boards as a safe treatment option (if no recycling/reuse is possible), and (4) by leaching nanoparticles (NP) from products to simulate land-filling conditions. To monitor the efficiency and sustainability of these treatment techniques, also the performance of current analytical methods was assessed and their capacity to detect and quantify the release, distribution and environmental fate of NM along their life cycle, but also to prevent severe shortages and economic risks for present and future applications. The generated new emission and exposure data associated with these final treatment techniques will create the needed scientific base for a more careful product design and sustainable use and management of selected NM but also for amending existing relevant legislation (e.g. on waste treatment or hazard classification).

A concise overview of the progress of work and results achieved within each work package (WP) and associated tasks during the whole project duration is given in the following, highlighting significant results and their possible impact.

5.1 Data gathering, generation, evaluation and validation (WP2)

Two project-specific databases, a (1) material and a (2) scientific literature database, have been established during the course of the project. The technical (material) database was developed to create a framework appropriate to systematically collect, evaluate and document the new and complex scientific data produced during the whole project period. For this, an Excel sheet and an online

structure were used to allow easy data transfer and access to all partners, and a correlation of the physicochemical (pc) data with all biological endpoints determined at the toxicological, eco-toxicological, exposure and LCA level. In addition to the established technical database, also Material Data Sheets (MDS) were prepared for the 4 selected nanomaterials (i.e. nanocellulose, nano-TiO2, CNT and nano-ZnO), to present main material properties partly delivered by manufacturers and partly generated by the project, together with a presentation of methods and analyses performed. The literature database was set up to regularly update the most recent research on topics covered by the project and to integrate and make the most recent knowledge on the selected nanoparticles constantly available to all project partners within their field of expertise (such as hazard, exposure, RA and LCA, disposal of selected NM). The validity of the produced and retained (pc and biological) data was continuously controlled by an internal advising expert group specialized in material measurement and testing, toxicology, RA or LCA, to evaluate and assess the quality and relevance of the results achieved, but in particular to plan and implement a comprehensive inter-laboratory comparison exercise using known reference standards, materials with unknown compositions, and materials that have been already tested within NanoSustain, to assess reproducibility and accuracy of measurement methods used prior to toxicological testing.

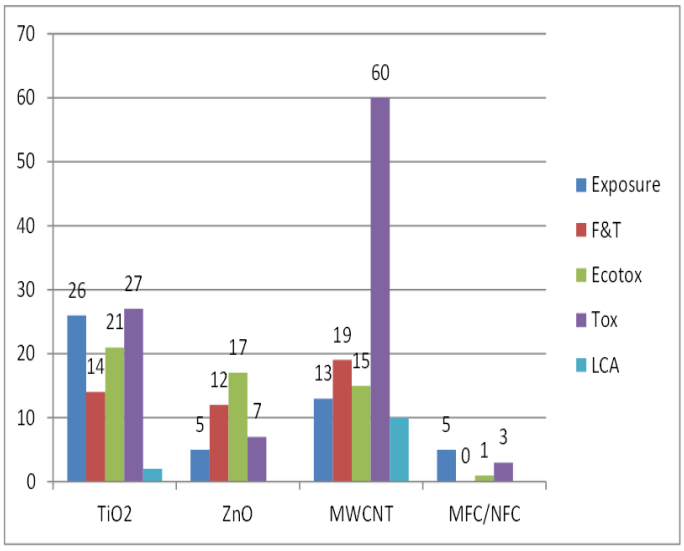

Figure 2: Last batch of publications (December 2012) to be processed by category and ENP (VN 2013)

National and international standardization committees, as well as several high level scientific journals, have begun to establish recommendations for adequate pc characterization data as a stringent requirement for toxicology testing of NM. NanoSustain has complied with these requirements and built up a toxicology database that stands on a sound pc characterization of the test materials. The established literature database was designed for 2 target groups: 1) the project partners to make high-quality information continuously available, and 2) the wider scientific community, to offer a common database on research topics relevant for and covered by the project. Almost 200 peer-reviewed papers have been reviewed covering all phases of the life cycle of selected nanomaterials published by January 2013 (see Figure 2+3).

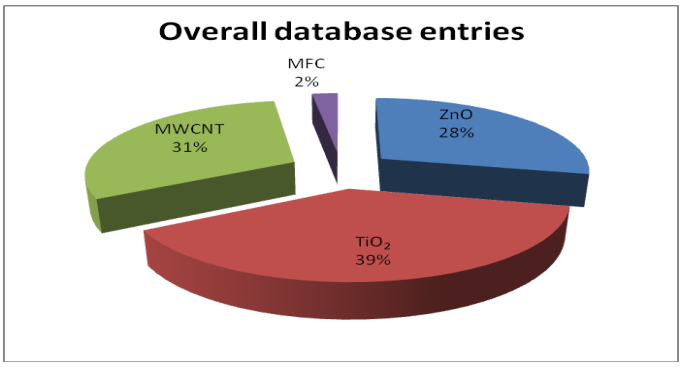

Figure 3: Entries in the NanoSustain literature database for the particles of interest (VN 2013)

One main outcome of the review was the fact that more and more studies document an increase in performing high-quality pc characterization of NP prior to toxicity testing, as increasingly demanded by the more important scientific journals. Most reviewed papers focus on ZnO and TiO2, but there is an increasing number of publications that addresses CNTs. For nanocellulose, however, the number of published papers is not high so far and no clear trend is in sight. Papers published during the last year (2012) mostly contain reviews on the synthesis and use of nanocellulose materials and on ongoing research, and only few papers deal with effects or exposure analysis, and none with fate and transport. Most frequently addressed pc properties include chemical composition, particle size and size distribution, morphology, surface area, and agglomeration/aggregation, which are increasingly measured also by using methods in combination. Core scientific findings of the reviewed papers have been extracted and summarized directly in the database and in descriptive tables.

The project results database that has been developed was using a structure that is compatible with databases developed and used by other Nanosafety Cluster (NSC) projects. The database was organized by using the following categories: physicochemical characteristics, toxicology, eco-toxicology, exposure, and LCA.

For each category and for each material, the specific pc characterization is reported, if available (e.g. size distribution in the cell culture media), and the different measured endpoints given. It is possible to specify the methodology used in a category to implement the measurement. In addition, the horizontal organization of the database specifies the different experimental setups used and reported for the various tests, as they may impact the different endpoints measured (e.g., a varying aggregation may be seen of particles in different solvents). The results database may be used as basis for other similar running or future projects and is available as an online version at the project website, for now still in the restricted area, as it is still reporting confidential data generated for each selected material. Also a link to the Material Data Sheets (MDS) (dossiers collecting the pc data, measurement and testing protocols) is given.

One of the most relevant steps taken to ensure a high quality of the generated data was the inter-laboratory comparison of methods that were used for pc characterization. By this it was possible to provide a measure on the comparability and validation of the different methods and instruments used by the participating laboratories. Data compared included the physical size of NP by TEM and the hydrodynamic diameter by DLS with supporting data obtained by SEM and AFM as well as by the determination of the average crystallite size by XRD. Test samples used included 3 NIST

traceable PSL particle size standards, 3 TiO_2 samples of which 2 were from the OECD WPMNM and 1 from NanoSustain itself, 2 ZnO samples of which 1 was again from the OECD WPMNM and 1 from NanoSustain itself. By including the NanoSustain powder samples, the generated analytical data, which was also included in the MDS, could be further used to generate "internal benchmark" values.

Nanosustain is prepared to share the generated pc data and expertise with other relevant EU FP7 projects and with the Database Working Group 4 of the NSC, to further develop common protocols and harmonize formats, to support the build-up of a central EU wide database on nanomaterials risk assessment.

5.2 *Hazard characterization and impact assessment (WP3)*

Another main goal of the NanoSustain project was to provide and test not only pure nanoparticles, but in particular life-cycle relevant test materials for hazard and exposure characterization. For this reason, the following test materials have been synthesized and treated and subsequently characterized and tested: (1) glass sheets coated with and without ZnO nanoparticles, (2) three different types of epoxy plates with and without CNTs, (3) two different paints applied on boards with and without nanoTiO_2, and paper prepared with and without nanocellulose. Powders from selected nanomaterials have been generated for subsequent testing and measuring source strengths of dust emissions to simulate handling and reworking of selected EN and products, and for assessing toxicologically and eco-toxicologically relevant pc properties and concentrations of NP in pure and after-production materials (composites), and when released to the environment (see overview of test materials in Table 1).

Reference product	**Nanoparticle containing product**	**Nanoparticle**
Paint with 36% Nabond TiO_2	Paint with 12% NanoAmor TiO_2 and 24% NaBond TiO_2	NanoAmor TiO_2 (139.1 m2/g) NaBond TiO_2 (28.2 m2/g)
Epoxy	Epoxy with 0.2% CNT	Nanocyl NC7000 CNT (245.6 m2/g)
Epoxyl (An epoxy formulation)	The same	
Glass treatment product without ZnO (100% polysiloxane matrix)	pro.Glass Barrier 401 (5% ZnO dispersion used for application on glass sheets)	ZnO (ZincoxTM 10)(53.6 m2/g)
Paper	Paper with nanocellulose	Nanocellulose suspension (2%)

Table 1: NanoSustain test materials: Pure nanoparticles and sanding dusts from products (with and without nanoparticles)

A comprehensive analytical characterization campaign on key properties of the obtained pure nanoparticles and of dust produced for exposure assessment was undertaken by using a variety of advanced analytical methods, such as SEM, TEM, XRD, AFM, SNOM, Nano-Raman, Micro-Raman, UV-VIS, SAXS, DLS, zeta-potential, FTIR and BET (see Table 2). To validate the used methods and the quality of the produced data, a number of standards and benchmark materials have been tested by an inter-lab comparison study (see WP2).

Physicochemical parameter	**Methodology**
Size	XRD, AFM, SEM, DLS,
Chemical composition	XRD, EDX, Raman, ICP-MS, UV-Vis, FTIR
Purity	XRD, Raman, TGA
Crystal structure	XRD, Raman, UV-Vis,
Surface area	BET
Porosity	BET
Zeta potential	ELS

Table 2: Measured physical-chemical properties and applied methodologies used in WP3

To assess the emission source potential and risk for workers during powder handling, data from simulated work activities (sanding) and dustiness testing (miniaturized EN15051 test) have been generated, characterized and evaluated. Also, work to evaluate the effect of weathering (VV treatment and temperature stress) and abrasion on the emission of selected ENM, and their release from glass sheets coated with and without nano-ZnO and from painted boards with and without nano-TiO_2, has been performed by sanding before and after exposure to weathering, in close collaboration with the EU FP7 NanoHouse project and Flügger Denmark.

The aim of the dustiness testing was to produce data on human exposure to nanoparticles that may occur during handling of after-production materials. Sanding dust was also generated and collected for toxicological testing and the emission of dust characterized during:

- sanding of epoxy (CNT) and paint products (TiO_2)
- breaking of coated glass sheets (ZnO)
- tearing (of paper) (nanocellulose)

Based on results received, a qualitative control banding and first order quantitative occupational exposure assessment model (Nanosafer) has been evolved and the data from the exposure assessment, dustiness and leaching experiments forwarded to WP4 for preparing the LCA of the tested nanomaterials.

An important task was to identify dose-response relationships for critical end-points of human health effects for NP-containing after-production dust in mice and comparison with the results obtained from pure materials. For this, 2 animal experiments were performed:

- Experiment I: Exposure to 5 different kinds of sanding dusts from NM containing products.

- Experiment II: Exposure to glass treatment product with and without nano-ZnO.

Mice were analyzed for:

1. DNA damage in lung and liver tissue
2. BAL cell differentiation
3. Liver (all mice) and lung histology (mice exposed to ZnO and glass treatment products) (performed at the Danish Food Institute).

For testing of pure ENM and lifecycle material, mice have been dosed by a single intra-tracheal instillation and analysis of pulmonary inflammation and DNA damage in lung and liver tissue has been performed from the two main experiments. Also RNA purification from lung and liver tissue has been performed and the mRNA expression of selected genes involved in inflammation and DNA repair has been analyzed, together with histological investigation of the tissue.

Another main focus in WP3 was to assess the eco-toxicity of nanoparticles and nanoparticle containing composites but also to evaluate the suitability of current eco-toxicity assays, such as the "Vibrio fischeri" bacterial test for environmental hazard and risk assessment of selected nanomaterials, namely CNT, ZnO, TiO2 and nanofibrillar cellulose (NFC). The eco-toxicity of samples from waste treatment of NFC (composting) and recycling of nanoparticles (ZnO) containing glass was evaluated and dose-response relationships of critical eco-toxicity endpoints have been established, e. g. for pure NM and contained in recycling materials obtained after composting (nanocellulose) or glass melting (ZnO).

NanoSustain also identified, reviewed and subjected existing methods, strategies and tools for RA used within the chemicals sector with the potential to manage the risk to NM for man and the environment to a critical analysis to learn how they work for NM, and to identify the type of information needed during all life cycle steps of a NM. Also the feasibility of these methodologies for potential users both inside and outside the manufacturing industry has been assessed in the light of uncertainties and gaps. In particular two methodologies have been identified and deemed as suitable for environmental RA of NM. These include the Swiss Precautionary Matrix for Synthetic Nanomaterials and the Environmental Defence and DuPont Framework. Both of them have been applied to real cases by using the four commercially available nanomaterials studied in the project. The applicability of these 2 methods to the NanoSustain test materials has been evaluated throughout the different life cycle stages. All materials studied are manufactured products containing nanoparticles. Summarizing, the "Swiss Precautionary Matrix" is a user friendly questionnaire, which might be very helpful as a first approach to establish the precautionary needs of the nanomaterials handled. In comparison with the "DuPont Nanorisk Framework", it is relatively quick and easy to use, and also provides visual results.

The sanding experiments simulating the release of NP during handling as well as tests simulating environmental stress (UV treatment, leaching and temperature stress) applied to boards and glass sheets treated with MN based products, namely TiO2 and ZnO, were used to develop and test an accurate and repeatable analytical method to detect and quantify one or more classes of NP in these LCA relevant materials, as well as environmental matrices and real samples. Quantification of the release of NP from these substrates under stressing conditions was successfully achieved via ICP-MS.

Also the type of prevalence and level of NP used in industry was taken into account and the resulting potential for exposure of human population. There was no general additive effect when adding nanoTiO2 to paints or CNT to epoxy boards and overall results suggest that the toxicity of TiO2 and CNT is masked when included in a paint or epoxy matrix, respectively. In contrast, the toxicity of ZnO was conserved when ZnO was included in a window-glass treatment product. Sanding experiments also indicated that there was no increased emission of nanoparticles during sanding of NP doped materials compared to reference materials without NM. Of the products tested, only nano-ZnO powder indicated a possible "high" risk due to the high dustiness and toxicity.

Standardized protocols (SOPs) have been established for nanoparticle measurement and pc characterization by an inter-laboratory comparison to assure consistency and quality control across all participating laboratories. However, the performed validation has to be seen as rather indicative, as involved labs were too few. Therefore, data quality was also checked by a data comparison, statistical treatment & analysis to determine their significance, precision, accuracy and reproducibility according to scientific best practices established and run at partner laboratories.

Summarizing results in WP3: The toxicity of pure nanomaterials was tested and of sanding dust received from complex products and composites containing embedded NM. No additive effect was observed when adding nano-TiO2 to paints or CNT to epoxy. Results suggest that the toxicity of nanomaterials is masked when embedded in a product (paint or epoxy) or matrix. In contrast, the toxicity of nano-ZnO was conserved when embedded in a complex window-glass treatment product. Concerning eco-toxicity, only ZnO powder showed a dose related response in acute toxicity tests by using "Vibrio fischeri" but no toxicity occurred for the 4 NFC (nanocellulose) samples: native NFC, slightly anionic, highly anionic and cationic, indicating (for NFC) that surface modification applied to the test samples did not have any toxic effect. On the basis of results received for the acute toxicity to "V. fischeri", nanoparticles can be ranked as follows: nano-ZnO: toxic; TiO2: not harmful; CNT: not harmful and NFC: not harmful. The latter is only applied to NFC samples tested in this project.

5.3 *Life Cycle Assessment (LCA) and preliminary Assessment (WP4)*

NanoSustain has assessed the environmental impact of the following organic + inorganic nanomaterials ("cradle-to-gate"-LCA) and (prospective) associated products along their whole life cycle ("cradle-to-grave"-LCA):

- nanocellulose used as paper additive,
- nano TiO2 used in paint application,
- nano ZnO used in glass coatings, and
- MWCNT used in epoxy plates.

Based on a comprehensive literature search and a questionnaire sent to all 3 involved manufacturing partners, specific process models for the application/use and end-of-life phases (recycling,

treatment and disposal) have been developed, including all relevant material and energy flows and important life cycle steps described and data sources investigated. Modelling, calculation, visualization, and evaluation of material and energy flows have been done by using the Umberto LCA software and results have been reported in 2 separate project deliverable reports.

The main bottleneck for LCA still consists in the lack of data for the selected ENM for after-use phases or regarding their possible environmental fate and impact. Individual life cycle steps were defined and described, data sources investigated, specific process models developed and provided in the LCA Umberto software. Also a literature survey on existing emission data has been completed to calculate prospective environmental concentrations needed for developing a specific exposure model. Based on the literature survey and established process models, data on all inputs and outputs were collected for the Life Cycle Inventory (LCI), and life cycle data and prospective environmental concentrations have been calculated.

Figure 4 gives an example to illustrate the results received for the global warming potential from the case study 'Life cycle modelling Nano-TiO2 in paint application'.

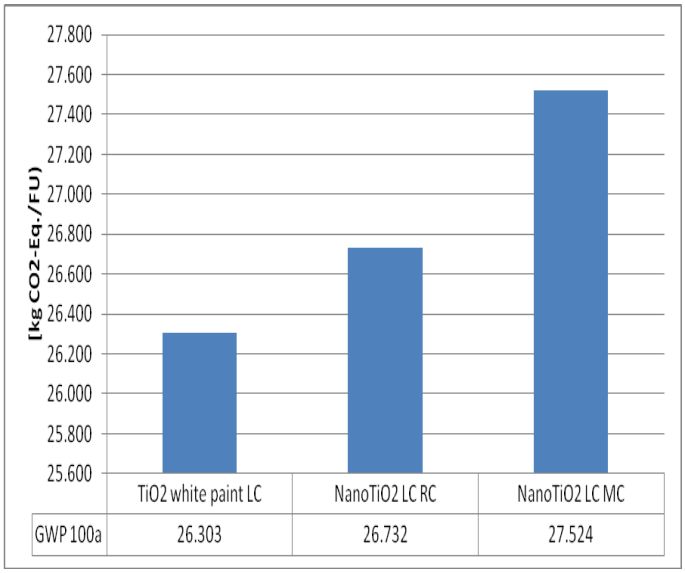

Figure 4: Global warming potential for Nano-TiO2 in paint application (UniHB 2013)

It can be seen that the global warming potential of the scenario "NanoTiO2 LC MC" is 4,6% higher than the scenario "TiO2 white paint LC" illustrating the influence of the different emission estimations in the application and use phase. The absolute difference between the two scenarios (TiO2 white paint LC versus NanoTiO2 LC MC) is 1221 kg CO2-Eq/FU.

In Figure 5 the predicted environmental concentrations (PEC) for surface waters have been extrapolated for nano-TiO2 from paint applications.

The use scenario has been based on current (optimistic) production/application estimates of nano-TiO2 in paint coatings, while the PECs of the total amount of nano-TiO2 reaching the aquatic environment from paint coatings has been recomputed as modelled by Gottschalk et al. 2009. The modelled PEC curves for nanoTiO2 from paint coatings show that the concentrations are approximately between 1 and 4 orders of magnitude smaller than the generic nanoTiO2 results obtained by Gottschalk et al. (2009) which may point to the plausibility of the achieved results, since only one individual application was investigated.

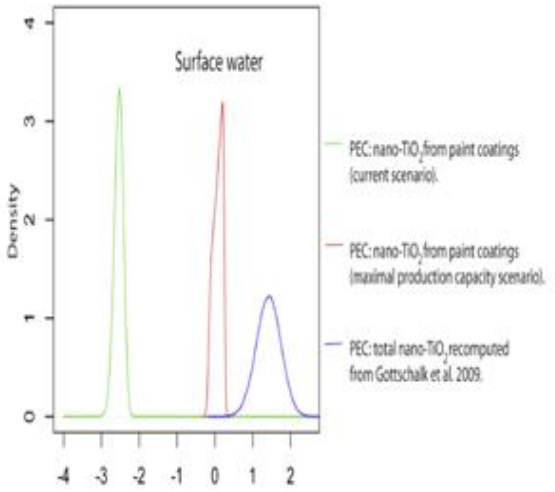

Figure 5: Probability distributions for predicted environmental concentrations (PEC) based on current production/use scenarios (green), future maximal production/use (red) and results recomputed from others (Gottschalk et al., 2009) (UniHB 2013)

For the precautionary design and for improved recyclability of ENM a comprehensive approach has been derived from the presented approaches and supplemented with environmental impact categories of the Life Cycle Assessment (see Table 3).

The concept included:

- Precautionary risk aspects,
- Resource aspects, and
- Environmental impact categories.

Categories and aspects	Data quality	Source
Precautionary risk aspects		
Decision tree of risk categorisation	Qualitative	German SRU to precautionary strategies for managing nanomaterials
Potential exposure of humans	Semi-quantitative	Swiss precautionary matrix for synthetic nanomaterials
Potential input into the environment	Semi-quantitative	Swiss precautionary matrix for synthetic nanomaterials
Potential of incident	Semi-quantitative	German ÖI Sustainability check, orientation on Swiss precautionary matrix
Ressource aspects		
Criticality	Qualitative, Semi-quantitative	EU concept of criticality
Recycling capability / tendency to dissipation	Qualitative	In orientation on German ÖI Sustainability check
Abiotic ressource requirement	Quantitative	Based on Life Cycle Assessment
Other LCA impact categories		
Energy requirement	Quantitative	Based on Life Cycle Assessment
Global warming potential	Quantitative	Based on Life Cycle Assessment
Toxicological potential, but not nanospecific	Quantitative	Based on Life Cycle Assessment
Ecotoxicological potential, but not nanospecific	Quantitative	Based on Life Cycle Assessment

Table 3: Criteria for precautionary design and for improved recyclability of engineered nanomaterials (Steinfeldt 2013)

The environmental impacts of the production of nanomaterials as obtained from the LCA case studies can be summarized as follows:

The final environmental impacts of the production of selected nanomaterials depend much on the type of manufacturing processes including energy demand, demand of operating supplies, yield and purification rate.

In the performed case studies, a great range of factors of environmental impacts can be seen for the production of nanomaterials when compared with micro-sized materials. For example, the environmental impacts of the production of nanocellulose are greater than of conventional sulfite pulp by factors of 1.5 to 4. The reason is the additional need for energy and chemicals for the several production routes of nanocellulose. This is also the reason for the differences between the various production methods of nanocellulose. In contrast, the environmental impacts of the production of nano-TiO2 are in the same order of magnitude than conventional TiO2. In some environmental impact categories, nano-TiO2 is better than conventional TiO2. The environmental impacts of the production of nano-ZnO are much higher than of conventional TiO2. When compared with the modeled production process of pulsation reactor, the factor range is between 8 and 68. However, when compared with the modeled laboratory process of flame pyrolysis, the impact factor range is much higher.

The influence of selected nanomaterials on the environmental impact of new (prospective) applications can be summarized as follows:

(1) The environmental impact of prospective paper applications with nanocellulose is primarily determined by the energy requirement of the kraft paper production and by the consumption of chemicals. For the production of nanocellulose, the cumulative energy demand may increase by 4.2% without the benefit of a reduction in weight. On the other side, the depletion of abiotic resources would only increase by 1.9% without the benefit of a possible reduction in weight.

(2) The environmental impact of nano-TiO2 containing paint applications is primarily determined by the preproduction of nano-TiO2 and of solvent chemicals. All other materials and processes have a very low impact and different benefits can be seen. For example, the global warming potential of the scenario "na-no-TiO2 LC MC" is by 4.6% higher than the scenario "TiO2 white paint LC" (see Fig. 4). On the other side, for the acidification potential, the 2 nano-TiO2 scenarios are better than the conventional "TiO2 white paint LC" scenario. The acidification potential of the scenario "nano-TiO2 LC RC" is 10.2% lower than the scenario "TiO2 white paint LC". The differences between the two nano-TiO2 scenarios illustrate the influence of the different emission estimations from the application and use phase.

(3) The environmental impact of nano-ZnO glass products is primarily determined by the preproduction of the glass with around 95%, the electricity demand of the coating production with 3.3 %, and the transport with around 1%. The production of the new pro.Glass Barrier 401 coating (including the higher energy demand of the production of nano-ZnO) has almost no influence on the balance, which means that the increase of the possible product life time will generate the improvement of the cumulative energy demand. The portions of the entire balance are extremely small. A cause for this is the small thickness of the coating of 2 x 1.6 µm in relation to the 3 mm thick glass. For example, the acidification potential of the scenario "Conv. product LC1.25" is 24.93% higher than for the "Nano-ZnO product" scenario.

(4) In the case study on 'Prospective MWCNT composite material - MWCNT in epoxy plates as rotor blades', the environmental impact is primarily determined by the energy requirement for the production of the wind power plant and by the demand of conventional electricity. In the comparison of scenario "WPP New0.15" and scenario "WPP old", the improvement of the global warm-ing potential is just around 4.5%. The preproduction of the MWCNT has a very low influence on the total balance, which means that the increase of the energy production efficiency of 0.15% is generating the improved global warming potential. The portions of the entire balance are very small partly due to the low content rate of 0.5% in the rotor blade in relation to the entire plant. Only 24.7 GJ-Eq is needed for manufacturing the 150 kg MWCNT

The potential and prospects of nanotechnology based applications to reduce the environmental load depends much on the type and level of innovation (nanotechnology generation, incremental vs. radical, end-of-pipe vs. integrated). Today most applications with a higher level of innovation are still in the development stage. A varying potential for gains in resource efficiency could be shown and quantified in the performed case studies (also from a life cycle perspective). On the other hand, there is still a clear lack of data hampering development and innovation.

For future applications with high environmental (sustainable) benefits, a very good combination of following characteristics will be needed:

- small content rate with better functionality,
- environmental benefit in the use phase (higher resource and/or energy efficiency),
- long-life (persistent) products, and
- nanomaterials integrated in the product matrix.

The executed computer simulations revealed that at present, when considered separately, none of the investigated applications (i.e. nano-TiO2 in paint coatings, nano-ZnO in glass coatings, MWCNT in epoxy material and cellulose) causes significant exposure to water, soil, or air systems. These findings apply even for model results computed for the maximal EN production and use scenarios. The highest nanoTiO2 PECs (2.2 ng/L) estimated for surface water represent an uncertainty factor of 500, which is smaller than the very conservative uncertainty factor of 1000 given by European guidelines (ECHA, 2008), and which reflects the predicted no effect concentrations (PNEC) used in a previous study (Gottschalk et al., 2009). An even clearer picture is obtained for nano-ZnO, where the near-zero water values (ng/L dimensions) could be compared to a conservative PNEC of 40ng/L. For the MWCNT application, the highest PECs (about 0.1ng/L) show an uncertainty factor that is 400,000 lower than the no effect level. Our PEC curves also include uncertainty factors for air and soils by orders of magnitude away from such rough eco-toxicological values.

The received findings may exclude, with some certainty, potential risks caused by these specific EN applications for the environment. However, a general and final positive conclusions can still not be drawn since only single EN applications have been investigated

that cover just a partial contribution to the total MN release and exposure to the environment (Gottschalk & Nowack, 2011). Consequently, for a general all-clear, exposure simulations are needed at a higher precision level, including levels for all relevant ENM applications. To do so, we need better data application and specific knowledge that can be only provided by a closer cooperation with industries involved in the production (and disposal) and sales of nanomaterial-based products and applications.

NanoSustain has generated completely new "Cradle-to-gate"-LCA data, (prospective) "Cradle-to-grave"- LCA data, and prospective environmental concentrations for the 4 selected nanomaterials and associated products. Today most nanotech-based applications are incremental innovations, with many applications having higher level of innovation still in the developmental phase. The performed LCA case studies could show a varying potential for gains in resource efficiency (also from a life cycle view). However, the current lack of real data is still hampering the LCA of EN.

For future applications with high environmental (sustainable) benefits, a very good combination of the following characteristics is highly needed:

- ✓ Small content rate with better functionality,
- ✓ Environmental benefit in the use phase (higher resource and/or energy efficiency),
- ✓ Long-life (persistent) product, and
- ✓ Nanomaterials integrated in the product matrix.

5.4 *Exploring technical solutions for recycling, treatment and disposal (WP5)*

The ultimate goal of the NanoSustain project was to explore and test the applicability of technical solutions (in WP5) for the safe and sustainable design, use, reuse/recycling, final treatment and disposal of selected nanomaterials and products. End-of-life phases occurring after the production and use phase have been the final target of the implemented research and development work. The experimental part designed to find practical and innovative solutions for handling waste containing nanomaterials in a safe and sustainable was supported by a comprehensive physicochemical and hazard characterization and exposure and impact assessment part.

For implementing the composting, biodegradability and recycling studies on nanocellulose and products, such as various papers, an internal nanocellulose standard was produced and characterized for quality control, validation, and reproducibility check. The suitability of nanocellulose-based materials for composting was studied and evaluated and both nanocellulose films and paper products containing nanocellulose were subjected to biodegradability tests according to EN 14046. During biodegradation, compost samples were taken to evaluate their eco-toxicity by the "V. fischeri" bioluminescence- test. Disintegration during composting was evaluated according to EN 14045. The obtained results suggest that all tested products can be considered as biodegradable under the used compost conditions and that they also disintegrate in the performed pilot-scale composting experiments. In addition, none of the compost samples was toxic towards "Vibrio fischeri". In aquatic environment, nanofibrillar cellulose (NFC) gels showed a strong tendency to agglomerate, which reduced the rate of biodegradability. However, the biodegradability test used was not optimal for gel-like products and biodegradability should be evaluated by using also other tests.

Multi-walled carbon nanotubes (MWCNT) containing epoxy composites are not suitable for direct recycling, why incineration (energy recycling) at laboratory scale furnace was carried out and the produced particles, bottom ash and gaseous effluents measured and characterized. Three different fuel compositions were mixed: wood chips with 20 wt. %, 5 wt. % and 0 wt. % of CNT containing composite. In the incineration experiments nanoparticles were observed in all combustion cases independent of the fuel composition according to the new nanoparticle definition by EU. Number concentration was highest for good combustion with 0 wt. % CNT containing composite and mass concentration for poor combustion with 5 wt-% CNT containing composite. No indication of CNT like tubular structures was found in the particle and bottom ash samples analysed by SEM/EDS. This was probably caused by the low amount of the CNT containing composite in the fuel mixture, the low amount of CNT in the CNT containing composite, and the formation of the large and hard, highly sintered bottom ash deposit that may "bind"/immobilise the species in the CNT composite in a non-volatile matrix. The Raman spectrum of the particles collected on filters during good and poor combustion of wood chips and 5 wt. % CNT composite did not indicate the presence of the CNTs. However, evidence of nano-structured carbon that was not MWCNT was found. The Raman spectra of the bottom ash collected after good combustion of wood chips and 20 wt. % CNT composite did not indicate the presence of CNTs. However, evidence of nanostructured carbon that was not MWCNT was again found. For 5 wt. % CNT composite, an indication of the presence of disordered amorphous carbon and graphitic carbon atoms was found, but no MWCNTs.

Several batches of coating material with and without ZnO-nanoparticles have been synthesized to test the recycling of nano-ZnO-coated glass. The emission potential of nanoparticles was investigated during heating and melting of different window glass samples: coated with nano-ZnO, coated with a sol-gel binder matrix, without any surface treatment, and from heating/melting of the empty furnace crucible without any glass sample. Particle number, mass concentration and number size distribution were measured during heating/melting to mimic the glass recycling process. Results indicated that particles are emitted during heating/melting of the glass, but their number, size and mass concentration do not depend on the coating, or on the type of coating. The amount of Zn was almost the same in the particles as in the pro. Glass Barrier 401 coated glass sample. Furthermore, no notable difference between the composition of the glass as provided and the corresponding melted glass sample was found.

Also the leaching of ZnO nanoparticles from nanomaterials containing waste material has been studied to explore the feasibility of land-filling as a final disposal option. Parameters affecting particles' leaching behaviour have been selected and methods evaluated, to detect the expected low concentrations required to draw conclusions regarding the release and final fate of nanomaterials in waste. Furthermore, a comparison of the release behaviour of nano-Zn and so-called non-nano-ZnO have has been carried out. NanoSustain evaluated the release behaviour of nano-ZnO from tests carried out with powder mixed with glass

beads under various laboratory conditions. The results were compared to tests carried out with micro-sized ZnO. Furthermore, additional tests were done with nano-ZnO coated glass. Testing of granular waste containing ZnO can be done with some modifications by standard test methods (such as the percolation test CEN/TS 14405, batch test EN 12457 and the pH dependence test (e.g. CEN/TS 14997)). However, the tank test procedure for surface leaching was not suitable for nano-ZnO coated glass, because the release of Zn is solubility controlled, why leaching was determined by percolation tests. The results indicated that the release of Zn and the toxicity of the eluates decreased with increasing L/S ratio. Results in particular from the pH-dependent test showed that the release of Zn from nano-ZnO coated glass is strongly solubility controlled and influenced by the pH of the test solution. Tests with DOC additions resulted in a higher release compared to tests without DOC most probably due to organic complex binding of the dissolved Zn^{2+} by humic acid. Furthermore, results emphasize that the salt concentration of the leachate hampers the release of Zn most probably due to increased agglomeration processes, emphasizing again the fact that agglomeration of nanoparticles strongly affect the fate (e.g. mobility and retardation) of nanomaterials in landfills. More research is needed on the long-term behavior of the formed agglomerates. In total, the release of Zn from nano-ZnO coated glass was strongly pH controlled being lowest at pH 9.2, but was also affected by other environmental master variables that also steered the experimental conditions. Leaching of Zn from nano-ZnO decreased with salt concentration due to increasing NP agglomeration and deposition and increased with the addition of DOC, which enhanced the dissolution and mobility of Zn.

To simulate the transport of water and contaminants in the saturated and unsaturated soils, a large number of models have been developed during the last decades. NanoSustain studied the release of ZnO in a sandy and clay-containing soil by a model focusing on the analysis of two mechanism of diffusion: diffusion without pressure applied on the surface and injection with a pressure of water solution containing nanoparticles. For both mechanisms profiles of the distribution have been evaluated.

Eco-toxicological studies of the eluates that used the acute toxicity *Vibrio fischeri* assay proved the close correlation between the toxicity of the eluates and the dissolved Zn concentration. However more tests are needed to evaluate the ecological relevance of long-term leaching under various landfill conditions as well as the bioavailability and ground water migration of Zn released from nano-ZnO coating.

5.5 *Dissemination and exploitation of results (WP6)*

Website and intranet:

Up to date (March 2014) the NanoSustain project website (www.nanosustain.eu) is still available and hosts newsletters and importantly project results. Registered users can access additional information such as the output of dissemination events and other presentations. A great deal of the produced data and results have been already published in various peer-reviewed journals and still more publications are planned by the various partners.

Project meetings:

Seven regular project meetings have been organized on a bi-annual basis to discuss the progress of work, problems and solutions among all project partners. In addition, regular online WPL meetings took place to follow up the work plan and specific physical WP with a focus on more detailed technical questions

Dissemination and exploitation of final results:

NanoSustain performed two training workshop, one on Life Cycle Analysis of nanomaterials held at the University of Bremen in September 2011, and an Autumn School on 'New Methods for Nanoparticle Characterization' held on 17 – 18th September 2012, and hosted by NanoSustain partner Kaunas University of Technology, Lithuania and focusing on emerging new trends and developments on characterization and measurement of the unique properties of engineered nanoparticles in relation to environmental, health and safety (EHS) aspects. Also three dissemination events were held in Glasgow/UK (May 2011), Venice/Italy (November 2011) and Barcelona (May 2013) to provide a platform for project partners to present results of their work, but also to interact with invited stakeholders.

A joint workshop in Barcelona was organized together with the EU FP7 NanoPolytox (www.nanopolytox.eu) and NanoFate (www.nanofate.eu) projects to share and discuss the outcomes of these three projects and to increase the final impact of each individual project. In addition to this 1-day workshop, a one-day training workshop was organized by the 2 projects for postgraduate / PhD students and young researchers on various nanosafety aspects, such as fabrication, characterization, toxicology, risk and life cycle assessment.

Case studies and facts sheets:

A key focus of the dissemination strategy was the production and distribution of CASE STUDIES and FACTSHEETS (downloadable at www.nanosustain.eu) that provide overviews of the challenges and processes involved in each of the project themes, including physicochemical analyses, hazard characterization (together with human health and environmental risks), life-cycle analyses, and the development of technical solutions for use, recycling and final treatment of the four selected nanomaterials based on the newly generated measurement and testing data. This information also describes the data evaluation and categorization processes used by NanoSustain according to different material and environmental attributes, such as toxicology, eco-toxicology, degradability, exposure and fate, and relevance for LCA, with the aim to guide the development of new sustainable products and industrial applications, and finally help to strengthen the competitiveness of the European nanotechnology industry.

The final main results have been presented by an invited oral presentation and a poster presentation at the EuroNanoForum international conference in Dublin 2013 (www.euronanoforum2013.eu).

6 Expected Impact

NanoSustain has produced an enormous amount of novel data that will help to improve our current knowledge on hazard, impact and sustainability of nanomaterials and products, in particular in relation to effects occurring during end-of-life stages. The produced new data will update and validate already existing databases on materials and methodologies required for reliable and accurate LCA and risk assessment of selected nanomaterials.

As almost nothing is known about the release, fate and impact of these nanomaterials during end-of-life processes, new solid scientific data on potential risks that may occur during reuse/recycling and final treatment or disposal has been produced and made available. For the first time innovative technical solutions used for waste recycling and disposal have been tested such as (1) recycling (by composting and melting), (2) incineration of nanowaste as a safe final treatment option, and (3) land-filling of nanoparticle containing products as final disposal. It is expected that the new knowledge produced will help to further develop these techniques and their applicability to engineered nanomaterials and so to overcome some still unresolved technical barriers towards a environmentally more safe and sustainable design, use and development of nanomaterials. Although not a main target, NanoSustain will also contribute to current standardization activities, such as CEN/TC 352 Nanotechnologies, ISO/TC 201 and 202 on Chemical composition, or ISO/TC 229 on Environmental partitioning and fate.

7 Directory

Table 4: Directory of people involved in this project

First Name	Last Name	Affiliation	Address	Email
Rambabu	Atluri	Nanologica AB	Drottning Kristinas väg 61, 11428 Stockholm	rambabu@nanologica.com
Ari	Auvinen	Technical Research Centre of Finland	PO Box 1000, Biologinkuja 7, FI-02044 VTT Espoo, Finland	ari.auvinen@vtt.fi
Andreas	Bhagwani	Nanologica AB	Drottning Kristinas Väg 61, 11428 Stockholm, Sweden	andreas@nanologica.om
Adina	Bragaru	National Institute for R&D in Microtechnologies	Erou Iancu Nicolae 126 A 077190, Bucharest, Romania	adina.bragaru@imt.ro
Agne	Beleisyte	Kaunas Technical University	Studentu 65, Kaunas 51369, Lithuania	Agne.beleisyte@yahoo.com
Rolf	Danzebrink	Nanogate AG	Zum Schacht 3 D-66287 Göttelborn, Germany	rolf.danzebrink@nanogate.com
Dan	Dascalu	National Institute for R&D in Microtechnologies	Erou Iancu Nicolae 126 A 077190, Bucharest, Romania	dascalu@nano-link.net
Alfonso	Garcia-Bennett	Nanologica AB	Drottning Kristinas Väg 61, 11428 Stockholm, Sweden	alfonso@nanologica.com
Arnim	von Gleich	University of Bremen	Badgasteiner Str. 1 28359 Bremen. Germany PO Box 330440	gleich@uni-bremen.de
Keld	Jensen	National Research Centre for the Working Environment	Lersø Parkallé 105, 2100 Copenhagen, Denmark	kaj@nrcwe.dk
Michael	Jung	Nanogate AG	Zum Schacht 3 D-66287 Göttelborn, Germany	Michael.jung@nanogate.com
Rune	Karlsson	NordMiljö AB	Duvenässätern, 68395 Sunnemo, Sweden	Rune.karlsson@enas-online.com
Ismo	Kalevi Koponen	National Research Centre for the Working Environment	Lerso Parkallé 105, DK-2100 Copenhagen, Denmark	ikk@arbejdsmiljoforskning.dk
Päivi	Korhonen	UPM Kymmene	Eteläesplanadi 2, Helsinki, FI-00100, Finland	Paivi.korhonen@upm.com
Alexander	Kurz	Nanogate AG	Zum Schacht 3 D-66287 Göttelborn, Germany	Alexander.kurz@nanogate.com
Mihaela	Kusko	National Institute for R&D in Microtechnologies	Erou Iancu Nicolae 126 A 077190, Bucharest, Romania	mihaela.kusko@imt.ro
Roberto	Labrador	Nanologica AB	Drottning Kristinas Väg 61, 11428	hanoi@nanologica.om

			Stockholm, Sweden	
Jussi	Lyyränen	Technical Research Centre of Finland	PO Box 1000, Biologinkuja 7, FI-02044 VTT Espoo, Finland	jussi.lyyranen@vtt.fi
Laura	Manadori	Veneto Nanotech	Via San Crispino 106 - 35129 Padova - Italy	laura.manodori@ venetonanotech.it
Christian	Micheletti	Veneto Nanotech	Via San Crispino 106 - 35129 Padova - Italy	christian.micheletti@venetonanotech.it
Mark	Morrison	Institute of Nanotechnology (ION)	Strathclyde University Incubator, Graham Hills Building, 50 Richmond Street, Glasgow G1 1XP	Mark.morrison@nano.org.uk
Ulla-Maija	Mroueh	Technical Research Centre of Finland	PO Box 1000, Biologinkuja 7, FI-02044 VTT Espoo, Finland	ulla-maija.mroueh@vtt.fi
Erik	Nilsson	Nanologica AB	Drottning Kristinas Väg 45, 11428 Stockholm, Sweden	erik@nanologica.om
Iolanda	Olivato	Veneto Nanotech	Via San Crispino 106 - 35129 Padova - Italy	iolanda.olivato@venetonanotech.it
Marialena	Pozzebon	Veneto Nanotech	Via San Crispino 106 - 35129 Padova - Italy	Marialena.pozzebon@venetonanotech.it
Stefano	Pozzi Mucelli	Veneto Nanotech	Via San Crispino 106 - 35129 Padova - Italy	stefano.pozzimucelli@venetonanotech.it
Rudolf	Reuther	Nordmiljö AB	Duvenässätern, 68395 Sunnemo, Sweden	rudolf.reuther@nordmiljo.se
David	Rickerby	European Commission Joint Research Centre, Institute for Environment and Sustainability	TP 272, 21027 Ispra, Italy	david.rickerby@jrc.ec.europa.eu
Iria	Rio-Echevarria	European Commission Joint Research Centre, Institute for Environment and Sustainability	TP 272, 21027 Ispra, Italy	Iria.rio@jrc.ec.europa.eu
Anne-Christine	Ritschkoff	Technical Research Centre of Finland	PO Box 1000, Biologinkuja 7, FI-02044 VTT Espoo, Finland	anne-christine.ritschkoff@vtt.fi
Monica	Simion	National Institute for R&D in Microtechnologies	Erou Iancu Nicolae 126 A 077190, Bucharest, Romania	monica.simion@imt.ro
Andreas	Skouloudis	European Commission Joint Research Centre, Institute for Environment and Sustainability	TP 272, 21027 Ispra, Italy	andreas.skouloudis@jrc.ec.europa.eu
Valentinas	Snitka	Kaunas Technical University	Studentu 65, Kaunas 51369, Lithuania	vsnitka@ktu.lt
Michael	Steinfeldt	University of Bremen	Badgasteiner Str. 1 28359 Bremen. Germany PO Box 330440	mstein@uni-bremen.de
Anne	Thoustrup Saber	National Research Centre for the Working Environment (NRCWE)	Lerso Parkallé 105, DK-2100 Copenhagen, Denmark	ats@nrcwe.dk
Lesley	Tobin	Institute of Nanotechnology (ION)	Strathclyde University Incubator, Graham Hills Building, 50 Richmond Street, Glasgow G1 1XP	Lesley.tobin@nano.org.uk
Sara	Totaro	Veneto Nanotech	Via San Crispino 106 - 35129 Padova - Italy	Sara.totaro@venetonanotech.it
Nicola	Trevisan	Veneto Nanotech	Via San Crispino 106 - 35129	nicola.trevisan@venetonanotec

			Padova - Italy	h.it
Irina	Tsitko	Technical Research Centre of Finland	PO Box 1000, Biologinkuja 7, FI-02044 VTT Espoo, Finland	irina.tsitko@vtt.fi
Jari	Vartiainen	Technical Research Centre of Finland	PO Box 1000, Biologinkuja 7, FI-02044 VTT Espoo, Finland	Jari.Vartiainen@vtt.fi>
Minna	Vikman	Technical Research Centre of Finland	PO Box 1000, Biologinkuja 7, FI-02044 VTT Espoo, Finland	minna.vikman@vtt.fi
Margareta	Wahlström	Technical Research Centre of Finland	PO Box 1000, Biologinkuja 7, FI-02044 VTT Espoo, Finland	margareta.wahlstrom@vtt.fi
Håkan	Wallin	National Research Centre for the Working Environment	Lersø Parkallé 105, 2100 Copenhagen, Denmark	hwa@nrcwe.dk

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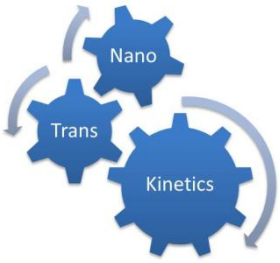
NanoSustain is a Collaborative Project (Small or medium-scale focused research projects) under the European Commission's 7th Framework Programme.

NanoTransKinetics

Modelling the basis and kinetics of nanoparticle cellular interaction and transport



Contract Agreement: NMP4-2010- 266737 Website: http://www.nanotranskinetics.eu
Coordinator: Kenneth Dawson, Centre for BioNano Interactions, University College Dublin, Belfield, Dublin 4, Ireland

Table 1 Consortium List.

No.	Beneficiary name	Short name	Country
1	National University of Ireland, Dublin / University College Dublin	NUID UCD	Ireland
2	Ludwig-Maximilians-Universität München [Ludwig-Maximilians University, Munich]	LMU	Germany
3	Universitat de Barcelona [University of Barcelona]	UB	Spain
4	Rice University*	RU	United States

Contents

1 Summary

NanoTransKinetics is a Small Collaborative Project funded by the European Commission 7th Framework Programme. The project started on November 1st 2011 and will run for 36 months. The project is paired with the US project "Nanoparticle Transport: From Cells to Organisms" funded via the the EPA STAR programme (EPA-G2010-STAR-N1 Fate, Transport, and Transformation). It is also linked with the ModNanoTox FP7 project via a memorandum of understanding, which agrees that the projects will have joint meetings, will share protocols and data, and will support the EU Nanosafety cluster efforts on modeling and databases.

NanoTransKinetics and ModNanoTox held a joint kick-off meeting in London on the 1st and 2nd of December 2011. The meeting was attended by representatives of all partners of both projects, as well as a representative of the US project that is paired with both (coordinated by Prof. Vicki Colvin from Rice University).

2 Background

Given that there is currently only limited data regarding the safety of nanomaterials (and validated data takes time to produce) *there is a need to develop approaches based on understanding and mechanism*, rather than overly rely on 'learning sets' which will require much longer to mature. Once this decision is made, it becomes essential to identify the key features and parameters of the arena and experience shows that *a combination of phenomenological and more detailed (semi-microscopic) models*, the latter being directly validated in a detailed manner by experiment is optimal. This approach allows the modeller-theorists to be in direct contact with the experimentalists (and their community) and creates a symbiotic relation that helps shape more usefully the experiments, and their reproducibility. In the lead up to this project, various path-finding efforts were implemented by the partners, and other networks, and the potential for useful interaction was immediately recognized.

Another aspect of higher level objectives and strategy was also considered. Thus, recognizing that a single such program cannot be a total solution, we have considered the issues that are considered most pressing in the field, and organized efforts toward the larger program in a manner *that key larger scale objectives will emerge first*. Understanding the nature of particles that most likely pass the BBB, and bioaccumulate inside non-immune cells are key examples addressed here.

3 Scientific and technological challenges

Besides crossing the traditional scientific domains (chemistry, physics, molecular and cell biology, biomedicine, engineering, and toxicology) this field *will above all require a radical shift of scientific paradigm such has rarely been seen in contiguous fields for a generation*. That is, whilst we can (and must) learn from what has been seen in the chemical and (small molecule) drug-organism interactions (for example ADME (Adsorption, Digestion, Metabolism, Excretion) approaches etc.), the underlying scientific processes in the nanoscale are so different as to render these as only of the most general guidance. The implications of this are deep, and can hardly be overstated, for the development of the program outlined here. Indeed, all the evidence we have suggests that we must return to fundamentals in this arena, and model these new processes at multiple levels of description (nanoparticles surface, cell, biological barriers) in order to develop a model that can usefully integrate emerging biological *in vitro* and *in vivo* data. We conclude that any attempt to press the nano-organism interaction into such a macroscopic ADME framework that is not founded on the appropriate microscopic principles will fail because the conceptual framework is the 'wrong shape'.

4 Objectives

From the analysis above, we conclude that it is now urgent to shift the focus of this discussion to a hierarchy of modelling elements that address the real issues of nanoparticle uptake, clearance, and translocation, and some application to examples of toxicology. Our work packages (WP2-4) are thus built around the need for such elements or modules, and involve:

- Modelling of the effect of NP physico-chemical characteristics on interaction with biological fluids – the protein corona (the mediator of biological interactions) as a means to classify nanoparticles;
- Modelling nanoparticle interaction with lipid membranes and extracellular matrix components – effects of NP charge / density / compressibility, lipid structure etc. and cell-cell interactions / degree of confluence etc.
- Modelling kinetics of cellular uptake and inter-compartmental transport and sub-cellular distribution of NPs;
- Modelling nanoparticle passage through biological barriers, including the Blood-brain barrier.

Without doubt, each element of the program is an attempt to reach far beyond the current state of the art. Indeed, we emphasize that the issues highlighted involve such radical paradigm shifts that research in the field is already very ambitious. There is no suggestion that one will be able to immediately produce a model that is predictive *in vivo* - indeed, we consider this an unrealistic short term objective of a single project in the field in its current state. However, we believe that the different elements presented will bring us a very considerable way towards this objective, leaving the way clear for adding in the research of other groups involved in this arena

Characterization of the 'Biological Identity' of the Nanoparticles
Perhaps one of the most striking (and unforeseen) aspects of the nanoparticles-cell interaction story, that clearly distinguishes nanomaterials from chemicals, is the issue of the 'protein corona'. This arena has been clarified by several authors (including Partner 1 and colleagues from FP6 NanoInteract),[1-4] and lead to the award of the 2007 Cozzarelli prize of the US National Academy of Sciences to Partner 1 for applications in this arena. In essence, chemicals (again making allowance for great generalizations) interact directly with biological elements, whereas nanoparticles are coated by strongly adhering proteins and lipids whose exchange times are so long that the effective biological identity of the particles is greatly influenced (in some cases likely completely determined) by the proteins, and not the materials. Figure 1 makes the issue clear by showing the uptake of silica with (and without) serum proteins. The relative amounts are enormous. It is important to note that uptake is dependant even on the type of serum used, and these differences have been studied and linked to different coronas. Clearly, the bare material surface is the wrong parameter. Similar observations are being made for many nanomaterials and situations. It is not possible to explain in great detail, but using new experimental methods it is also now possible to 'read' the corona around particles in organelles inside the cell. Evidently we need to shift considerably towards modelling of the particle and its adhering proteins, and the interaction of this object with biological membranes and barriers in the current program.

Uptake of nanoparticles into cells

Small molecules typically distribute across living organisms such that molecules 'dissolve and distribute' in organs (very crudely speaking) according to near-to-equilibrium physiochemical principles in which *quasi* equilibrium rate constants dominate. Whilst this is a great over simplification, it carries with it the heart of the matter. For example, a small molecule dye will essentially 'dissolve' (diffuse) across a biological membrane. When the source is removed, if there are no highly specific and high affinity interactions in the environment (for example, inside a cell) to retain the molecules, there will be a rapid flow out of the cell (across the cellular membrane again) according to chemical potential considerations. This is all nicely illustrated in a very simple *in vitro* cell model in Figure 2A where uptake and export of a molecular dye are tracked by fluorescence flow cytometry.[5]

Trafficking and clearance of nanoparticles at cellular level

Here again, radically new paradigms emerge, for unlike chemicals (which may have wide and distributed access to the intra-cellular space by similar dissolution processes) nanoparticles have limited and managed access using endogenous cellular pathways used to transport proteins and other biomolecules. In some cases these processes lead to nanoparticles being localized at very high concentrations in particular organelles (for example lysosome is typical, as shown in Figure 2C, and later on). Transport occurs only along prescribed pathways, for which appropriate particle surface signals are available - for example, in Figure 2D we show that nanoparticles of a very similar substance to the dye in Figure 2A (but in nanoparticulate form) are not cleared upon removal of the extracellular nanoparticles source, but instead are trapped (as far as we can tell 'permanently') inside lysosomes. This may be visualized in a sequence of confocal fluorescence and EM images from silica nanoparticles (see Figure 3) in which we see events of uptake, and internalization, and final localization into lysosomes. This is a very general paradigm we have seen in many particles, cell types (and higher levels) that must be accommodated in any model.

5 Progress and Outcomes to date

The premise of NanoTransKinetics is that the prediction of all toxicological and biological impacts has, as its basic pre-requisite, the correct prediction of the sites of action and localization of nanoparticles in living organisms. Based on this information, toxicological impacts can be deduced. It is quite clear that poor quality experimental data leads to low impact models with little or no predictive capacity. Ensuring the quality of the data to be utilised in the establishment of the NanoTransKinetics phenomenological and semi-microscopic models is critical to the success of the project.

Efforts towards modelling the interactions of nanoparticles with proteins and biofluids (WP2) have included development of an approach to describe universally the adsorption behaviour of proteins to nanoparticles.

Fluorescence Correlation Spectroscopy (FCS) was used to study the binding of fluorescently-labelled serum proteins (i.e., transferrin, Tf) to unlabelled polystyrene NPs. We observed that in many cases the Langmuir adsorption isotherm fails to correctly describe the protein adsorption (Figure 5a), and it does not allow distinction between hard and soft corona. Conversely, we observed universal adsorption behaviour as a function of molar protein/NP ratio. This model, termed the "Strong Binding Model", assumes a strong interaction until all free space on the NP surface is fully coated (Figure 5b). NanoTransKinetics partners are now assessing the applicability of the model to a wider range of proteins.

The University of Barcelona partner is using molecular dynamic simulations to study competitive protein adsorption on a nanoparticle (NP), i.e. the non-monotonic behavior of the amount of protein adsorbed to a NP in contact with plasma as a function of contact time and plasma concentration. To achieve this goal UB are developing a phenomenological model that is based on recent experimental results from LMU and UCD, and builds on a model UB-UCD have adopted to describe competitive adsorption on flat surfaces. Because these models have water included in an effective way, to improve our description we are developing, at the same time, a coarse-grain water model that will allow us to include water in an explicit way in our studies for nanoparticle interaction with protein solutions. Part of this work has been published already, acknowledging funding from NanoTransKinetics.[9-11]

Inspired by recently published experiments from UCD,[12] a model for the effect of cell division on NP uptake was significantly extended (WP3).

It is now clear that Nanoparticle uptake must be understood within a framework where cells are continuously progressing along their respective cell cycles, dividing at the end of the cycle and thereby splitting their NP load among the daughter cells. An analytical model to describe this was developed and validated against experimental data,[13] as shown in Figure 6.

For NPs that do not affect cell cycle progression, the model is complete as is. For Nanoparticles that *do* affect cell cycle progression (including causing cell death), the model can in principle be extended, which we have also demonstrated.

6 Expected Impact

The expected impacts of the *NanoTransKinetics* project can be listed as follows:

Contribution to the development of robust systems for evaluating the health and environmental impact of engineered nanomaterials; Modelling of nanoparticle interactions with living systems, with an end-goal of prediction of impacts and safety evaluation is a potential "hot-spot" in terms of risk assessment knowledge gaps, and the impact of many common, in-market or near to market nanoparticles on the human and environmental health is almost entirely unknown. Building a secure foundation, based on key microscopic and mechanistic issues has the most secure chance of being sufficiently robust.

Reduction of the need for empirical testing (reduction of costs, reduced need for animal testing); There are believed to be roughly 30,000 nanoparticle types under investigation, potentially seeking a place in the market. The program described here could form the basis of a future screening strategy to predict the likely impact of new nanoparticles. *NanoTransKinetics* is the only program (of its scale and understanding) in the world addressing the specific (emergent) issues of modelling nanoparticles as biological entities that are trafficked and transported around cells in an actively processed manner, and is thus the only program credibly able to contribute to the issue of future measures on the time scale necessary.

Contribution to predictive models for designing and engineering nanomaterials that are safe by design; A reductionist approach, based on interactions and mechanisms, gives the capacity to identify and evolve the key characteristics (size, bare zeta potential, corona composition) of NPs leading to different impacts, and above all, to clearly identify the causal link between them. This link is the key to safety by design.

7 Directory

Table 1 Directory of people involved in this project.

First Name	Last Name	Affiliation	Address	e-mail
Vicki	Colvin	Rice University	Houston, TX 77005-1827 USA	colvin@rice.edu
Kenneth	Dawson	National University of Ireland, Dublin / University College Dublin	Belfield, IE-4 Dublin, Ireland	kenneth.a.dawson@cbni.ucd.ie

First Name	Last Name	Affiliation	Address	e-mail
Giancarlo	Franzese	University of Barcelona	Av. Diagonal 647 Barcelona 08028, Spain	gfranzese@ub.edu
Karen	Griffin	National University of Ireland, Dublin / University College Dublin	Belfield, IE-4 Dublin, Ireland	Karen.Griffin@cbni.eu
Joachim	Rädler	Ludwig-Maximilians University	Geschwister-Scholl-Platz 1 Munich 80539, Germany	joachim.raedler@lmu.de

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NanoValid

Development of reference methods for hazard identification, risk assessment and LCA of engineered nanomaterials

Project number: 263147 Website: www.nanovalid.eu
Coordinator: Rudolf Reuther, NordMiljö AB, Sunnemo, Sweden

No.	Beneficiary name	Short name	Country
1	NordMiljö AB [Environmental Assessments]	NOMI	Sweden
2	The Institute of Nanotechnology	ION	United Kingdom
3	European Commission Joint Research Centre, Institute for Environment and Sustainability	EC-JRC	Belgium
4	University of Tampare	UTA	Finland
5	University of Salzburg	PLUS	Austria
6	University of Zaragoza	UNIZAR	Spain
7	University of Namur	FUNDP	Belgium
8	University of Ljubliana	UNILJ	Slovenia
9	University of Birmingham	UOB	United Kingdom
10	Fraunhofer Gesellschaft	FHG	Germany
11	Helmholtz Centre for Environmental Research	UFZ	Germany
12	National Institute for R&D in Microtechnologies	IMT	Romania
13	National Research Centre for the Working Environment	NRCWE	Denmark
14	Federal Institute for Occupational Safety and Health	BAUA	Germany
15	Eidgenössische Anstalt für Wasserversorgung, Abwasserreinigung und Gewässerschutz	EAWAG	Switzerland
16	National Institute of Chemical Physics and Biophysics	NICPB	Estonia
17	Federal Institute for Materials Research and Testing	BAM	Germany
18	German Institute for Standardization	DIN	Germany
19	The National Institute of Metrology, Standardization and Industrial Quality	INMETRO	Brazil
20	Federal University of Minas Gerais	UFMG	Brazil
21	Centre for Cellular and Molecular Biology (CSIR)	CCMB	India
22	McGill University	MCGU	Canada
23	Veneto Nanotech	VN	Italy
24	Nanologica AB	NLAB	Sweden
25	StratiCell	STC	Belgium
26	Grimm Aerosol Technologies	GAT	Germany
27	QUANTIS	QUANTIS	Switzerland
28	Centro Ricerche FIAT	CRF	Italy
29	Ahmedabad University	UOA	India
30	Vlaamse Instelling voor Technologisch Onderzoek NV	VITO	Belgium
31	USEPA (no full member of the NanoValid consortium but associated by a LOI to the project)	USEPA	US

Contents

1 Summary

Project Duration: 1 November 2011 – 31 October 2015

Project Funding: 9.6 Mio. EUR

The growing development, production and use of engineered nanomaterials and associated products will increase exposure of humans and ecosystems to these new materials. However, current knowledge is still incomplete and established test methods inappropriate to reliably assess exposure and risk of materials at the nano-scale. As a result, there is an urgent need to further develop these methods to overcome limitations of current hazard and risk assessment schemes and to generate the data needed for regulative requirements and for safeguarding production, application and disposal of nanomaterials.

The main goal of the NanoValid project is to develop validated reference methods and materials for measuring and testing of relevant manufactured nanomaterials. Current analytical and toxicity test methods and models have been subjected to a rigorous intercalibration and validation campaign and where necessary modified, adapted and further developed. The feasibility of validated measurement, characterization and test methods is further assessed by case studies to test the performance and robustness of the developed validated methodology under real conditions and so to improve existing exposure monitoring systems as well as risk management and reduction strategies.

As a first step, the existing literature on protocols for synthesis and characterization of EN was reviewed in WP2 to identify knowledge gaps and needs. Two sets of nanomaterials were selected and characterized by a variety of methods to find out candidate materials and methods (with a high level of consistency) for further validation. Test samples included in-house silica, gold and titanium dioxide, and externally produced silver and carbon nanotubes. A 3rd set of test materials (palladium, fullerenes, CuO, ZnO) is in preparation. A special silica prototype was synthesized and proved suitable as CRM candidate. Existing standard operating procedures (SOPs) for the synthesis and characterization of SiO_2, Au and TiO_2 have been optimized and their applicability assessed in different media, as particle properties may change, e.g. by the acidity of the test solution or the presence of salts causing nanoparticles (NP) to dissolve or aggregate. Also standard bioassays were tested for their suitability as rapid screening methods.

Results from WP2 were used in WP3 to implement first inter-lab comparisons for a variety of measurement techniques and by considering measures to control dispersion and solubility processes. For human toxicity testing, protocols have been adapted from the EU FP7 QualityNano project by using a human cell-line (A549) and results show a high reproducibility among participating laboratories. To monitor the release of NP aerosols at work places or from accidents, a specific NP aerosol generator and self-cleaning dispersion room have been tested and first measurements performed. Also fate and effect studies in aquatic and terrestrial organisms have started focusing on methods to assess uptake, bio-persistence and bioaccumulation of EN in laboratory models.

WP4 is addressing the question whether validated methods will support accurate risk (RA) and life-cycle assessment (LCA) of EN. For this, the available literature was reviewed and results show that current test schemes for hazard identification may be applicable as simple and rapid screening methods. In particular, studies on the inner ear proved successful as a model for assessing human risks associated with EN. But results also suggest that tests for specific EN should not be waived. Assessing the performance of LCA schemes is still ongoing including a comprehensive data collection and evaluation of material flows. In particular, data from industrial partners, such as FIAT and CINKARNA, a Slovenian producer of TiO_2 NP, will help to evaluate the impact of EN along the whole product value chain and the performance of the developed LCA.

Results of previous WPs are used in WP5 to develop reference methods and CRMs. Due to homogeneous size and shape, studies on the 12 nm silica CRM candidate material confirm that it can be used to validate methods for these 2 measurands. Upscaling of its synthesis is underway and first inter-lab studies started with this particular silica. Work is also planned to address more complex multi-layered EN (such as quantum dots). Similarly, different dispersion methods have been tested and recommendations given for future inter-lab comparisons to reduce variability. To develop reference labeling methods, radioisotopes & fluorescent molecules are used to trace NP during testing. For developing validated bioassays, different human cell-lines and animal models have been selected and studies on model soil-plant systems and aquatic organisms performed to assess effects for a number of ENs.

WP6 will test how robust the validated reference methods developed with well defined EN properties and known test media will work in real-life. A number of field studies are prepared including on-site occupational exposure measurements at various production sites, and a transport accident scenario. To monitor real personal exposure to EN, stationary and portable NP detectors are used at workplaces including automotive sites at the industrial partner FIAT.

2 Scientific / industry needs and problems addressed

Current knowledge is still incomplete and established test methods inappropriate to reliably assess human and ecosystem exposure to and risk from materials at the nano-scale. There is an urgent need to further develop appropriate methods to overcome limitations of current hazard and risk assessment schemes. NanoValid will address this need through a comprehensive assessment of industrially relevant engineered nanomaterials (ENMs), with particular focus on the development of appropriate reference materials and methods, to manage and reduce associated risks.

The core idea and concept for the project is based on the observation that:

(1) physicochemical properties of nano-sized particles, and hence their biological activity, are often unique and distinct from those of the same bulk materials and often unpredictable,

(2) existing standard methods for measuring and testing that have been developed for macro- and micro-scale material properties may not be applicable to nanoparticles.

These points taken together explain why many current analytical and toxicology protocols developed for bulk materials may not be suitable for dealing with ENMs, and why a large proportion of the published data may be inaccurate in the context of ENMs and may lead to the drawing of scientifically invalid conclusions.

NanoValid will make new progress in the following fields:

1. Nanomaterials fabrication and characterization

State-of-the-art: At the moment a large proportion of the relevant literature involves poorly characterized, often industrially produced ENPs. The methods for the suspension, preparation and characterization of ENMs prior to biological testing are currently not standardized. Consequently, results from current toxicological tests are not comparable and do not provide the technical framework needed by stakeholders and policy makers as the basis for control and regulatory measures.

It is widely accepted today that the surface chemistry of ENPs is extremely important for possible toxicological effects. Unfortunately, only a few publications are available on the chemical characterization of ENM surfaces, such as nanotubes or core-shell particles. In addition, these studies do not consider standardization or metrology issues that are needed to ensure safe industrial application of ENMs and still no inter-laboratory comparisons or references samples or CRMs exist.

Progress: NanoValid is producing well defined ENPs for toxicology testing by optimizing and extending current synthesis protocols for selected nanomaterials (see priority lists below) and by including multiple particle sizes, different structural forms, shapes and surface modifications. Synthesis and processing methods will be stringently defined and followed to ensure minimal deviation in the physicochemical properties of the ENMs between batches. Batches of selected ENMs will be also characterized by using a battery of different measurement tools to ensure that any variations are kept within well defined and allowed limits. They will be used in sets of experiments to accurately identify the physicochemical determinants of toxicology and eco-toxicology.

Likewise, the properties of ENP aerosols will be accurately characterized as a function of the ambient conditions to understand the dynamics of aggregation and propagation that govern their behavior and at the same time limit the effectiveness of control methods, which is decisive in the management of possible risks.

NanoValid is developing and using highly sensitive EN labeling and tracing methods and is designing and using controlled atmosphere dispersion chambers that allow a more precise and reliable monitoring of the behavior of released nanoparticles.

Initial test materials have been selected from those listed below, in close coordination with relevant standardization bodies (DIN an CEN/TC 352) and programs (OECD/WPMN) and with other relevant projects such as MARINA, NanoDefine, NanoSustain or QualityNano):

Priority 1 test materials: metal oxides (SiO_2, TiO_2, ZnO, CuO), metals (Ag, Au and Pd), CNTs (SWCNTs and MWCNTs) and fullerenes.

Priority 2 test materials: quantum dots (CdSe, CdS, CeO_2), salts (Ca-phosphates, PbS), nanocellulosic materials, polystyrene, dendrimers, ceramics, nanoclays.

2. Human health *in vivo* and *in vitro* models

State-of-the-art: Cellular stress and immune activation have both been reported following exposure to ENMs. However, the lack of validated methods makes it difficult to interpret available experimental and field results. Similar studies sometimes report contradictory effects and often material and methods are insufficiently described to allow a scientifically sound evaluation of data, also regarding exposure to and contamination with bacterial compounds and other stressors.

Progress: NanoValid is establishing and implementing the following specific tools to reduce these uncertainties:

1) Perform analytical centrifugation and Scanning Electron Microscopy (SEM) on all dispersed ENPs, regardless of the dispersion protocol used and before any *in vitro* and *in vivo* testing.

2) Create standardized protocols (SOPs) to follow *in vivo* uptake, interaction, traffic, storage and elimination of ENPs by cells; to study *in vivo* uptake in the lung and elimination through the kidney and the reticuloendothelial system; to define novel endpoints, and to compare *in vivo* effects following uptake by oral, dermal and pulmonary routes.

3) Further develop and adapt current cytotoxicity tests to tridimensional tissues, such as reconstructed epidermis, reconstructed lung epithelium or intestine epithelium, to study the possibility of ENP-induced impacts. Progress will be controlled by monitoring correct positive and negative tests and by adapting and modifying further tests. Also existing nanotoxicology tests from classical hepatotoxicology on monolayers of HepG2 cells will be adapted to test and verify effects of ENPs. Other cytotoxicity assays will be optimized to exclude false positives and false negatives more efficiently as compared with current test systems, and novel methods designed to quantify uptake of ENP into cells, together with new protocols to test and assess potential sources of errors.

Although recent publications have shown that fullerenes, ZnO and TiO2 nanoparticles possess a significant genotoxic potential to human cells, existing data on cellular and molecular interactions of ENPs with mammalian and bacterial systems are still scarce and inadequate. NanoValid will help to elucidate the exact mechanism of toxicity of ENPs to understand their *in vivo* response in various model systems. In this context, NanoValid is using the most efficient cell-based assays as a basis for biological testing to reliably monitor work place safety in the industry, which has been exclusively based so far on physical measurements of particle size distributions and concentrations.

A panel of human reporter cell lines is currently developed to specifically test *in vitro* ENP effects on cellular stress and inflammation and use in novel *in vitro* reconstructed tissue-based and single-animal models.

Also a new collection device for direct on-site measurements will be developed which will include a novel Biomodule to allow biological tests for nanotoxicity on-site and which can be used by already available personnel without requiring biological experts.

3. Eco-toxicity

State-of-the-art: Although ENPs are used in many consumer products and industrial applications, their real environmental fate and effect potential throughout their entire life cycle is largely unexplored and reliable quantitative data on toxicological effects of ENPs still scarce even at the single organism level. Eco-toxicological studies on ENPs that have been conducted so far include *in vitro* exposure assessment of vertebrate cells, as well as vertebrates (fish), invertebrates, algae, plants and bacteria. Recent laboratory studies show that even aggregated ENPs can be toxic due to solubilization and other specific properties and mechanisms. But there is still no reliable and validated scheme available for eco-toxicological risk assessment of ENPs. One of the key operational bottlenecks is the lack of reliable methods for the characterization of ENPs in exposure test media to account for bioavailability, bio-persistence and bioaccumulation. Another deficiency is the lack of a mechanistic understanding of how physicochemical differences are manifested, which requires well defined cellular systems.

Progress: NanoValid will help to close these gaps by generating a comprehensive knowledge database on ENPs regarding their life cycle impact on a large range of organisms, which will allow comparison and identification of common mechanisms of effects that are specific for certain types of ENPs. Particular light will be shed on the behavior of ENPs in exposure media used in OECD and other well recognized regulatory test schemes. Recent studies show that analytical centrifugation needs to be performed before any *in vitro* and *in vivo* testing. NanoValid is using this method to examine the compatibility of various exposure media with *in vitro* test models or to determine if and to what degree ENPs are agglomerated, after different treatments, such as gentle sonication, centrifugation or using biocompatible dispersant agents.

NanoValid is developing a specific validated model based on fish cell lines to study bioavailability, persistence and bioaccumulation mechanisms in relation to the toxicity of ENPs in fish. By following up and characterizing uptake mechanisms and developing methods for quantification of particle uptake, existing exposure assessment methods will be improved and refined. In addition, interlinking and comparing *in vivo* with *in vitro* results will allow the validation and further development of powerful *in vitro* and *in silico* methods as alternatives to animal testing.

4. Improvement of analytical detection and labeling systems

State-of-the-art: Existing analytical methods have detection limits that are too high to be able to reliably detect low concentrations of ENPs, despite their large surface area conferring a chemical reactivity equivalent to that of a much greater mass concentration of chemically identical, but larger-sized particles. Due to these limitations and challenges, which we face today when working with different ENP characterization techniques, almost nothing is known on the mobility of ENPs in natural environments.

Progress: NanoValid is developing new approaches to increase precision and reproducibility of current analytical detection systems designed for nanomaterials at low concentration in biological and environmental samples, including methods to determine their chemistry, size and morphology, e.g. by advanced secondary electron and optical imaging and spectroscopic techniques. By using these improvements, NanoValid is also assessing the applicability of a new system of respiratory exposure assessment that is based on mathematical turbulence models.

State-of-the-art: Also information on reliability and comparability of current biodistribution and bioaccumulation data of nanoparticles is scarce and severely affected by many factors, such as the status of tested nanomaterials, the labelling methods used and sample preparation from animal organs/tissues, which calls for standardized protocols for ENPs labelling, tracing and quantification.

Progress: NanoValid is developing reliable sample preparation and isotope (radiogenic and stable) labeling protocols for selected ENMs, and related analytical protocols for reliably detecting/tracing various ENPs in different animal organs/tissues.

3 Scope and objectives

The main objective of NanoValid is the development of a set of reliable reference methods and certified reference materials (CRM) including methods for dispersion control and the labeling of ENMs. Based on a comprehensive and critical literature and data survey, the most suitable test materials and methods are selected and tested, and specific nanomaterials synthesized, characterized and stabilized for final method validation.

Existing industrial or newly designed nanomaterials (ENMs) are submitted to comprehensive inter-laboratory validation campaigns that include currently most advanced methods and instruments for measuring and characterizing of ENMs, to generate accurate and reproducible material data and standardized method protocols, also for labeling, tracing and quantifying of nanoparticles in relation to their size/size distribution, morphology, material identification and other standard physicochemical (pc) properties. The stability and behavior of selected ENPs is monitored and tested in a variety of relevant biological and environmental samples and test media under both normal and extreme conditions to derive optimum and reproducible fabrication, measurement and test conditions.

The tested pc methods derived from extensive intercalibration and inter-comparison of selected methods and materials are used to

design well-defined certified reference materials, which in turn are employed to adapt, modify and validate measurement methods and current biological approaches (*in vitro*, *in vivo* and *in silico*) for assessing the toxicity of ENMs that may pose risks to human health and the environment. The effects of chronic exposure and of exposure under real-life conditions, where ENPs are likely to act as components of complex mixtures will be taken into account. Finally, appropriate reference methods will be established based on the validated pc and biological methods, and their applicability assessed to a variety of industrially relevant ENMs by means of case studies.

Specific objectives are to:

(1) Test, compare and validate current methods to measure and characterize physicochemical properties of selected ENMs

(2) Monitor and control their dispersion and stability in various test media and environmental matrices by novel labeling methods

(3) Generate panels of well-characterized and reproducibly synthesized ENMs, engineered nanoparticles (ENPs) and associated products, designed for further (eco-) toxicological testing

(4) Test, compare and validate current *in vitro* and *in vivo* methods (for toxicity and ecotoxicity testing) to early identify potential hazards, assess human health effects, including acute and chronic toxicity (oral, inhalation, dermal), and effects to the environment

(5) Develop a standard test panel according to the mode of action and interaction of ENMs and ENPs with experimental media as used in OECD and other standardized tests

(6) Identify responsive biomarkers for potential cytotoxic, genotoxic and immunotoxic effects

(7) Develop further validated methods and materials to reference methods and materials, including Certified Reference Materials (CRMs), for more reliable risk and life cycle assessment (RA and LCA)

(8) Demonstrate feasibility of validated and established reference methods by means of case studies to assess and improve the performance of methods and systems both during normal operations and for management of accidental risks, evaluation of risk reduction strategies and field detection systems, and for monitoring hazard and exposure to ENPs

(9) Establish a database on hazard properties of selected ENPs that could be used to support the REACH hazard assessment system

(10) Build a comprehensive knowledge hub and database to improve existing models on transport and fate of ENPs in the environment, including bioaccumulation, persistence, bioavailability and life cycle impacts onto all forms of biota

(11) Initiate and support focused efforts to achieve international standardization in cooperation with national (e.g. DIN) and international (e.g. OECD WGMN) organizations.

4 Technical approach and work description

NanoValid's overall strategy is based on (1) a continuous and comprehensive critical review and integration of the existing scientific literature and of relevant material databases, and on (2) a rigorous intercalibration campaign including outstanding test laboratories in Europe and world-wide that participate in the project, to compare and validate current methods and test schemes that have been developed for hazard characterization as well as exposure and risk assessment of bulk chemicals. New methods and schemes are developed and validated by using relevant and representative industrial and newly synthesized NPs and benchmark materials, and by testing the impact of relevant test media and environmental conditions.

NanoValid is organized in five technical Work Packages (WPs) and three non-technical (management, coordination and dissemination) WPs, as follows:

WP1 Project management

WP2 Fabrication of test materials and selection of test methods

WP3 Validation of pc methods, *in vitro*, *in vivo* and computational methods (*in silico*)

WP4 Application of validated methods to risk (RA) and life cycle assessment (LCA)

WP5 Development of reference methods and certified reference materials

WP6 Case studies to assess the feasibility of validated methods

WP7 Dissemination, exploitation, training, networking and clustering

WP8 Scientific coordination

Although each individual WP has its own distinct focus, function, objectives, tasks, deliverables and milestones, all WPs will closely interact with, support and complement each other in an overarching holistic approach required to match the complexity and multidisciplinary nature of the proposed project. A bottom up approach will be used to gradually link tasks that start with a lower level of complexity (e.g. primary data generation, method and material survey and selection in WP2) with tasks of increasingly higher levels of mutual interaction (e.g. validation of methods and testing their applicability to RA and LCA (in WP3 and WP4), until the intended objectives (verified by specific deliverables and milestones) are achieved and results generated (e.g., establishing reference methods and materials in WP5 and proving their applicability in WP6). A global dissemination and exploitation strategy (WP7) including internet-based interfaces for all relevant stakeholders (academia, industry, regulatory authorities policy-makers, the public) and events organized at different levels around the project is facilitating the take-up and exploitation of project results already during the course of the project.

5 Status of the project

NanoValid has started on 1 November 2011 and was launched by a kick-off meeting in Rome, Italy, on 16-17 November 2011, together with MARINA (www.www.marina-fp7.eu), another large-scale EU FP7 nanosafety project. Both NanoValid and MARINA are collaborating by streamlining their R&D activities to use synergies, share data and test materials, and so increase the overall outcome and impact of these 2 projects. The progress of the work of NanoValid has been successfully reviewed by the EU Commission

after the first 18 months and the project has now passed midterm (March 2014).

The ultimate goal of the NanoValid project is to establish reference methods and certified reference materials (CRM) for the physicochemical (pc) and (eco-) toxicological characterization and manufacture of selected engineered nanomaterials (EN). To reach this main goal, various analytical, experimental and desktop studies have been initiated and completed during the first 18 months, including comprehensive literature surveys and reviews, synthesis, supply and characterization of appropriate test materials, testing the suitability of methods to assess human and (eco-) toxicity and exposure to selected EN, and launching first inter-lab comparisons (round robins) to validate selected measurement and testing methods.

The following project objectives have been achieved during the 1st reporting period (M1-18):

- ✓ Generation of 2 sets of newly and reproducibly synthesized ENs: SiO2, Ag, Au, TiO2 and CNTs, and ongoing delivery of a 3rd set of test materials including Pd, C60, CuO and ZnO
- ✓ Selection and use of appropriate parameters + methods to measure/characterize physicochemical (pc) properties of selected ENs and their detection in various media
- ✓ Full physicochemical (pc) characterization of selected EN
- ✓ Review of available dispersion control methods for EN in various media with recommendations
- ✓ Evaluation and preliminary testing of labeling methods for EN
- ✓ Launching of round robins to validate the applicability of existing measurement and *in vitro* and *in vivo* methods to assess the (eco-) toxicity and identify human health and environmental effects caused by nanoparticles (NP), including acute + chronic (oral, inhalation, dermal) effects
- ✓ Develop rapid screening methods for biological profiling of EN
- ✓ Establishing candidates for certified reference materials
- ✓ Review and critical evaluation of the performance of current approaches for RA and LCA of EN
- ✓ Initiating case studies to assess the feasibility of validated methods under real life conditions
- ✓ Bringing research results to standardization (project liaison with CEN TC 352).

In the following, a short overview is given on the work done and results achieved so far in WPs 2-6 during the first 2 project years (M24).

5.1 *Materials fabrication and methods selection (WP2)*

As a first step, existing protocols have been reviewed and optimized in WP2 for the synthesis of 2 prototypes of nano-SiO2 (in-house fabricated by NLAB), 1 prototype of nano-TiO2 (in-house fabricated by CCMB) and 1 prototype of Au (in-house fabricated by INMETRO and dispersed in H2O). Based on the NanoValid priority list (see chapter 2 above), a 1st set of test materials was prepared and distributed to project partners in WP2, 3, 5 and 6, including the 2 SiO2 prototypes and a prototype of Ag (fabricated by MARINA partner Colorobia). In the 2nd half year of the project, another 2nd set of test materials was prepared and characterized by partners from WP2 and WP3, including prototypes of Au (from INMETRO), TiO2 (from CCMB) and CNTs (from MARINA partner Nanocyl).

Table 1 below summarizes methods and parameters used for the pc characterization of selected EN in WP2. Partners BAM and NLAB agreed to upscale the synthesis of a 12 nm SiO2 prototype by BAM that showed a high potential for being further developed as certified reference material (CRM), and so to provide the project with a first CRM. For this, up-scaling tests have been carried out at NLAB, to check reproducibility and homogeneity, and to upscale the synthesis protocol from 50 ml up to 500 ml. After synthesis and full characterization, this particular SiO2 prototype will be distributed to all partners involved in method validation.

Method	Parameter
X-ray Diffraction (XRD)	Crystalline phase
Scanning Electron Microscopy (SEM)	Morphology, particle size (dry)
Tunneling Electron Microscopy (TEM)	Crystalline phase, symmetry of porous SiO_2, chemical composition
Dynamic Light Scattering (DLS)	Particle size (dry), agglomeration/aggregation
Z-potential	Electrical charge
Brunauer–Emmett–Teller (BET)	Surface area, and porosity
Inductively coupled plasma mass spectrometry (ICP-MS)	Chemical composition

Table 1: PC characterization techniques used in WP2 for the 2nd set of test materials (Au, TiO2, CNTs)

To date (March 2014), a total of 10 prototypes has been selected and provided by the project, including 2 SiO2, 1 Ag, 1 Au, 1 TiO2, 1 CNTs, 1 Pd and 1 C60. A 3rd set of test materials is in preparation (CuO, ZnO). Five test materials (2 SiO2 prototypes, Au, TiO2, CuO) have been produced in-house and the SiO2, Ag, Au, TiO2 and CNT prototypes fully characterized by following OECD standards including most important parameters, such as size, structure, shape and porosity and by using XRD, SEM, DLS, BET, z-pot, TEM, ICP, EDX (see Table 1). Four draft SOPs on characterization methods (XRD, TEM, DLS, BET) have been prepared and two methods (for DLS and BET) validated by WP3 as potential candidates for reference methods. Also the Au prototype sample proved as a good candidate for a reference material for pc characterization (see also WP5 below), while one of the 2 SiO2 prototypes looks much promising to serve as a good reference material candidate for BET measurements. An overview report was prepared on how existing protocols for synthesis have been optimized and on SOPs established for synthesis and pc characterization methods, with results achieved so far (M24).

To support the ongoing identification and validation of appropriate fabrication and characterization methods and the selection of suitable test materials, the expertise available within NanoValid on toxicity testing was reviewed and the most relevant methods tested for their potential to serve as screening methods (biological profiling) and to provide a preliminary hazard analysis of selected EN. Samples from the 1st set of test materials were used and existing SOPs adapted and modified encompassing 15 different bioassays and 13 different organisms/cell types. Results showed that the SiO2 prototypes are not toxic in most test systems up to 100 mg/L, while Ag NP proved to be toxic in all tested bioassays

including bacteria, protozoa, crustaceans, plants, yeast and mammalian cells. Experiments also showed that Ag NP toxicity was very similar to that of soluble Ag ($AgNO_3$) suggesting that particle dissolution may play a major role. More detailed studies are under way to elucidate the exact mechanisms behind the observed toxic action of Ag NPs. The selected (eco-) toxicity tests have been applied to a wide range of ecologically relevant organisms and included *in vitro* tests with human cells. Obtained results reflect very well the amplitude and variability of possible responses that may occur when nanomaterials are released and end up in ecosystems.

Before testing, the test samples (powders) need to be dispersed in various test media and a SOP for the various dispersions was prepared. Summarizing results, Ag NP formed very stable dispersions in all toxicological test media and the average hydrodynamic diameter was between 111 nm and 130 nm (by DLS), in contrast to SiO_2 NP that formed large aggregates in de-ionized water with a hydrodynamic diameter around 1 µm in all media tested. These large SiO_2 aggregates could not be broken down into smaller particles even after extensive ultra-sonication and thus aggregates settled rapidly during the toxicity testing procedure (see: Bondarenko et al. 2013, Toxicity of Ag, CuO and ZnO nanoparticles to selected environmentally relevant test organisms and mammalian cells in vitro: a critical review, *Arch. Toxicol.* 87:1181-1200). So far, 18 preliminary SOPs have been issued for sample preparation and toxicity testing of nanoparticles and are currently reviewed and validated within the project. Since different nanoparticles may need different sample preparation protocols (e.g. Ag NPs vs carbon-based NP), additional SOPs for sample preparation will be added to the current 18 SOPs.

To collect, organize and make the data available for all project partners, a project-specific online results database is presently prepared in WP2 and appropriate structures and templates have been drafted. Uploading of the data produced so far on pc characterization and toxicological testing of selected NP will start in April 2014.

5.2 *Method validation (WP3)*

WP3 aims to generate validated SOPs on measurement and testing of NP that can be used to improve current RA and LCA schemes (WP 4), to develop reference methods/particles (WP5), and to perform case studies to assess the feasibility of the validated methods under real conditions (WP 6). The resources in WP3 have been devoted to establish validated SOPs to (a) characterize nanoparticles and (b) conduct cytotoxicity studies using various cell lines and end point assays. To test the efficacy of the methods, partners from other EU funded projects were also participating in inter-lab comparison studies. For human toxicity testing, protocols have been adapted from the EU FP7 QualityNano project by using the human cell-line (A549), and results showed a high reproducibility among participating laboratories. To monitor the release of NP aerosols at work places or from accidents, a specific NP aerosol generator and self-cleaning dispersion room have been tested and first measurements performed. Also a variety of fate and effect studies in aquatic and terrestrial organisms are currently run focusing on the validation of tests used to assess uptake, bio-persistence and bioaccumulation of EN in laboratory models.

Four prototypes of ENMs i.e. mesoporous silica, gold, silver and MWCNT have been characterized (viz. size, size distribution, shape, surface charge, surface chemistry, purity, surface area, hydrodynamic size etc.) using a range of analytical techniques through a series of 2 inter-laboratory tests (see Figure 1 and Table 2). The Round-Robin tests were conducted by research partners from NanoValid and MARINA. Methods validated by these inter-laboratory comparison studies have led to the formulation of validated SOPs that are made available to all partners through the project website. At the same time, three different test media (1mM $NaNO_3$, DMEM cell culture and artificial fresh water) have been used to test the reactivity of ENMs and to identify appropriate media and test conditions to control suspension stability of ENMs and dispersion processes (see publication: Meissner et al. 2014, Dispersion of ENMs used in toxicological studies: a comparison of sonication approaches demonstrated on TiO_2 P25, Journal on Nanoparticle Research 16:1-13).

	SILICA	SILVER	GOLD	MWCNTs
Source	Synthesised by NLAB	Purchased from Colorobia	Synthesised by INMETRO	Synthesised by NANOCYL
Form	Powders and uncapped. Mesoporous	4 wt.% aqueous suspension. PVP used as a surfactant.	Citrate capped NPs. Aqueous suspension of 0.006 %	Powders
TEM size	212.8±52.1 nm	21±8 nm	13.28±0.81 nm	10.16±0.46 nm (dia) > 1 µm (length)
DLS size	600.5±115.4 nm	117±24 nm	18.18±1.8 nm	-nd-
NTA size	157±30 nm	89±5 nm	35.7±1.7 nm	-nd-
AFM size	195±85 nm	13±9 nm	-na-	-na-
BET surface area	909.8±42.2 m^2/g	-nd-	-nd-	264.5±17.69 m^2/g
Phase	Amorphous	Crystalline (ICDD: 00-004-0783)	-nd-	Graphitic-like domains represent intratube structure of MWCNTs.
Zeta potential (pH 5.5-6)	-30.7±9.3 mV	-19±9 mV	-37.8±8.5 mV	-nd-
XPS	Predominantly Si and O was detected.	High concentration of C and O was picked on the surface of the particles.	C, O Cl and Na were present. Au 4f XPS spectrum showed the presence of metallic Au.	No other elements apart from C and O was detected. Similar with EDS

Table 2: Summary of physic-chemical characterization of various ENMs used in WP3 RR tests

A state-of-the-art fluidized bed aerosol generator (FBAG) was designed at the University of Zaragoza, Spain, to assess exposure to NP. Aerosols of SiO_2 nanoparticles have been obtained by using this device, in which both airborne nanoparticle concentration and particle size distribution can be effectively controlled. The aerosol generation from the FBAG is based on a long-lasting detachment of 100-nm nanoparticles upon fluidization at different flow rates. Moreover, the development of the different devices for aerosol NP sampling is carried out to determine the morphology of released NP by means of SEM and TEM, together with other selected spectroscopic techniques. Also, a novel self-cleaning test chamber for the dispersion of ENMs aerosols was developed to simulate the behaviour of NP aerosols in either particle-free or controlled environments, and to simulate indoor conditions at different locations. Equipment and places for the dispersion of ENP aerosols have been made available to validate the performance of aerosol characterization methods, such as CPC + SMPS, OPC and NPS500.

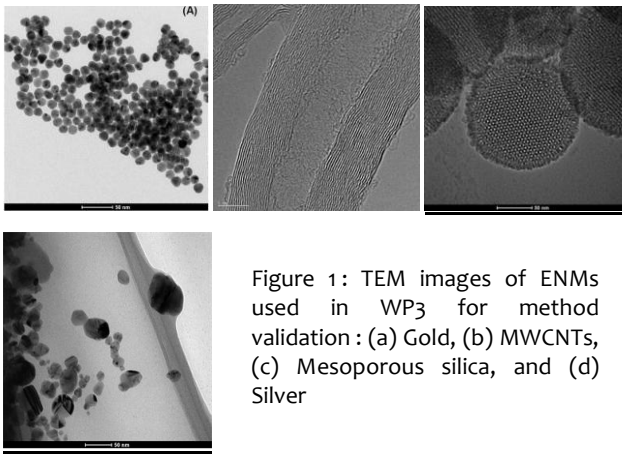

Figure 1: TEM images of ENMs used in WP3 for method validation : (a) Gold, (b) MWCNTs, (c) Mesoporous silica, and (d) Silver

Inter-lab comparison studies have also been performed to assess the cytotoxicity of Ag NPs on A549 and THP-1 cell lines. These experiments are conducted by several partners with an objective of preparing validated SOPs for several cell lines, and selected assays (e.g. MTS, ATP, Caspase, cytokines). SOPs have been created so far from RR performed to assess the effect of cell culture conditions, the growth of A549 cells, and potential mycoplasma contamination on human toxicity testing by using the MTS assay and Ag NP. To validate the applicability of the used methods, also interferences occurring in the assay from the nanoparticles themselves are studied.

5.3 *Applicability of validated methods to RA and LCA (WP4)*

Main objectives of WP 4 are: Identification of hazard posed by ENM to humans and the environment, to assess the risk of ENMs to both human and the environment by critically evaluating the suitability of current test methods and test strategies, and in particular of the validated methods developed and of results obtained within the various NanoValid work packages; and creating a life cycle assessment method for ENM.

As first steps, risk assessment strategies specifically developed for nanomaterials were critically evaluated and hazard assessment on specific nanomaterials (e.g. CNT, Ag, Cu) was performed based on data from the scientific literature. This has resulted in the publication of a review paper on "Bioaccumulation and eco-toxicity of Carbon Nanotubes" (Jackson et al. 2013, *Chemistry Central Journal* 2013, 7:154). For the planned life cycle analysis and life cycle impact assessment, specific data collection sheets have been developed and are used to collect, compile and store all relevant data generated during the project.

WP 4 partners will continue to work on hazard and exposure assessment of nanomaterials and have started to integrate the data obtained from the characterization and biological screening of the 1st and 2nd set of test materials from WP2 and WP3, to develop the framework for a new risk assessment strategy for both human and environmental health. In this context, the available toxicity data on Ag NPs are currently evaluated over WP boundaries by comparing results from NanoValid partners obtained with the same type of particles but in various organisms and cell lines. The assessment and comparison of the toxic effects was aimed at gaining a comprehensive overview on silver effects with regard to both, variations in test design and species sensitivity.

A second main activity brought forward within WP4, involves the creation of decision trees. The decision tree approach aims at harmonizing test procedures for nanomaterials, by guiding in the deduction of a nanomaterial specific characterisation and testing regime. Specifically, material identity, physical-chemical characterisation, choice of test conditions and test systems, and behaviour during the tests were considered. The decision trees are further refined and extended by integrating the relevant data and knowledge produced within NanoValid.

In line with that, a list of criteria for good quality nanotoxicity data to be used for RA and LCA was developed. The criteria are allocated to four different data categories, being (1) physical-chemical characterisation, (2) sample preparation (3) toxicity testing, and (4) general aspects. These criteria are based on the facts that nanomaterials differ in their properties and behaviour substantially from conventional chemicals and that one material may exist at the same time in various, substantially different nanoforms. The diversity of the nanoforms of a substance may hence lead to different testing requirements. Accordingly, for data evaluation and judgement on appropriate test procedures, several parameters specifically applying to nanomaterials have to be taken into account.

For the planned life cycle analysis and life cycle impact assessment, specific data collection sheets have been developed and are currently used to collect, compile and store relevant data, an effort involving many NanoValid partners. The life cycle inventory analysis requires an extensive data collection process to get all relevant data already available or continuously generated by NanoValid partners. In particular the engaged participation of CINKARNA (Slovenia), a nano-TiO2 manufacturer, allows the use and assessment of real "direct" production and process data.

5.4 *Developing reference methods and materials (WP5)*

Methods identified under WP2 and validated under WP3 will be further evaluated in WP5 for their potential to be developed as reference methods. Also, test materials selected and/or newly fabricated in WP2 and validated in WP3 will be used to develop appropriate RMs and CRMs. A principal milestone will be the preparation and characterization of stable nanoparticle suspensions that can be directly used for in vitro testing, analysis of the particles behavior in physiological media and environmental matrices, and for verification of these approaches by various size measurement methods.

Materials supplied by WP2 have been also characterized in WP5 by means of T-SEM and XPS in terms of size distribution and chemistry. In addition to the 1st set of test materials (SiO2, Ag), a ca. 10 nm nano-silica test sample has been developed by BAM with a high potential to become a certified reference materials (CRM). Also a nano-Ag CRM (developed by BAM but not within NanoValid) was made available to be used as internal benchmark standard.

Together with WPL2 and WPL3, WPL5 BAM has developed an internal set of standard criteria for the specification of test samples that can be used for toxicity testing. This ENP specification form was combined with a template form developed

within the German NanoGem project and is now available at the NanoValid website to be used for all NPs tested within the project. The document has been recently presented at a ISO/TC 201 meeting and will be considered for standard development.

A literature study on existing methods for the dispersion of nanomaterials in water as well as in cell culture media has been prepared by FHG (see WP3) and first results of validation measurements that use different dispersing methods for standard nano-TiO_2 and nano-SiO_2 have been obtained. Based on this, a SOP on dispersion has been developed. Drafting of a protocol and an appropriate strategy for determining the uncertainty of measurement as part of an inter-laboratory comparison on dispersion is in preparation and an appropriate strategy for the establishment of uncertainty budgets has been designed.

Dispersion monitoring and control will be done both in aerosols and in suspensions. For the development of an SOP, tested and validated methods are used by counting and sizing ENPs in aerosols through optical and condensation particle counters, in combination with size classification and separation by means of a Differential Mobility Analyzer. A SOP for dispersion of NPs in water will be developed by a RR test performed among all involved laboratories, for their proper handling in toxicological and environmental media and particle size measurements in appropriate concentrations will be done by using dynamic light scattering and centrifugal methods and qualified by zeta potential determination (microelectrophoresis).

A report has been prepared to provide a clear definition of what is a Reference Method established for pc testing methods. In addition, a standardized protocol for inter-laboratory comparison of pc measurement methods has been prepared and a first round robin on pc methods (size measurement and zeta potential) in cooperation with MARINA is in preparation.

NanoValid will establish two classes of reference nanomaterials: (1) a CRM representing a well defined, "certified" parameter with an uncertainty, and measurands such as size, BET surface, etc. And (2) reference materials (RM) useful for eco-toxicity testing and application. These type of RM will be supported by a set of relevant specification data, which are valid and delivered together with the NanoValid ENP Specification Form [www.nanovalid.eu/templates].

The following 2 CRM candidates are presently under development: the BAM nano-SiO_2 and a nano-Au prototype prepared by INMETRO. In addition, a survey on specified RMs available a bigger batches and useful for ecotox testing has been prepared.

The synthesized BAM nano-SiO_2 is almost monodisperse and size is approximately 15 nm. Due to the homogeneity in size and in shape, this prototype silica is an appropriate candidate to be further developed as CRM. The BAM Certification Committee is aware about this development and principal approval has been reached. It is planned to use traceable SAXS for certification. The Au NPs synthesized by NanoValid partner INMETRO were characterized by SEM, MET, DLS, Zeta, XPS, AFM (see Fig. 2). An inter-laboratory comparison on size determination was performed. The homogeneity of the batch has been investigated and found to be sufficient.

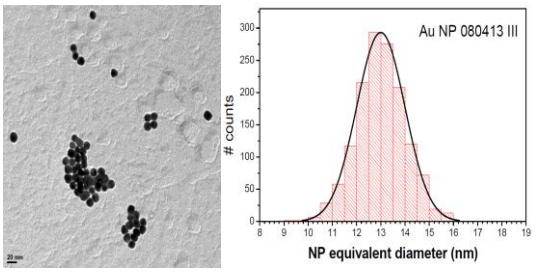

Figure 2: TEM image and particle size distribution of gold particles (INMETRO).

Inter-laboratory comparisons are in preparation:

- on size distribution and zeta potential measurements with data collected from partners and appropriate data reduction approaches.
- on BET measurements for surface area measurement with titania nanoparticles selected as test material.

To establish reference methods for NPs labelling, different optical labelling of NP have been investigated. Synthesis methods are further modified to reduce particles size but maintaining the capacity of labelled ENPs detection. After analysing a survey of the labelled nanomaterials developed within NanoValid, lanthanide-containing Y_2O_3 nanoparticles were selected as candidate for a round robin (RR) test. The conditions for the preliminary tests have been established for the quantification of the labelled NP to set up the threshold detection limits. A draft SOP for labelling is under preparation. Hollow Au nanospheres and SPIONs have been synthesized and tested as contrast agents (imaging) to evaluate the biodistribution and effects of NPs in the human body. To assess the usefulness of these labeled NP as type 2 reference material, the batch homogeneity has been tested after several synthesis sets and found to be sufficient.

To establish reference methods for assessing uptake and distribution of nanomaterials in the body, the nanotoxicity of the 2 SiO_2 prototypes and the Ag delivered by WP2, as well as of carbon nanowhisker delivered by MARINA was tested by UTA *in vitro* and evaluated by using WST-1 (or MTT), ATP, and nuclear membrane permeability using propidium iodide. To study changes in the function of biological barriers, such as skin, mucosa and brain, nano-Ag, SPIONs, nano-Au, MARINA nanoparticle-carbon nanowhisker, and C60 were tested in vivo by using the inner rat ear as a multifunctional model and evaluated by imaging, auditory function and histology evaluation studies.

UTA could visualize the distribution of SPIONs and Nano-liposomes containing gadolinium-tetra-azacyclo-dodecane-tetra-acetic acid (LPS+Gd-DOTA) in the body by MRI. DMSA@SPIONs enter the inner rat ear more efficiently than POA@SPIONs after intra-tympanic administration, as visualized by MRI, and stayed in the inner ear for at least 7 d. Hollow Au NPs impaired the middle ear mucosa but not the inner ear barrier (see Fig. 3), while uptake and distribution of Ag NPs in the body were detected by micro CT. The distribution of Ag NP and LPS+Gd-DOTA on the biological barrier function and auditory function has been assessed by gadolinium-enhanced MRI. It was also possible to track uptake and distribution of ENMs in the body using near infrared (NIR) imaging in vivo and the concept was proved in mouse ear using NIR dyes. In addition a method was developed to evaluate possible toxicity of ENM on kidney filtration. For the evaluation of the toxicity, *in vitro* tests

generally proved to be more than 1000 times more sensitive than *in vivo* studies. Also hyaluronan and glycosaminoglycan metabolism in the rat inner ear and kidney were measured after exposure to Ag NPs. Ag NPs induced accumulation of hyaluronan and glycosaminoglycan in the inner ear and kidney.

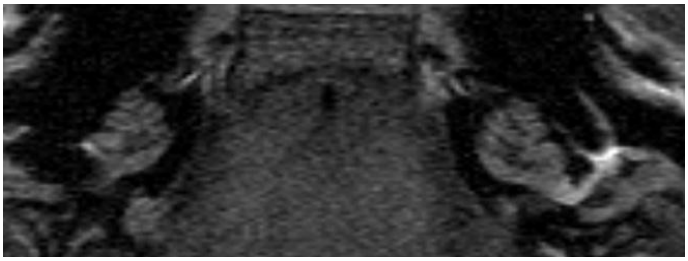

Figure 3: Hollow Au NPs enhanced passage of Gd-DOTA across the biological barrier of the middle ear mucosa 5 h after transtympanic injection at a concentration of 1 mg/ml (UTA).

In addition, the performance of various eco-toxicological assays (aquatic and terrestrial) has been tested by partner NICPB and others and will be further modified and optimized, to finally identify and develop candidate reference methods. Results so far indicate that nano-Ag may have the greatest impact. SOPs for a series of eco-toxicological test organisms (bacteria, protozoa, crustaceans) as required by REACH have been established with crustaceans being most sensitive towards Ag NPs, why well characterized samples of this nanomaterial will have a high priority for future investigations.

Protocol templates specific for (1) particle synthesis/production, (2) particle characterization, and (3) toxicity testing have been compiled and preliminary and validated SOPs provided by adopting the template available in EU FP7 Nanommune Handbook.

Different activities to develop reference methods for eco-tox testing are presently performed in WP5 (see the following examples):

- ✓ micro-biological tests proved to have the potential to flag possible risks posed by NP to soil microflora with evidence for nano-TiO2 to induce acute cytotoxicity to the agronomically beneficial nitrogen fixing bacteria *Sinorhizobium meliloti*
- ✓ Initial cone plant growth tests have been performed and proved helpful to reduce the material amount by > 50 % compared with pots. The plant response to toxicants in cones and pots is similar, but the sample variance was greater in cone tests.
- ✓ a review is in preparation to identify toxicological and ecotoxicological key organisms that are relevant nanotoxicity testing.

Novel approaches are under development to measure the size of NPs attached onto a surface by using a quartz crystal microbalance with dissipation (QCM-D) monitoring and a standard calibration method that allows to use Ag ion selective electrodes down to a Ag+ activity 10^{-14}.

As the development of reference toxicological and eco-toxicological methods are one of the priorities of the Nanovalid project, different toxicological methods are currently under validation to be used for hazard assessment of ENMs. Also exposure assessment methods that provide evidence that nanoparticles are in contact with biological systems are currently selected and criteria developed for reliable exposure assessment in biological systems including man and the aquatic and terrestrial environment.

To transfer relevant project results to method and materials standardization, NanoValid has signed a project liaison with CEN and is represented by the metrology and standardization project partners DIN and BAM in various CEN and ISO/TCs. An internal survey identified possible work items and TCs that appear most interesting where NanoValid could contribute to current standardization efforts, including CEN/TC 352 Nanotechnologies, CEN/TC 137 Assessment of workplace exposure to chemical and biological agents, CEN/TC 201 Surface chemical analysis, ISO/TC 202 Microbeam analysis, ISO/TC 229 Nanotechnologies and ISO/TC 24/SC 4 Particle characterization.

5.5 *Case studies (WP6)*

Case studies are used to translate the knowledge gained through validated methods in WP2-5 to the real world. They are related to work place safety assessment (WP6.1 + 6.2), the environment (WP6.3), accidents (WP6.4.), reducing risks for occupational handling (WP6.5) and monitoring NPs in the automotive industry (WP6.6). One challenge is to take the real world situations with so many unknowns into account, so laboratory practice needs to be replaced by situations that correspond to actual exposures. Another challenge is the robustness of assays, which need to deal with materials that are by definition not clean and are at least initially also not well-defined.

This WP started with a focus on code of procedures (WP6.5), which were defined based on an open workshop (BAUA Berlin, 27-28 November 2012), to ensure safe and standardized handling of NPs in all partner labs and beyond. The proceedings of the workshop are available on the project website as a best practice example. A standardized procedure for field studies to assess the feasibility of the developed guidelines has been prepared and field studies are currently running at various research and small production sites to assess the occupational exposure to NM during manufacturing and handling.

Monitoring work place environments can take two forms: Assays using collected materials and on-site testing. For the first approach, samples collected at industrial sites in Denmark have been provided and distributed to partners for testing. At the same time, an intermediate approach is followed by PLUS (WPL6), which produces mixtures of clean NPs with selected compounds (the pro-inflammatory bacterial compounds LPS, MDP, ieDAP, CpG-DNA and flagellin, plus the allergenic proteins Bet v 1 and Der p 1). In this case the type and extent of contamination is known and the mixture can be used to validate the robustness of biological assays (WP6.1). Also a novel sampling device (hot gas sampler) has been produced by GAT and planning for on-site measurements is ongoing.

For the purpose of on-site testing (in WP6.1 + WP6.2), PLUS has developed a panel of reporter cell lines expressing Red Fluorescent Proteins (RFP) under control of promoters indicating cell stress or inflammation, i.e. unspecific and early signals of the body indicating irritation. The reporter cells are based on the human lung epithelial cell lines A549. Figure 4 shows detection of IL-8 gene expression using RFP.

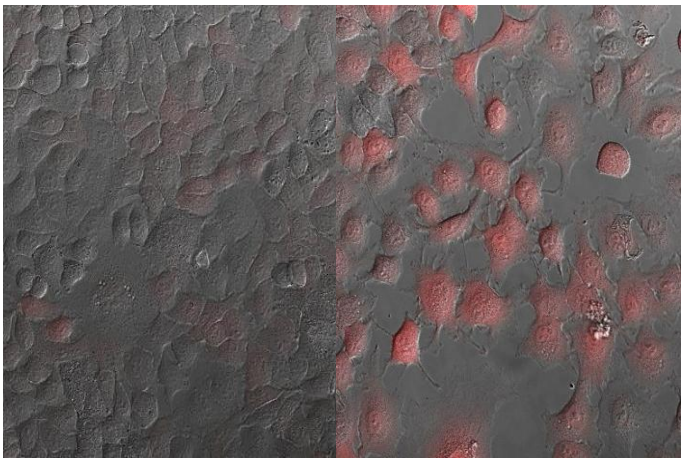

Figure 4: Detection of gene expression using RFP (PLUS)

The reporter cells developed here will be used during 2014 in test facilities of partners UNIZAR and VITO, where simulated work places are established. A further modulation to make these tests more realistic is the use of human lung fluid. Such fluid is collected during some clinical procedures (bronchoalveolar lavage fluid, BAL), and PLUS is testing whether the addition of human BAL gives different effects than the conventionally used FCS, which of course contains different proteins and other compounds compared to human lung. Work in case study WP6.1 shows that use of human BALfluid to coat particles affects inflammatory signals. Figure 5 below show again data for the inflammation marker IL-8. FCS-coated NPs induce higher amounts of IL-8 compared to NPs in protein-free medium. Interestingly, BAL data are variable, but highly reproducible for individual persons. Note that BAL from donor A results in a high IL-8 response, indicating that there is substantial person to person variation. The BAL are from clinically treated patients, not from healthy donors, but the high prevalence of people with lung conditions like COPD or allergic asthma in the work force suggests that risk persons have to be taken into account for safety assessment.

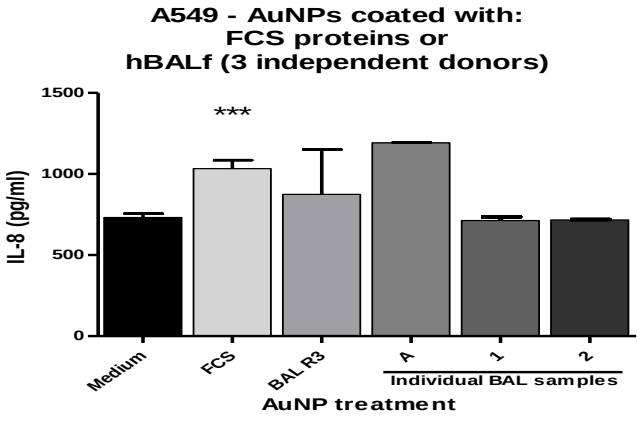

Figure 5: Preliminary results of studies on inflammatory effect of BAL and CFS coated Au NPs (PLUS)

In case study WP6.3 (lead by EAWAG), three methodologies are explored: (1) particle measuring devices to characterize the properties and fate of selected ENPs in various natural and standardized test media in relation to dispersion, agglomeration, aggregation and solubility under realistic conditions (GAT); (2) a novel *in vitro*, multi-compartment fish epithelial barrier model employed to determine bioavailability, persistence and toxicity of ENMs to fish (EAWAG); and (3) an *in vivo* isopod-based assay used to assess the uptake of ENM via food in a representative terrestrial organism (UNILJ). Performance of these methods will assessed and validated by testing the behavior and impact of well characterized NP under environmentally realistic conditions.

In case of accidents, exposure to NPs may be limited by body barriers and uptake ability, since the amount of NPs can be in principle indefinitely high. Case study 6.4, which is led by UNIZAR, considers two accident scenarios: 1) An industrial accident leading to an explosion or massive release of an NP-containing cloud within a processing plant, and 2) a transport accident leading to a massive spill of NPs on a public waterway (river or lake). The release of NPs in accidents and their time/space distribution is at present modelled by a subcontractor and results will be provided on accidents involving massive release of NPs based both on modelling and experimental data.

The description of a specific scenario for a potential industrial site accident should allow the development of a hazard and operability (HAZOP) study implemented to identify safety problems at an industrial site and to improve its operability. Several companies have been contacted by UNIZAR to support the study but no real commitment could be obtained for a HAZOP-type study. For this reason, a transport accident scenario has been selected as the data needed seem to be more accessible and because of the lack of information on regulation of transport of ENMs within the ADR (European Agreement concerning the International Carriage of Dangerous Goods by Road); the inadequacy or incompleteness of MSDS for ENMs; and because an analysis of the storage container for ENMs is needed. One of the largest chemical-processing companies in Slovenia, CINKARNA Celje, already working with NanoValid on LCA (see WP4), was again prepared to cooperate and to support the planned transport accident study. Simulation experiments on the formation of NP aerosol plumes that may occur after transportation accidents are under development. Experimental data on the aerosol generation using several materials will provide support to the diffusion models proposed. Also, an active collaboration with MARINA partners IOM, IUTA and RIVM has been started on (i) exposure assessment and (ii) accidental release of ENMS. For this UNIZAR has hosted a joint meeting between NANOVALID and MARINA in July 2012, on accidental risk management and the planned case studies.

The automotive case study (WP6.6) carried out by CRF-FIAT concentrates on nanocomposites, where NPs are embedded within polymeric matrices in order to give them new functionalities for improving the performances for the automotive applications. The case study will be useful in order to evaluate the NPs (e.g. ferrite NPs) environmental impact during the life cycle of the vehicle. The two case study materials are:

- new modified e-coating for improving corrosion resistance of metallic automotive components.
- new modified adhesives activatable by radiofrequency (RF) fields with reversibility properties.

To modify the traditional systems by NPs to supply new functionalities and improvements of performances to the final applications, experimental work has been performed both for coating and adhesive applications. For the coating application, a detailed selection of potential NPs has been done; further specific indications of the parameters to take into account for the final

choice of NPs have been elaborated and dispersion tests of NPs within the e-coating performed for the optimization of the final product. For the adhesive application, different typologies of characterization were carried out on unaltered adhesive and on NPs in order to define the main process parameters for innovative adhesive manufacturing. Procedures to ensure safe use of reagents, chemicals and NPs have been defined. Finally, installation of NP counters, and preliminary set of tests, in particular NPs detections for the adhesive case study, have need performed. An illustration of the setup and an example of data obtained is shown in Figure 6.

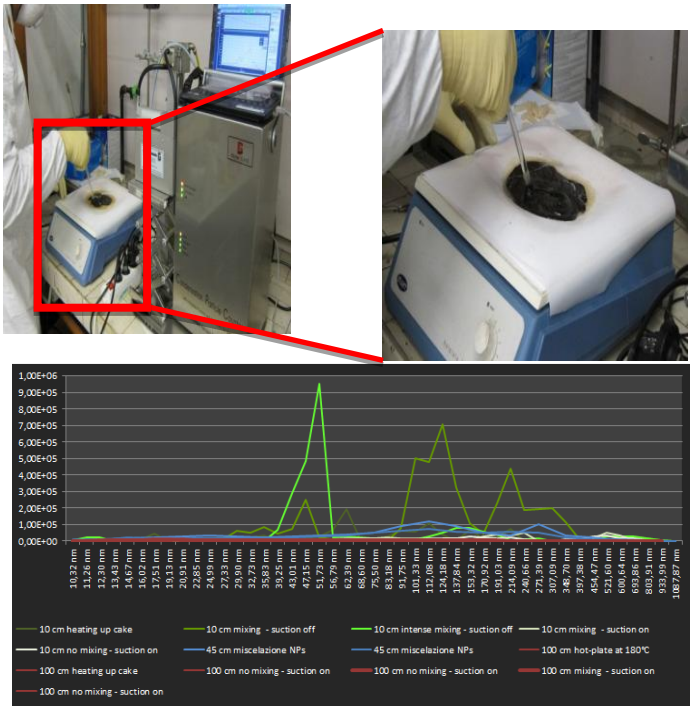

Fig. 6 : NPs detection test from the automotive case study for the adhesive application (CRF-FIAT)

6 Expected impact

NanoValid will generate validated methods used for hazard and exposure testing, dispersion control, labelling and pc characterization of engineered nanomaterials. This will be achieved by comprehensive inter-lab comparison campaigns between all participating laboratories and by implementing a set of case studies. This will be a first step in the generation of reliable quantitative data on the pc properties, toxicology and eco-toxicology of nanomaterials and in the development of accurate methods required to generate accurate data. The validated methods and new knowledge developed will help to identify, manage and reduce possible health, safety and environmental risks associated with nanomaterials and associated products to meet existing regulatory needs and develop new legal requirements for safe, responsible and sustainable development.

New knowledge will be generated on the toxicity of ENMs to particle-ingesting organisms and to those not internalizing NPs, and on mechanisms that control particle solubility and bioavailability, which will help to improve the applicability of current RA and LCA systems to NPs. The detection and evaluation of a wide range of different ENMs with different properties and under various laboratory and field conditions will enhance and extend our current understanding of the true nature of the solution, dispersion and agglomeration/aggregation behaviour, persistence and fate of ENMs. NanoValid is developing validated procedures for labelling and dispersion control of nanoparticles prior to toxicological testing, which will allow accurate tracing and the conversion of test materials that have been developed and fabricated into standardized stable nanoparticle aerosols and suspensions.

As a major outcome, the project will provide a set of reliable (pre-standardization) protocols and reference methods that are applicable to a wide range of NPs for early hazard identification, risk and life cycle assessment and the design of safe materials. NanoValid is generating a comprehensive knowledge database to indentify key intrinsic properties of relevant nanomaterials and so help to better understand their behaviour in various test media, physiological solutions and environmental matrices. The generation of scientifically sound and well-defined reference tools and materials will support standardization and regulatory efforts to assess potential effects of ENM, which in turn will improve current test schemes for risk management, reduction and monitoring. Providing reliable methods including new *in vitro* cell panels and other models to assess bioaccumulation, persistence and bioavailability will help to early identify problematic materials and stimulate the development of safe production processes, properties and products („green nanotechnology“). Results from NP testing will contribute to new conceptual and experimental standards for toxicity testing by *in vitro* test systems, which in turn will help to reduce animal testing.

The development of reference methods and materials will support pre and co-normative activities, such as those required for the implementation of REACH and other relevant EU legislation. It will assist current policy and future decision making, to meet increasing regulative requirements in nanotechnology and the need of relevant stakeholders, such as public authorities, industry, researchers and citizens. Finally, a more reliable measurement, characterization and toxicological assessment of ENMs will support good governance in research and industry and contribute to the future definition of appropriate measures and guidelines in line with the precautionary principle.

7 Dissemination and exploitation strategy

So far, NanoValid has implemented a specific training workshop at the University of Zaragoza (UNIZAR), organized by the Institute of Nanoscience of Aragon (INA), on 16-20 September 2013 on “Advanced Characterization of Nanomaterials” and a summer school will take place in Tallinn, Estonia, organized by the National Institute of Chemical Physics and Biophysics (NICPB) on 16-17 June 2014 on “Synthesis, characterization and aquatic ecotoxicity testing of nanoparticles.” Both events are specifically dedicated to students and young researchers. The project has issued so far 7 newsletters and numerous publications including several peer-reviewed scientific papers have been produced.

As a major outcome of the generation of validated methods, NanoValid develops a series of new collecting, characterizing, and

testing methodologies, some of them having clear commercialization opportunities. Through the participation of industrial end-users that have an established background in instrument commercialization, and of partners with a focus on regulatory nased research, the project will lead to:

- ✓ new standardized test materials (e.g. CRM) that can be used by different industries to validate the physicochemical properties of ENMs they manufacture or purchase;
- ✓ novel ENP samplers, e.g. to collect hot gases;
- ✓ real-time collection and assessment devices that collect airborne ENPs and assay them on an integrated biomodule, thus integrating both particle bioactivity and transport, and so avoiding any loss or change of reactivity or physical properties;
- ✓ a multi-compartment fish cell barrier model to mimic physiological systems and provide a more realistic evaluation of ENP fate and effect in living organisms;
- ✓ novel environmental models to determine the fate and effect of ENMs to post consumer, that can be employed by different manufacturers to comply with new regulations;
- ✓ guidance and training manual for the safe handling of nanomaterials at work places.

A dedicated project website (www.nanovalid.eu) is continuously informing on latest project news and developments, as well as announcements and output of dissemination events, training workshops and summer schools. NanoValid dissemination activities directly target various stakeholder groups through a regular newsletter also hosted on the website. NanoValid has also edited and produced the first two NanoSafety Cluster Newsletters, registered almost 300 newsletter subscribers, and established an NSC LinkedIn group with 50 members signing up in its first week. The aim of these activities is to increase communication between project counterparts to enhance complementarity and raise awareness of project ouput. As a number of project partners are also involved in affiliated NanoSafety Cluster initiatives and projects, these links are exploited to extend the outreach possibilities within the RTD community and wider community of stakeholders. To ensure all relevant stakeholders are reached, the consortium partners employ the leverage of their own substantial networks and individual membership of international committees and associations. This takes advantage of the range represented in the consortium: academic organizations, industrial SMEs and large-scale manufacturers, standards organizations, material testing institutes, networks, associations, and consultancy firms. With regard to exploitation of results, NanoValid will assess the exploitable commercialization potential of developed diagnostic tools by means of an exploitation seminar, and will identify and propose new work items for method and material standardization by the active participation of NanoValid partner DIN (who is a member of the consortium) in relevant ISO/CEN and OECD technical committees.

8 Directory

Table 3: Directory of people involved in this project.

First Name	Last Name	Affiliation	Address	email
Miriam	Baron	Federal Institute for Occupational Safety and Health	Friedrich-Henkel-Weg 1-25, 44149 Dortmund, Germany	Baron.miriam[at]baua.bund.de
Andreas	Bhagwani	Nanologica AB	Drottning Kristinas Väg 45, 11428 Stockholm, Sweden	andreas@nanologica.com>
Irina	Blinova	National Institute of Chemical Physics and Biophysics (NICPB)	Akadeemia tee 23, Tallinn 12618, Estonia.	irina.blinova@kbfi.ee
Olesja	Bondarenko	National Institute of Chemical Physics and Biophysics (NICPB)	Akadeemia tee 23, Tallinn 12618, Estonia.	olesja.bondarenko@kbfi.ee
Steffi	Böhme	Helmholtz Centre for Environmental Research (UFZ)	Permoser Str. 15, 04318 Leipzig, Germany	Steffi.boehme@ufz.de
Fayçal	Boureima	QUANTIS	PSE-D, EPFL, 1015-Lausanne, Switzerland	fayçal.boureima@quantis-intl.com
Adina	Bragaru	National Institute for R&D in Microtechnologies	Erou Iancu Nicolae 126 A, 077190 Bucharest, Romania	adina.bragaru@imt.ro
Wibke	Busch	Helmholtz Centre for Environmental Research	Permoser Str. 15, 04318 Leipzig, Germany	Wibke.busch@ufz.de
Irene	Cannavaro	Centro Ricerche FIAT	Strada Torino 50 - 10043 Orbassano (TO) - Italy	irene.cannavaro@tirocinanti.crf.it
Andreas	Comouth	Grimm Aerosol Technology	Dorfstrasse 9, D-83404 Ainring Germany	ac@grimm-aerosol.com
Thierry	Decharneux	StratiCell	Crealys Science Park, 10 rue Jean Sonet 5032 ISNES, Belgium	tdecharneux@straticell.com
Mihai	Danila	National Institute for R&D in Microtechnologies	Erou Iancu Nicolae 126 A, 077190, Bucharest, Romania	Mihai.danila@imt.ro

Arnaud	Dauriat	QUANTIS	PSE-D, EPFL, 1015-Lausanne, Switzerland	arnaud.dauriat@quantis-intl.com
Alok	Dhawan	Institute of Life Sciences (before: Indian Institute for Toxicology Research)	Ahmedabad University, AES Bungalow no. 2, Commerce Six Roads, Navrangpura, Ahmedabad – 380009, Gujarat INDIA (before: P.O. BOX - 80, M.G. Marg, Lucknow - 226 001, India)	alok.dhawan@ahduni.edu.in dhawanalok@hotmail.com (before: alokdhawan@iitr.res.in)
Jean-Michel	Dogne	University of Namur (FUNDP)	Pharmacy Dpt., Rue de Bruxelles 61, B-5000 Namur, Belgium	jean-michel.dogne@fundp.ac.be
Sandra	Domingo	StratiCell	Crealys Science Park, 10 rue Jean Sonet 5032 ISNES, Belgium	sdomingo@straticell.com
Damjana	Drobne	Department of Biology, Biotechnical faculty, University of Ljubljana	Vecna pot 111, 1111 Ljubljana, SLOVENIA	damjana.drobne@bf.uni-lj.si
Albert	Duschl	University of Salzburg, Department of Molecular Biology	Hellbrunner Strasse 34, 5020 Salzburg, Austria	Albert.duschl@sbg.ac.at
Christophe	Falce	ARKEMA FRANCE	Direction Recherche Développement - 420 rue d'Estienne d'Orves 92705 COLOMBES CEDEX (France)	christophe.falce@arkema.com
Mark	Geppert	Eawag, Swiss Federal Institute of Aquatic Science and Technology	Überlandstrasse 133, 8600 Dübendorf, Switzerland	mark.geppert@eawag.ch
Jose Mauro	Granjeiro	Instituto Nacional de Metrologia, Normalização e Controle de Qualidade (INMETRTO)	Av. N.S. das Graças 50, Xerém, 25 250-020, Duque de Caxias, RJ, Brasil	jmgranjeiro@gmail.com
Maureen	Gwinn	US Environmental Protection Agency Effects Identification and Characterization Group, National Center for Environmental Assessment	1200 Pennsylvania Ave NW Mailcode 8623-P Washington, DC 20460	Gwinn.Maureen@epamail.epa.gov
Roberto	Hanoi	Nanologica AB	Drottning Kristinas väg 45, 11428 Stockholm	hanoi@nanologica.com
William	Hendershot	McGill University, Department of Natural Resource Sciences	McGill University Macdonald Campus, 21,111 Lakeshore Drive, Ste Anne de Bellevue, Quebec H9X 3V9, Canada	William.hendershot@mcgill.ca
Martin	Himly	University of Salzburg (PLUS) Department of Molecular Biology	Hellbrunner Strasse 34, 5020 Salzburg, Austria	Martin.Himly@sbg.ac.at
Vasile-Dan	Hodoroaba	Federal Institute for Materials Research and Testing (BAM)	Unter den Eichen 87, 12205 Berlin, Germany	dan.hodoroaba@bam.de
Silvia	Irusta	University of Zaragoza	Zaragoza, Spain	sirusta@unizar.es
Angela	Ivask	National Institute of Chemical Physics and Biophysics (NICPB)	Akadeemia tee 23, Tallinn 12618, Estonia	angela.ivask@kbfi.ee
Keld Alstrup	Jensen	National Research Centre for the Working Environment (NRCWE)	Lersø Parkallé 105, 2100 Copenhagen, Denmark	kaj@nrcwe.dk, kaj@arbejdsmiljoforskning.dk
Olivier	Jolliet	QUANTIS	PSE-D, EPFL, 1015 Lausanne, Switzerland	olivier.jolliet@quantis-intl.com
Anne	Kahru	National Institute of Chemical Physics and Biophysics (NICPB)	Akadeemia tee 23, Tallinn 12618, Estonia	anne.kahru@kbfi.ee
Henryk	Kalbe	Federal Institute for Materials Research and Testing (BAM)	Unter den Eichen 87, 12205 Berlin	henryk.kalbe@bam.de
Rune	Karlsson	NordMiljö AB [Environmental Assessments]	Duvenässätern, 68395 Sunnemo, Sweden	rune.karlsson@enas-online.com

Kaja	Kasemets	National Institute of Chemical Physics and Biophysics (NICPB)	Akadeemia tee 23, Tallinn 12618, Estonia.	kaja.kasemets@kbfi.ee
Lothar	Keck	Grimm Aerosol Technology	Dorfstrasse 9, 83404 Ainring, Germany	lk@grimm-aerosol.com
Ismo K.	Koponen	National Research Centre for the Working Environment (NRCWE)	Lersø Parkallé 105, 2100 Copenhagen, Denmark	ikk@nrcwe.dk
Anna	Kouninna	QUANTIS	PSE-D, EPFL, 1015 Lausanne, Switzerland	anna.kounina@quantis-intl.com
Dana	Kühnel	Helmholtz Centre for Environmental Research (UFZ)	Permoserstrasse 15, 04318 Leipzig, Germany	Dana.kuehnel@ufz.de
Mihaela	Kusko	National Institute for R&D in Microtechnologies (IMT)	Erou Iancu Nicolae 126 A 077190, Bucharest, Romania	mihaela.kusko@imt.ro
M. Pilar	Lobera	University of Zaragoza	Zaragoza, Spain	plobera@unizar.es
Stephane	Lucas	University of Namur (FUNDP)	NARILIS, Rue de Bruxelles 61, B-5000 Namur, Belgium	stephane.lucas@fundp.ac.be
Laura	Manodori	Veneto Nanotech	Via San Crispino 106 - 35129 Padova - Italy	laura.manodori@ecsin.eu
Idris	Mohd	Centre for Chemical and Molecular Biology (CSIR)	Hyderabad, India	idris@ccmb.res.in
Brunetto	Martorana	Centro Ricerche FIAT	Strata Torino 50, 10043 Orbassano, Italy	Brunetto.martorana@crf.it
Heather	McShane	McGill University, Department of Natural Resource Sciences	McGill University Macdonald Campus, 21,111 Lakeshore Drive, Ste Anne de Bellevue, Quebec H9X 3V9, Canada	Heather.mcshane@mail.mcgill.ca
Tobias	Meissner	Fraunhofer Institute for Ceramic Technologies and Systems (IKTS)	Winterbergstrasse 28, 01277 Dresden, Germany	Tobias.meissner@ikts.fraunhofer.de
Superb	Misra	University of Birmingham School of Geography Earth & Environmental Sciences (GEES)	Edgbaston Birmingham B15 2TT UK"	s.misra@nhm.ac.uk; s.misra@bham.ac.uk
Mark	Morrison	Institute of Nanotechnology	Strathclyde University Incubator, Graham Hills Building, 50 Richmond Street, Glasgow G1 1XP	Mark.morrison@nano.org.uk
Monika	Mortimer	National Institute of Chemical Physics and Biophysics (NICPB)	Akadeemia tee 23, Tallinn 12618, Estonia.	monika.mortimer@kbfi.ee
Stefano Pozzi	Mucelli	Veneto Nanotech	Via San Crispino 106, 35129 Padova, Italy	Stefano.pozzimucelli@venetonanotech.it
Werner	Oesterle	BAM Federal Institute for Materials Research and Testing	Unter den Eichen 87, 12205 Berlin Germany	werner.oesterle@bam.de
Iolanda	Olivato	Veneto Nanotech	Via San Crispino 106, 35129 Padova, Italy	iolanda.olivato@venetonanotech.it
Rolf	Packroff	Federal Institute for Occupational Safety and Health (BAUA)	Freidrich-Henkel-Weg 1-25, 44149 Dortmund, Germany	packgroff[at]baua.bund.de
Markus	Pesch	Grimm Aerosol Technology	Dorfstrasse 9, 83404 Ainring, Germany	mp@grimm-aerosol.com
Caroline	Peyrot	Université de Montréal	C.P. 6128 Succ. Centre-Ville Montreal, QC H3C3J7	Caroline.Peyrot@umontreal.ca
Marcos Assuncao	Pimenta	National Institute of Science and Technology on Nanomaterials and Carbon, Universidade Federal de Minas Gerais	Avenida Antonio Carlos 6627, Belo Horizonte, MG, Brazil	mpimenta@fisica.ufmg.br
Jean-Pascal	Piret	University of Namur (FUNDP)	URBC, Rue de Bruxelles 61, B-5000 Namur, Belgium	jppiret@fundp.ac.be
Annegret	Potthoff	Fraunhofer Institute for Ceramic Technology and Systems (IKTS)	Winterbergstrasse 28, 01277 Dresden, Germany	annegret.potthoff@ikts.fraunhofer.de
Ilmari	Pyykkö	Hearing and Balance Research Unit Field of Oto-rhino-laryngology School of Medicine, University of Tampere	Medisiinarinkatu 3, Room C2165a, 33520 Tampare, Finland	ilmari.pyykko@uta.fi

Steffi	Rades	Federal Institute for Materials Research and Testing (BAM)	Unter den Eichen 87, 12205 Berlin, Germany	Steffi.rades@bam.de
Niklas	Raun-Jacobsen	National Research Centre for the Working Environment (NRCWE)	Lersø Parkallé 105, 2100 Copenhagen, Denmark	nrj@arbejdsmiljoforskning.dk
Rudolf	Reuther	NordMiljö AB [Environmental Assessments]	Duvenässätern, 68395 Sunnemo, Sweden	Rudolf.reuther@enas-online.com
David	Rickerby	European Commission Joint Research Centre Institute for Environment and Sustainability	21027 Ispra, Italy	david.rickerby@jrc.ec.europa.eu
Roman	Ruettinger	Deutsches Institut für Normung (DIN)	Burggrafenstrasse 6, 10787 Berlin, Germany	roman.ruettinger@din.de
Davide	Roncato	Centro Ricerche FIAT	Strada Torino 50 - 10043 Orbassano (TO) - Italy	davide.roncato@crf.it
Michel	Salmon	StratiCell	Crealys Science Park, 10 rue Jean Sonet 5032 ISNES, Belgium	msalmon@straticell.com
Jesus	Santamaria	University of Zaragoza	Zaragoza, Spain	Jesus.santamaria@unizar.es
Christelle	Saout	University of Namur (FUNDP)	URBC, Rue de Bruxelles 61, 5000 Namur, Belgium	christelle.saout@fundp.ac.be
Kristin	Schirmer	Eawag, Swiss Federal Institute of Aquatic Science and Technology	Überlandstrasse 133, 8600 Dübendorf, Switzerland	kristin.schirmer@eawag.ch
Michael	Schmitt	Deutsches Institut für Normung (DIN)	Burggrafenstrasse 6, 10787 Berlin, Germany	Michael.schmitt@din.de
Volker	Seibicke	Deutsches Institut für Normung (DIN)	Burggrafenstrasse 6, 10787 Berlin, Germany	Volker.seibicke@din.de
Rishi	Shanker	Indian Institute of Toxicology Research (CSIR)	P.O. BOX - 80, M.G. Marg, Lucknow, India	rishi@iitr.res.in
Laura	Sigg	Eawag, Swiss Federal Institute of Aquatic Science and Technology	Überlandstrasse 133, 8600 Dübendorf, Switzerland	Laura.Sigg@eawag.ch
Monica	Simion	National Institute for R&D in Microtechnologies	Erou Iancu Nicolae 126 A, 077190 Bucharest, Romania	Monica.simion@imt.ro
Shashi	Singh	Centre for Chemical and Molecular Biology (CSIR)	Hyderabad, India	shashis@ccmb.res.in
Mariliis	Sithmae	National Institute of Chemical Physics and Biophysics (NICPB)	Akadeemia tee 23, Tallinn 12618, Estonia.	mariliis.sihtmae@kbfi.ee
Andreas	Skouloudis	European Commission Joint Research Centre Institute for Environment and Sustainability	21027 Ispra, Italy	Andreas.skouloudis@jrc.ec.europa.eu
Caroline	Smal	StratiCELL SA	Crealys Science Park, 10 rue Jean Sonet 5032 ISNES, Belgium	csmal@straticell.com
Geoffrey	Sunahara	Life Sciences, NRC Aquatic and Crop Development Resource Portfolio, National Research Council Canada	6100 Royalmount Ave. , Montreal, Quebec H4P 2R2, Canada	Geoffrey.sunahara@c nrc-nrc.gc.ca
Mauro Martins	Teixeira	Department of Biochemistry and Immunology, Institute of Biological Sciences, Federal University of Minas Gerais	Av. Presidente Antônio Carlos 6627 Belo Horizonte, MG, Brazil	mmtex.ufmg@gmail.com
Lesley	Tobin	Institute of Nanotechnology	Strathclyde University Incubator, Graham Hills Building, 50 Richmond Street, Glasgow G1 1XP	Lesley.tobin@nano.org.uk
Olivier	Toussaint	University of Namur (FUNDP)	URBC, Rue de Bruxelles 61, 5000 Namur, Belgium	olivier.toussaint@fundp.ac.be
Nicola	Trevisan	Veneto Nanotech	Via San Crispino 106, 35129 Padova, Italy	Nicola.trevisan@venetonanotech.it

Teresa	Trott	Grimm Aerosol Technik GmbH & Co. KG	Dorfstrasse 9, D-83404 Ainring, Germany	tt@grimm
Nathalie	Tufenkji	McGill University, Department of Chemical Engineering	3610 University St, Montreal, Quebec H3A 2B2, Canada	nathalie.tufenkji@mcgill.ca
Wolfgang	Unger	Federal Institute for Materials Research and Testing (BAM)	Unter den Eichen 87, 12205 Berlin, Germany	Wolfgang.unger@bam.de
Janez	Valant	University of Lublijana, Department of Biology, Biotechnical Faculty	Vecna pot 111, 1111 Lublijana, Slovenia	Janez.valant@bf.uni-lj.si
Eva	Valsami-Jones	University of Birmingham School of Geography Earth & Environmental Sciences (GEES)	Edgbaston Birmingham B15 2TT UK"	e.valsamijones@bham.ac.uk
Håkan	Wallin	National Research Centre for the Working Environment (NRCWE)	Lersø Parkallé 105, 2100 Copenhagen, Denmark	hwa@nrcwe.dk
Joann	Whalen	McGill University, Department of Natural Resource Sciences	McGill University Macdonald Campus, 21,111 Lakeshore Drive, Ste Anne de Bellevue, Quebec H9X 3V9, Canada	Joann.whalen@mcgill.ca
Kevin	Wilkinson	Université de Montréal	C.P. 6128 Succ. Centre-Ville Montreal, QC H3C3J	Kj.wilkinson@umontreal.ca
Jing	Zou	Hearing and Balance Research Unit Field of Oto-rhino-laryngology School of Medicine, University of Tampere	Medisiinarinkatu 3, Room C2165a, 33520 Tampare, Finland	Jing.Zou@uta.fi

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nanOxiMet

Oxidant generating capacity as a **met**ric to allow grouping of nanomaterials and prediction of human health effects

Contract Agreement: via SIINN ERA-NET Website: http://www.nanoximet.eu
Coordinator: Dr. T. Kuhlbusch, Institute of Energy and Environmental Technology (IUTA) e.V., Germany

Table 1 Consortium List.

No.	Beneficiary name	Short name	Country
1	Institute of Energy and Environmental Technology e.V.	IUTA	Germany
2	Leibniz Research Institute for Environmental Medicine	IUF	Germany
3	University Paris Diderot, Unité de Biologie Fonctionnelle et Adaptative	UPD-BFA	France

Contents

1 Summary

Project Duration: 01.06.2013 – 31.05.2016

Project Funding: 818.000 €

Several recent studies show that a determination of the pathogenic characteristics of manufactured nanomaterials (MNM) per se is not feasible and in consequence a comprehensive knowledge of the material itself and its (inter-)reaction with the environment is always needed. Studies also indicate that the surface area MNMs and their ability to generate oxidants are promising predictive metrics in nanotoxicology. The particle surface area is assumed to be a relevant metric to describe the particle environment interaction in general. The ability of the particle to generate oxidants is seen as an indicator of how reactive the particle surface area is. The major aim of this project is to test the hypothesis that the surface area and the oxidative potential of particles are suitable estimation metrics for toxicological effects of MNM. Therefore, general physical MNM properties are investigated, combined and linked with toxicological outcomes in MNM exposed cell culture models.

Consequently, as starting point selected MNM suspensions are characterised e.g. for the size, zeta potential, morphology, oxidant generation potency (e.g. including reactive oxygen species - ROS) and surface area in different liquids. It has to be noted that different pathways of oxidant generation and particle reactivity exist and hence a variety of measurement methods have been introduced. In view of possible standardisation, three different detection methods (electron paramagnetic resonance spectroscopy, fluorescence spectroscopy using redox sensitive probes, antioxidant depletion) will be compared. As it is still a challenge to determine the surface area of MNM in suspension also different surface area detecting and calculating approaches will be compared using scanning electron microscopy, dynamic light scattering, spray characterisation and nuclear magnetic resonance spectroscopy. The investigations are planned for twelve different materials representing different types of MNM and hence allow the investigation of possible grouping. The list of the materials contains (noble) metals, metal oxides and carbonaceous materials. Two of these materials will be applied in two primary particle sizes (both < 100 nm primary particle size) to test if surface activity per surface area and toxicological outcomes differ. All these investigations will allow setting up a data base with well characterised materials ready for correlation studies and detailed analyses.

Parallel to the characterisation approach, toxicological in vitro studies on oxidative stress and different stages of oxidative cellular responses (induction of antioxidants, inflammatory mediators and cellular toxicity) are performed in a number of relevant types of lung cells (i.e. bronchial- and alveolar epithelial cell lines, two macrophage- cell lines and primary cells) in a dose and time dependent manner. The induction of oxidative stress includes the evaluation of redox state changes by the detection of specific ROS using several techniques and antioxidant depletion as well the oxidative damages to relevant macromolecules (DNA, proteins and lipids). In addition, the induction of oxidative cellular

responses by activation of specific signalling pathways according to the level of oxidative stress will be investigated.

A systematic sensitivity investigation of two target cell types to the different MNM will be performed with a broad range of reactivity analyses by using a large panel of biological endpoints ranging from the initial intracellular ROS production to oxidative damages going through the hierarchical oxidative stress response.
Finally, all results will be summarised and evaluated in view of potential MNM induced adverse health effects and a possible grouping of nanomaterials. Beside the straight forward statistical approach, an evaluation based on reactivity per surface area will be used to link "particle reactivity" with toxicological outcomes.

The results will be made publicly available and actively distributed among national and international stakeholders including OECD, nanoSafetyCluster, national and international FP7 projects.

2 Background

For risk assessment of MNM, as well as for worker and consumer safety, it is of major interest to have validated assays and metrics allowing discrimination of different degrees of toxic potencies of MNMs. So far, no unifying particle characteristic or screening tool has been identified. This confirms the currently still poorly understood complexity of cellular and molecular events at the MNM-biological interface level, leading or not to toxicity. Several types of MNM have been demonstrated to cause adverse effects to cells, tissues, organs and organisms when compared to their larger pendants. Such toxic effects have been associated with various different intrinsic physical-chemical characteristics of MNM, which include size, specific surface area, and surface reactivity (Oberdörster et al., 2005; Borm et al., 2006).

The determination of some of these characteristics in liquids is still a challenge due to several limitations of preparation and detection techniques. Common techniques applied to determine particle size distributions in liquid are e.g. dynamic light scattering (DLS), tracking nanosight technology, field-flow fractionation or spray characterisation. All of the instruments have advantages and disadvantages in view of particle size distribution measurements and subsequent calculation of surface area concentrations (e.g. extreme time consuming method development, low needed concentrations or use of appropriate algorithm and/or mode). Being aware of these limitations a combination of DLS, spray characterisation and scanning electron microscopy analysis will be employed and compared. A direct surface area detection method is not established, but a new instrument based on nuclear magnetic resonance spectroscopy, is now available for this project, which is suggested to detect the surface area of particles directly in liquids. This instrument will also be tested for its applicability for MNM suspensions and the results compared to those obtained by the other methods. The zeta potential will be determined together with the particle size by an electrophoresis based approach in a Zetasizer.

From decades of inhalation toxicology research it has been emerged that the formation of ROS and the associated induction of oxidative stress may represent a key pathway in the causation of adverse health effects of ambient micro- and nanosize particles (Xia et al., 2006, Borm et al., 2007, Ayres et al., 2008). Hence, the oxidant generating capacity of MNM has been forwarded as novel metric to predict their hazard (Borm et al., 2007, Ayres et al., 2008). The potency of MNM to generate oxidants will be tested and intercompared using the redox sensitive probe 2',7'-dichlorofluorescin (DCFH) (Foucaud et al. 2007), oxidation of antioxidants in artificial lung lining fluid (Mudway et al. 2004) and electron paramagnetic resonance (EPR) spectroscopy with oxidant-trapping agents (Shi et al., 2003; Borm et al., 2007; Papageorgiou et al., 2007). An inter-comparison of these methods detecting the so-called "oxidant generation capacity" is crucial to enable the assessment of which of the different reactive mechanisms and species are more health relevant and are the most predictive to toxic effects, but has not been conducted so far. This is urgently needed, since both particle reactivity and the potential to form reactive oxygen species have been identified as relevant physical-chemical endpoints by the OECD working Party on MNMs.

Apart from the intrinsic (surface area related) ROS generation of MNM, it is also possible to evaluate the generation of ROS and associated redox changes in MNM exposed target cells (Ayres et al., 2008) using, beside others, similar methods as used in the cell free approaches (Karlsson et al., 2006, Singh et al., 2007). The intrinsic oxidant generating capacity of MNM and/or the ability of the material to generate ROS upon interaction with a target cell can cause potential changes in the intracellular redox status and hence possibly lead to oxidative stress.

The concept of oxidative stress describes the imbalance between oxidants and antioxidants, which might result in cell or tissue damage (Sies, 1997). According to the extent of the oxidative stress, cellular responses vary from adaptation to death. In relation to this, for MNM exposed cells a model of biological response has been proposed that defines oxidative stress in a hierarchical manner with involvement of specific cell signalling pathways and responses at different tiers (Li et al., 2003; Ayres et al., 2008). A low ROS production triggers anti-oxidant (heme-oxygenase) and xenobiotic metabolizing (glutathion-S-transferase, NADPH quinone-oxidoreductase) enzyme synthesis resulting from the activation of the Nrf-2 transcription factor. In condition of redox homeostasis Nrf-2 is protein-binded in the cytosol. Upon ROS insult, Nrf-2 becomes mobile, can translocate to the nucleus and bind to genes expressing the anti-oxidant responsive element (ARE) in their regulating sequences. If the protection afforded during this step is insufficient, an inflammatory response occurs, characterised by the activation and secretion of pro-inflammatory cytokines and related mediators. Similarly to the previous step, it results from the activation of signalling pathways, such as MAPK pathways, leading to the activation of the transcription factors NF-kB and AP-1. Finally, if ROS reach an even higher level, cell death may happen by apoptosis or necrosis (Xia et al., 2008; Ayres et al., 2008). Thus, each of these three tiers of oxidative stress response are characterised by specific cellular changes that can be measured in MNM toxicity screening assays. In this project, a detailed characterisation of changes in each of the tiers of oxidative stress responses will be evaluated after MNM treatment of human cells (epithelial and macrophage type cell lines and primary cells) in a detailed dose- and time dependent manner. Under the same conditions, the cells will also be evaluated for oxidant related intracellular redox changes, as well as associated oxidative attack effects on the cellular proteins, the DNA and lipids.

Taken together, this will enable a concise evaluation of the level of oxidant related cellular changes (ranging from mild and reversible

to irreversible/toxic) in relation to the applied surface area dose and calculated surface area related oxidant generating capacity.

3 Scientific and technological challenges

For the assessment of possible risks associated with MNM, and their use in different products, it is essential to correctly understand and predict a possible hazard by a flawless discrimination between MNM with lower and higher hazardous potency. So far, no unifying particle characteristic or screening tool has been identified that can be used for such purpose in a straightforward manner. Both the acellular and the cell based oxidant detection assays have been considered to be promising tools for such hazard identification and potential grouping of MNM. However, recent studies demonstrated that specific particles can induce oxidative stress responses in the absence of any intrinsic oxidant capacity (e.g. Hussain et al., 2010; Gerloff et al., 2012). On the other hand, specific MNM may also display considerable intrinsic oxidative properties which, depending on dosimetry issues and cellular antioxidant defence mechanisms, may not necessarily lead to adverse oxidative stress responses (Unfried et al., 2007; Ayres et al., 2008). As such, these observations reflect the fundamental problems on the sensitivity and specificity of the oxidant generation metric with regard to its usefulness in hazard ranking and categorisation of MNM. Still, no comprehensive study evaluating particle bound reactivities by a single or a concise set of different techniques have been conducted so far. Especially this bridging of different reactivity measures, including a detailed physical-chemical description of particle surface area concentrations and distribution, with different cell toxicity tests will allow the development and establishment for such links and extrapolation to possible human health effects.

Another current problem is the determination of particle size distributions and surface area concentrations of nanomaterials in liquids. No easy-to-use and accessible method exists. Two techniques, one based on NMR measurements in water and another so-called spray characterisation (detection after aerosolisation) will be investigated and their usefulness evaluated. Results of these measurements and those obtained based on DLS size distribution measurements will allow for a first inter-comparison and assessment of the accuracy of the data.

By combining the information on MNM surface area concentration and particle reactivity, specific particle reactivity per surface area can be calculated. Moreover it can be tested in view of linking the dose-response relationship between different MNM if a correct model can be developed.

4 Objectives

The main objective of this project is to prove the hypothesis that the *nanomaterial reactivity, determined by one or a combination of different measurement methods, can be linked to in vitro cell toxicity test outcomes and hence can be used as a valid tool for the detection of a possible hazard of such materials*. Additionally, the toxicity test results may allow prediction of possible human health effects.

Interim goals during the project to reach the main objective are:

- Unified preparation, surface area, zeta potential and size distribution characterisation and comparability of MNM suspensions in deionised H2O and cell (like) media,

- Production of a nanomaterial in two different sizes (Aluminium-oxide or Silver with pirmary particle diameter in a nano-range)

- Assessment of the use of particle size distribution measurements after aerosolisation (spray characterisation) to determine surface area concentrations,

- Comparison and evaluation of different oxidant generation capacity detection methods - intrinsic as well as on cellular level,

- Evaluation of intracellular ROS production, oxidative stress and damage markers to determine the level of oxidative stress,

- Evaluation of ROS related responses to define the role of oxidative stress in different tiers of oxidative stress responses,

- Establishment of dose–effect relationship for the different MNM for different toxicological endpoints,

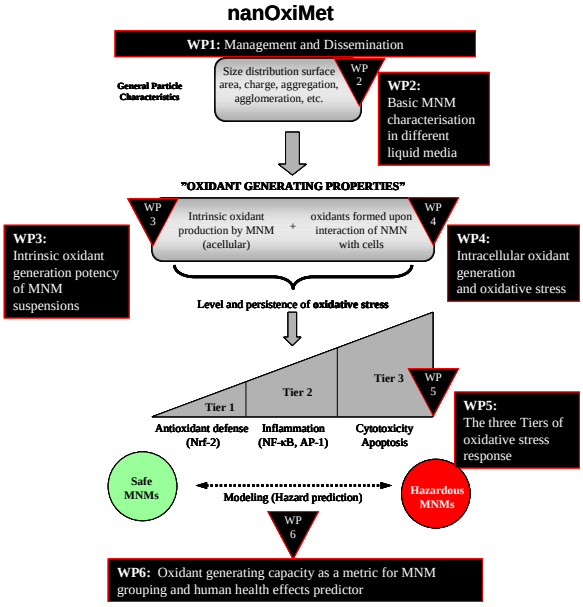
- Evaluation of MNM intrinsic ROS as an alternative, novel metric to assess their potential hazard,

- Generation of a ROS-based MNM data base to allow grouping of nanomaterials according to the hazard potential,

- Production of a guidance manual on how the relevant data are measured or obtained and how the data can be interpreted for practical purposes for different users.

5 Organisation

The project is in total divided into six work packages (WP) as illustrated in



. WP1 deals with the management of the project and its dissemination activities. In WP2 the MNM characterisation in liquids will be performed. In WP3 and WP4 the intrinsic, acellular and cellular oxidant generation potency / oxidative stress are detected, respectively. WP5 will go into detail to the level of oxidative stress applying the three tiers of oxidative stress response approach. Finally, in WP6all data will be collated, processed and interpreted for a possible grouping and prediction of health effects of MNM based on oxidant generation capacity.

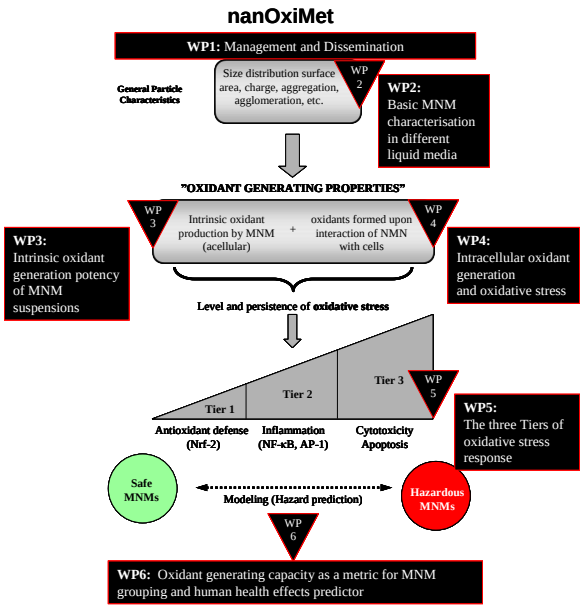


Figure 1 : Organizational structure of the nanOxiMet project

6 Expected Impact

The potential toxicity of nanomaterials is discussed and tested with a set of 59 endpoints of which 17 are related to the physical-chemical characterization and 9 to mammalian toxicology (OECD WPMN 2010). This huge list of testing endpoints is more or less mandatory to assess the potential risk of new nanomaterials.

With the outcomes of this project, a significant contribution to the development of such a consolidated framework addressing nano-related risks is anticipated. Furthermore, with the possibility of nanomaterial grouping and studying particle size influences information on potential adverse health effects can be given early and alternative materials or different structures can be proposed that minimize risks for humans and the environment.

Distributed to the stakeholders, the information will also significantly enhance the knowledge with regard to hazard and risk assessment. It will fill some of the current knowledge gaps and, hence, allow the development of sustainable and safe nano-technology.

Furthermore, the development towards fewer endpoints to be tested will lead to significant cost reductions during the test phase of new nanomaterials and new products and will be a guide on how to avoid or minimise risks for workers and consumers.

The development and implementation of the expected outcomes and results of this project will also strengthen the position of nanotechnology in the EU, since it allows the implementation of a relatively early warning signal of potentially hazardous new materials.

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8 Directory

Table 1 Directory of people involved in this project.

First Name	Last Name	Affiliation	Address	e-mail
Thomas	Kuhlbusch	IUTA	Bliersheimer Str. 58-60, 47229 Duisburg, Germany	tky@iuta.de
Carmen	Nickel	IUTA	Bliersheimer Str. 58-60, 47229 Duisburg, Germany	nickel@iuta.de
Heinz	Kaminski	IUTA	Bliersheimer Str. 58-60, 47229 Duisburg, Germany	kaminski@iuta.de
Bryan	Hellack	IUTA	Bliersheimer Str. 58-60, 47229 Duisburg, Germany	hellack@iuta.de
Roel	Schins	IUF	Auf´mHennekamp 50, 40225 Duesseldorf, Germany	roel.schins@iuf-duesseldorf.de
Catrin	Albrecht	IUF	Auf´mHennekamp 50, 40225 Duesseldorf, Germany	catrin.albrecht@iuf-duesseldorf.de
Andrea	Neumeyer	IUF	Auf´mHennekamp 50, 40225 Duesseldorf, Germany	andrea.neumeyer@iuf-duesseldorf.de
Gabriele	Wick	IUF	Auf´mHennekamp 50, 40225 Duesseldorf, Germany	gabriele.wick@iuf-duesseldorf.de
Christel	Weishaupt	IUF	Auf´mHennekamp 50, 40225 Duesseldorf, Germany	christel.weishaupt@iuf-duesseldorf.de
Armelle	Baeza-Squiban	UPD-BFA	case 7073, 5 rue Thomas Mann 75 205 Paris cedex 13 France	baeza@univ-paris-diderot.fr
Sonja	Boland	UPD-BFA	case 7073, 5 rue Thomas Mann 75 205 Paris cedex 13 France	boland@univ-paris-diderot.fr
Leticia	Aragao-Santiago	UPD-BFA	case 7073, 5 rue Thomas Mann 75 205 Paris cedex 13 France	leticia.aragao-santiago@univ-paris-diderot.fr
Karine	Andreau	UPD-BFA	case 7073, 5 rue Thomas Mann 75 205 Paris cedex 13 France	karine.andreau@univ-paris-diderot.fr
Julien	Dairou	UPD-BFA	case 7073, 5 rue Thomas Mann 75 205 Paris cedex 13 France	julien.dairou@univ-paris-diderot.fr
Linh	Chi Bui	UPD-BFA	case 7073, 5 rue Thomas Mann 75 205 Paris cedex 13 France	linh-chi.bui@univ-paris-diderot.fr

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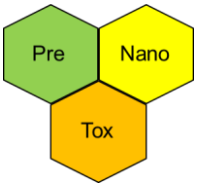
PreNanoTox

Predictive toxicology of engineered nanoparticles



Contract Agreement: NMP.2012.1.3-2- 309666 Website: http://www.prenanotox.eu
Coordinator: Rafi Korenstein, Dept. of Physiology and Pharmacology, Faculty of Medicine, Tel-Aviv University, Tel-Aviv, Israel

Table 1 Consortium List.

No.	Beneficiary name	Short name	Country
1	TEL-AVIV UNIVERSITY	TAU	Israel
2	ISTITUTO DI RICERCHE FARMACOLOGICHE MARIO NEGRI	IRFMN	Italy
3	FORSCHUNGSZENTRUM JUELICH GMBH	JUELICH	Germany

Contents

1 Summary

Project Duration: 36 months

Project Funding: EC

The production of many consumer products based on manufactured nanoparticles (NPs) has led to growing public and regulatory concern about the safety of nanomaterials. Since experimental toxicological testing of NPs is costly and time-consuming, it is necessary to develop a new research field based on knowledge, methods and tools to reach the goal of predictive nanotoxicology. The PreNanoTox consortium addresses three currently missing critical elements needed to develop a platform for predictive nanotoxicology: (1) There is a current lack of a unified large database, leading to our suggestion to form such database by applying cutting edge information extraction tools on a large repository of scientific articles; (2) There is a need for better understanding of the underlying mechanisms of the primary interaction of NP with the cell membrane. It is suggested to apply appropriate theory and simulation assuming that the surface chemistry of a NP (affecting e.g. NP's surface reactivity, hydrophobicity, or surface electrostatics) as well as other physical properties (e.g. size and shape) determine the extent of adsorption of NPs to a cell surface, leading eventually to an uptake of the NPs; (3) There is a need to extend the traditional QSAR paradigm to the field of nanotoxicology. This will be carried out by linking appropriate NP descriptors, with emphasis on those which determine the extent of adsorption of NPs to cells, with biological responses. The PreNanoTox consortium is made up of four research groups (from three scientific organizations), which lead in information technology, soft matter modelling, computational chemistry and in-vitro toxicology, yielding a synergetic output.

2 Background

The possibility to predict the properties (e.g. solubility) and activities (e.g. toxicity) of unknown chemicals has been successfully addressed within the frame of theoretical models known as Quantitative Structure-Activity Relationships (QSARs) for the last several decades. A Structure-Activity Relationship (SAR) rests upon the proven hypothesis that chemicals with similar molecular structures have similar effects in physical and biological systems. Therefore, they allow predicting the potential of a chemical containing the substructure to exhibit a certain biological effect if the chemical substructure is known. To describe the variations of the extent of an effect with variations in molecular structure, the QSAR relates a quantitative measure of a structure (e.g. a physicochemical property) to a biological effect (e.g. a toxicological endpoint). To extend the traditional QSAR paradigm to NPs, one needs descriptors (numerical parameters representation) for NPs' physicochemical properties. These descriptors might, in principle, be either computed or obtained experimentally. However, at the present stage we do possess restricted computational means for the exact calculation of the physico-chemical properties of NPs. Therefore, we intend to concentrate on simulating the most relevant properties, such as adhesion strength and its effect on the magnitude of wrapping the NP by the cell membrane, which depends also on particle size. This activity will contribute important elements to the construction of Quantitative Nanostructure-Activity Relationship (QNAR).

An essential element in the successful application of QNAR for the prediction of biological effects induced by NPs is to have a mechanistic understanding of the interaction of NPs with cells. We have identified the strength of interaction of NPs with the cell membrane as the primary rate-limiting event that determines the consequent ones, such as direct effect on the cell membrane or effect on the rate of uptake. In addition, depending on the distance between neighbouring NPs bound to the

membrane surface, further changes in cell membrane deformation may occur causing local and global shape changes of the cells. The inward local curvature (negative curvature), induced beyond certain threshold of adhesion-strength to a site on the cell surface, will develop into a larger invagination which is expected to undergo fission, setting off enhanced uptake of NPs by the cell through well-established or unknown endocytic pathways. The adsorption and uptake of the NPs may elicit different insults such as change in cell membrane permeability, oxidative stress, and other yet unidentified toxicological routes, which could lead eventually to programmed and non-programmed cell death. Thus, we intend to model the adsorption and wrapping of the NPs by the cell membrane by combining analytical and simulation approaches. This will be addressed using coarse-grained models which are appropriate for systems on the supra-molecular level. By a systematic investigation of the dependence of nanoparticle adsorption and wrapping on the size, shape, and surface chemistry of the nanoparticle we expect to achieve an understanding of the role of these NP parameters as well as membrane properties on the initiation of NP uptake. The suggested paradigm, based on our working hypothesis, is expected to identify the appropriate quantitative physico-chemical properties (descriptors).

In the present project, we suggest a novel generic approach for providing a central and critical element for QNAR activity: a large database on the relation between the physic-chemical properties of NPs and the biological outcome following exposure to them. This requirement will be addressed in the present project by forming a database from the large corpus of peer-refereed articles, employing automated information extraction procedures. The database will be formed for selected NPs including silica and polystyrene. On the basis of the data acquired, the available experimental data on the physicochemical characteristics of the evaluated NPs, such as chemical composition, size, shape, surface area, surface charge, and solubility-aggregation, will be identified and matched with the biological effects induced by these NPs and the results from the theoretical modelling. This information will be the input for the specific QNAR approach and the general data-mining one.

Some of the major findings emerging from modelling and molecular dynamics simulations of adhesion and wrapping of the NPs, together with the outcome from QNAR modelling will be experimentally validated by in-vitro studies. These studies will employ cell lines representing one of the primary exposed human organs to NPs such as lung. Overall we suggest providing a new platform for predictive nanotoxicology.

3 Scientific and technological challenges

In order to develope a platform for predictive nanotoxicology, the following issues need to be addressed :

(1) The current lack of centralized database severely limits the capability of the informatics experts to envision and analyze the relationships between physico-chemical properties of NPs and their different effects when interacting with living organisms and the toxicological endpoints induced by them. To address this need, we suggest developing approaches of automatic information extraction in the domain of nanosafety, which will enable to form a very large and unified database from peer-refereed scientific articles, and provide the basis for the following QNAR step. To the best of our knowledge this is the first attempt of its kind to construct a unified data-base from scientific literature in the field of nanosafety .

(2) Extension of the traditional QSAR paradigm from chemicals to NPs by identifying and calculating appropriate NPs' descriptors and by building externally validated QNAR models linking NPs descriptors and the respectively observed biological responses.

(3) The ability to apply proper QNAR and data-mining modeling requires a mechanistic understanding on the relationship between the physico-chemical properties of NPs and their respective toxicity. Currently there are very few models of nanoparticle toxicity, especially those relating to dissolution of metal and metal-oxide NPs due to the inverse relationship between their surface activity and their size. For NPs, which do not undergo dissolution we suggest a model where NPs' toxicity depends among others on the strength of adsorption of a NP to a cell surface. We intend to lay down the theory and simulate the adsorption and wrapping the NPs by the cell membrane, thereby providing a mechanistic basis for NP's toxicity, for the successful application of QNAR and data-mining modelling.

The overall layout of PreNanoTox is based on three pillars and approaches. The first pillar of PreNanoTox will be a unified database, based on the data extracted from scientific publications, as text or graphs. We will attempt to reach this goal by further developing some of the tools previously employed in the FP7 NHECD project for text mining and by adapting existing graph extraction tools towards **automated information extraction from the formats of original peer refereed scientific publications**. The suggested approach is based on experience we have already acquired in the ongoing FP7 NHECD project on "Creation of commented database on health, safety and environmental impact of nanoparticles" (http://www.nhecd-fp7.eu/), where we have formed a repository of peer refereed scientific articles which have been commented (categorized and ranked). In this project we intent to further develop the approaches and tools of automatic data extraction, beyond those applied in the NHECD project, which will enable us to apply efficient text mining for information extraction from free text, figure labels and graphs. We will normalize the various streams and formats of incoming data (from the various sources) into a single unified database. This is a prerequisite for the utilization and development of the modelling tools. The immerging information from this database will be the physicochemical properties NPs of the different NPs and their biological effects.

As the success of applying QNAR and data-mining tools for predicting the biological/toxicological outcome of NPs interaction with cells depends on the knowledge of the underlying mechanisms of interaction, we intend to develop a theory and simulate the interaction of NPs with the cell membrane as a function of NP's size and surface chemistry. Our proposed working hypothesis was that the **surface chemistry of a NP** (affecting NP's surface reactivity, hydrophobicity, or surface electrostatics) **as well as its other physical properties** (e.g. size and shape) **determine the strength of adsorption of NPs to a cell surface**. Depending on both the strength of interaction and the **distance between neighbouring NPs bound to the membrane surface** (a function of NP concentrations in the extracellular medium), further changes in cell membrane deformation may occur causing local and global shape changes of the cell. This analytical and simulation effort constitutes the second pillar of PreNanoTox.

On the basis of the toxicological unified database on selected groups of NPs they will be scrutinized to identify available physico-chemical parameters and to add descriptors based on quantum-mechanics calculation to obtain a more complete characterization of NP properties. QNAR and data mining methods will be explored to seek structure activity relationships for in-vitro and in-vivo toxicity. The methods to be used will primarily depend on the amount of information (in terms of number of NPs and their associated parameters) and type of effect (quantitative or qualitative) to be addressed. If data is scarce, simpler methods such as multilinear regression, hierarchical and non-hierarchical classification methods and clustering will be used. In cases where data is more abundant, more sophisticated methods will also be evaluated; for instance neural networks or random forest. These activities form the third pillar for PreNanoTox activity.

4 Objectives

The overall goal of PreNanoTox is providing tools for establishing the necessary theoretical and in-silico based knowledge for the construction of a platform for predictive nanotoxicity which will be offered to industry and to health and environmental regulation authorities. A special effort will be dedicated to harmonize the activity of PreNanoTox with the other EC

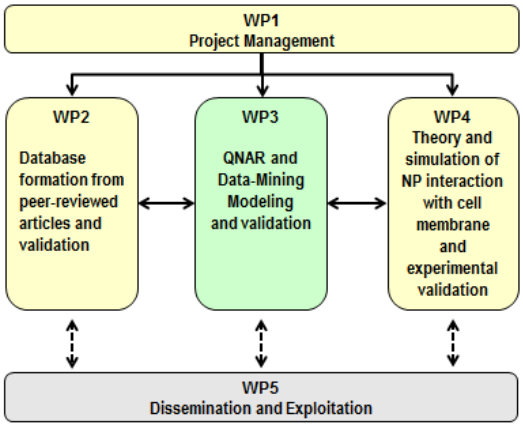
funded modelling consortia. In order to achieve this goal it is intended to address the following objectives:

- Formation of a large nanotoxicological database of in-vitro and in-vivo studies of selected NPs by developing and applying methodologies of automated information extraction on a large repository of peer-refereed scientific articles, and validate its quality.

- Develop, adapt and validate novel methodologies for establishing QNAR modeling as a tool for predicting biological effects of selected engineered NPs. QNAR will be applied on a large toxicological database of selected NPs to be formed from the **scientific literature.**

- Develop theory and simulation programs on nanoparticle adsorption and wrapping by the cell membrane and validate experimentally their predictions. Emphasis will be on uncovering of the physico-chemical properties that affect the interaction of selected NPs with cell membrane and lead to their consequent intracellular uptake.

In order to reach the above objectives it is intended to employ the following research strategy. The first phase of the project has been the generation of specific requirements about the exact needs of the PreNanoTox platform, including scientific and technological requirements (such as selection of tools). This enables us to embark on the research and development tasks (Database formation in WP2, QNAR/Data-Mining Modelling in WP3 and theory-simulation of the interaction of NPs with cell membrane in WP4). The dissemination of the outcomes of these three WPs and their exploitation will be carried out in WP5, which will communicate the PreNanoTox findings, progress, best practice and knowledge outputs to the full range of stakeholders, including the regulatory bodies and policy makers, industry, and the general public. The overall scheme of the interrelations among the different WPs is shown in the following scheme:



5 Progress and Outcomes to date

The interaction of nanoparticles with membranes is a necessary first step for any toxic effects of nanoparticles in living cells. While very small nanoparticles can penetrate a membrane, larger nanoparticles with diameters above 20nm either bind and attach to the lipid bilayer membrane or get wrapped by the membrane. We describe wrapping of nanoparticles using a continuum membrane model, where the curvature-elastic properties of the membrane are taken into account by a set of curvature-elastic constants. Particularly important is the bending rigidity that characterizes the membrane's resistance to deformations. Contrary to molecular dynamics simulations with a lipid-scale modeling, the continuum model allows the description of particles with sizes of a few hundred nanometers with still reasonable computational resources.

Spherical and cylindrical nanoparticles are two limiting cases for elongated nanoparticles, with either both axes with equal length (and therefore no elongation) or with an infinite extension in one direction. For a membrane with bending rigidity only, the deformation energy cost for wrapping a nanoparticle is proportional to its squared curvature, which in case of spherical and cylindrical particles is homogeneous everywhere on the surface of the particle. We have studied wrapping of spherical and cylindrical nanoparticles by two-component membranes in the strong segregation limit. In this limit, one component forms a domain (either a macroscopic domain or a lipid raft) to which the particle attaches. Due to the different lipid composition, the membrane of the domain has different curvature-elastic properties than the surrounding membrane. In addition, there is a line tension for the border of the domain where the two different lipid phases are in contact.

In the first twelve months of the project, we have calculated phase diagrams for wrapping of spherical and cylindrical nanoparticles for various values of membrane tension, membrane bending rigidity, line tension, and adhesion strength between particle and membrane. We have calculated phase diagrams that delineate regions in parameter space where the particle is unbound, partially attached, or completely wrapped. For a particle attaching to a homogeneous membrane, the deformation energy cost per membrane area for a cylindrical particle is only a quarter of the costs required to wrap a spherical particle. For a two-component membrane, the wrapping phase behavior is more complex: When a spherical particle attaches to a lipid domain that is larger than the particle surface area, the length of the contact line shrinks when the membrane wraps around the particle, which supports wrapping. Less favorable membrane properties for wrapping for the surrounding membrane and a domain size that is smaller than the particle surface area leading to partially-wrapped states. In addition, we have studied wrapping of nanoparticles with more complex shapes, such as ellipsoids and super-ellipsoids. Illustration is shown in the following Fig. 1.

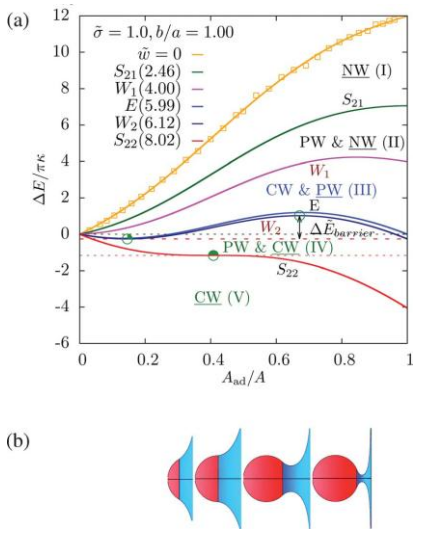


Fig. 1 (a) Energies for wrapping a spherical particle as a function of the wrapping fraction Aad/A for reduced membrane tension ~s ¼ 1. The figure shows the wrapping energy profiles for six adhesion strengths: the numerically calculated data for zero adhesion strength and the corresponding fit function, E, with equal energy for the non-wrapped and the completely wrapped state (~w ¼ 5.99), the binding transitionW1 between the unwrapped and the partially wrapped state (~w ¼ 4.00), the binodal W2 between the partially wrapped and the completely wrapped state (~w ¼ 6.12), and the spinodals S21 and S22 that are associated with W2 (for ~w ¼ 2.46 and ~w ¼ 8.02 respectively). The phase boundaries separate 5 regimes in the phase diagram with stable and metastable completely wrapped (CW), partially wrapped (PW), and non-wrapped (NW) states; the stable state is underlined. The wrapping fractions that are plotted in Fig. 1 are marked by circles. (b) Sketches for spherical particles with wrapping fractions of 0.25, 0.50, 0.85 and 0.96, with the adhered

membrane in red and part of the free membrane in blue. More details can be found in two recently published articles:

1. Dasgupta, S., Auth, T., Gompper G. (*2013*) Wrapping of ellipsoidal nano-particles by fluid membanes. ***Soft Matter***, Vol. 9, pp. 5473-5482.

2. Dasgupta, S., Auth, T., Gompper G. (*2014*) Shape and orientation matter for cellular uptake of non-spherical particles. ***Nano Letters***, DOI: 10.1021/nl403949h.

In addition we have integrated an information extraction framework where we identified, based on theoretical considerations, the most important parameters to be extracted for the NPs, the biological models and the biological endpoints. The extracted data was carried out from text of "Abstract" and "Materials and methods" sections and included the following steps: Extract text from document, Parse text with Stanford parser, Identify Named entities, Extract Attributes of identified entities, using Stanford dependencies and to convert objects to database structure.

An initial corpus of 50 papers was prepared for further testing the PreNanoTox automated data extraction facility. We plan to tune the resources and iterate until reaching the desired performance compared to the manually prepared corpus.

Within harmonization activity with other modeling consortia a joint effort of data curation through manual extraction of information from peer refereed articles on Silica was initiated. For this purpose PreNanoTox established a data-base and tools for the deposition of manually extracted data.

6 Expected Impact

PreNanoTox intends to pave the way to construct a large database from the published peer-refereed literature which is, to the best of our knowledge, a first attempt of its kind in the field of nanosafety. The ability to access the knowledge in scientific literature of nanosafety through the formation of a database, that will enable applying bioinformatic and chemoinformatic tools to it, is expected to serve as an example for other scientific domains. More specifically, this project is expected to contribute to the development of robust systems for evaluating the health and environmental impact of engineered nanoparticles by enabling the analysis of the formed large database using new approaches for QNARs which will serve for regulatory purposes. For the scientific activity it will be of invaluable tool to identify specific information in the vast number of peer refereed published articles.

To apply effectively QNAR and data-mining modeling, it is important to find and verify mechanisms that interlink between the physico-chemical properties of the NPs and their interaction with cells. We believe that a possibility to make significant progress is a systematic investigation, starting from a relatively simple system and including additional complexity step by step. We are focusing in our modeling approach on NP adsorption and wrapping by the cell membrane, i.e. the process of the uptake of the NP that is the crucial step for consequent damage to occur. This process is controlled by the surface chemistry and the size-shape properties of the NPs, as well as by the properties of the cell membrane. This will contribute to develop the existing bottom-up modeling approaches to reach maturity and to be predictive for membranes as complex as the cell membrane. Thus the major foreseen impact is by enabling to improve the quality and reliability of the predictive tool of QSAR and data-mining, thereby enhancing the use of predictive toxicology both for regulators and industry.

7 Directory

Table 1 Directory of people involved in this project.

First Name	Last Name	Affiliation	Address	e-mail
Rafi	Korenstein	Tel Aviv University	69978 Tel Aviv, Israel	korens@tau.ac.il
Oded	Maimon	Tel-Aviv University	69978 Tel-Aviv, Israel	maimon@tau.ac.il
Emilio	Benfenati	Mario Negri Institute	Via La Masa 19 - 20156 Milano, Italy	emilio.benfenati@marionegri.it
Gerhard	Gompper	Research Centre Juelich	52425 Juelich, Germany	g.gompper@fz-juelich.de
Thorsten	Auth	Research Centre Juelich	52425 Juelich, Germany	t.auth@fz-juelich.de

8 Copyright

QualityNano

A pan-European infrastructure for quality in nanomaterials safety testing

Contract Agreement: SP4-Capacities-2010-262163 Website: http://www.qualitynano.eu
Coordinator: Kenneth Dawson, Centre for BioNano Interactions, University College Dublin, Belfield, Dublin 4, Ireland

No.	Beneficiary name	Short name	Country
1	National University of Ireland, Dublin / University College Dublin	NUID UCD	Ireland
2	Natural History Museum	NHM	United Kingdom
3	Institute of Occupational Medicine	IOM	United Kingdom
4	Joint Research Centre of the European Commission	JRC	Italy
5	Federal Institute for Risk Assessment	BfR	Germany
6	Karlsruhe Institute of Technology	KIT	Germany
7	Facultes Universitaires Notre-Dame De La Paix	FUNDP	Belgium
8	Institute of Work and Health	IST	Switzerland
9	University of Leeds	UL	United Kingdom
10	Norwegian Institute for Air Research	NILU	Norway
11	Helmholz Centre Munich	HMGU	Germany
12	Ludwig-Maximilians Universität, München	LMU	Germany
13	Centro de Investigación Cooperativa en Biomateriales	CIC	Spain
14	Upsalla University	UU	Sweden
15	Institut Català de Nanotecnologia - Consejo Superior de Investigaciones Científicas	ICN	Spain
16	Stichting Dienslandbouwkundig Onderzoek	DLO	Netherlands
17	Wageningen University	WU	Netherlands
18	Deutsche Gesetzliche Unfallversicherung	BGIA	Germany
19	Tel Aviv University	TAU	Israel
20	Slovak Medical University	SMU	Slovenia
21	Vlaamse Instelling voor Technologisch Onderzoek	VITO	Belgium
22	Trinity College Dublin	TCD	Ireland
23	Finnish Institute of Occupational Health	FIOH	Finland
24	University of Exeter	UOE	United Kingdom
~~25~~	~~Edinburgh Napier University~~	~~ENU~~	~~United Kingdom~~
26	University Paris Sud	UPS	France
27	L'Institut National de l'environnement industriel et des risques	INERIS	France
28	Heriot Watt University*	HWU	United Kingdom
29	University of Birmingham*	UBham	United Kingdom
30	Rijiksinstituut voor Volksgezondheid en Milieu*	RIVM	Netherlands

* Grant agreement amended in 2012.

Note: On 1st February 2013, QNano was re-launched as QualityNano to highlight its role in elevating the overall quality of research in the field via development of best practice (JRA) and provision of Access (TA) and training (NA) to the research community.

Contents

1 Summary

Nanoscale objects interact with living organisms in a fundamentally new manner, ensuring that a fruitful marriage of nanotechnology and biology will long outlast short term imperatives. Therefore, investment in an infrastructure to drive scientific knowledge of the highest quality will have both immediate benefits of supporting the safety assessment of legacy nanomaterials, as well as pointing towards future (safe) applications with the lasting benefits to society. There are immediate priorities, for few doubts that serious damage to confidence in nanotechnology, unless averted, could result in missed opportunities to benefit society for a generation, or more. QualityNano, as an infrastructure for analysis of nanomaterials for biological safety assessment, will materially affect the outcome, at this pivotal moment of nanotechnology implementation.

The overall vision of the QNano Research Infrastructure for nanosafety assessment is the creation of a 'neutral' scientific & technical space in which all stakeholder groups can engage, develop and share scientific best practice in the field. Initially it will harness resources from across Europe and develop efficient, transparent and effective processes.

Nanoscience constitutes a new scientific frontier in which we can, for the first time, engineer materials on the length scale of some millionths of a millimetre. The potential applications of nanotechnology for the benefit of mankind range from information technology, energy storage and harvesting, to radically new medical technologies. The projected market for nanotechnology incorporated in manufactured goods may be worth US$ 1.6 Trillion in the forecast period (2009-2013).[1]

The scientific issues are fundamental, and durable. Much of the internal processing, passing of signals, and other key functions of living organisms use endogenous processes operating on the nanometer scale. Engineered nanoscale objects (nanomaterials) therefore can interact with organisms in a fundamentally new way (compared to micron scale materials of identical composition), ensuring that the fruitful marriage of nanotechnology and biology

will long outlast short term imperatives.[2] As such, our ability to generate fundamental scientific knowledge of the highest quality to support the safety assessment of nanomaterials for humans and for the environment will be an investment in the infrastructure, and the future, with lasting positive impact. All steps must therefore be taken, as quickly as possible, to ensure that the field is guided towards success, with responsibility. Few doubt that serious damage to confidence in the technology could result in missed opportunities to benefit society for a generation, or more.

Thereby it will enable provision of services to its Users, and the broader community, all in the context of a best-practice ethos. This will encourage evidence-based dialogue to prosper between all stakeholders. However, QualityNano will also pro-actively seek to drive, develop and promote the highest quality research and practices via its Joint Research Activities (JRA), Networking Activities (NA) and provision of Transnational Access (TA) functions, with a global perspective and mode of implementation.

QualityNano is also looking to the future, beyond the current issues, and promote the growth and development of the science of nanoscale interactions with living organisms. By working with new and emerging scientific research communities from medicine, biology, energy, materials and others, it will seek to forge new directions leading to new (safe, responsible, economically viable) technologies for the benefit of European society.

The QualityNano project commenced on 1st February 2011 and will run for 48 months. It is just at the mid-term point now.

2 Background

Despite significant R&D investment over the last 10 years,[3] several critical road-blocks to rapid implementation and commercialisation in a safe and responsible manner, acknowledged by all stakeholders, were not fully foreseen. The real (and perceived) unknown hazards and risks of nanomaterials, allied to concerns about the reliability of current testing approaches have been highlighted in all dimensions from science, media, and even to the highest levels of government.[4] Furthermore, discussions between stakeholders have not always been easy and to some degree the discussion has become polarised, based on opinions, and some erosion of trust has occurred.[5]

Additional complicating issues have arisen because manufacturing standards, and workplace practices, of nanomaterials are not uniform across market sectors, and in different parts of the world. It is clear that, in the absence of an understanding of what constitutes (useful) standards, the reputation of nanotechnology could be affected by the weakest players. Serious issues have already arisen, for example, from issues of impurities, unconventionally sequestered in nanomaterials. The political sensitivity of these issues in a global market, and the need to address them via infrastructural developments such as we propose here, is universally acknowledged.

Compounding this, very significant variability of reported biological and toxicity outcomes on nominally identical materials has caused controversy in science, and the media, and could, if not urgently

reversed, lead to a loss of confidence in science that single force capable of unifying societal views on this topic. Solid, disciplined, evidence based dialogue is urgently required to resolve these issues. The need for scientific opinion, whether academic research, regulatory or industrial, to converge on basic results within a cohesive framework of structured research (in part based on blind round robin tests) is now critical. Indeed, there are few more urgent or compelling cases to be made than the need for infrastructure now to transform and drive the transition required.

QualityNano will:

1. Create a neutral ethos of excellence where all nanotechnology stakeholders can focus on concrete science-based outcomes;
2. Establish a core infrastructure to address the critical issues currently hampering the industrial deployment of nanotechnologies across a range of industry sectors;
3. Provide Users with a full range of services from standard nanomaterials, tuition in best practice, laboratory support and training, and a suite of protocols for all aspects of nanomaterials processing and characterisation in a biological context;
4. Push beyond the state of the art in nanomaterials processing, labelling and identification and characterisation *in situ;*
5. Develop novel analytical approaches and tools where most urgently needed to enhance understanding of health and safety issues in nanotechnology;
6. Create a hub to drive the development and implementation of standards across all aspects of nanosafety evaluation and to link with other EU actions (RTD, ERANET, Nanosafety Cluster, OECD, ISO) and international stakeholders;
7. Look to the future - framed with new scientific communities, and new industry sectors, forging new (safe and responsible) applications of nanoscience and implementations of nanotechnology.

QualityNano will qualitatively change the outcome and potential for successful commercialisation of nano-enabled products at this critical period of nano-implementation.

3 Project Description and Organisation

The vision of QualityNano is the creation of a 'neutral' scientific and technical space in which scientists from all stakeholder groups can engage, develop, and share the scientific best practices in the field. It is understood that such an organization cannot resolve all of the challenges, nor even address all the important areas of the science, especially at the beginning. In the early days its aspiration must be limited to the creation of an ethos, development of processes, and harnessing of the resources, to allow evidence based dialogue in critical areas to flower. The program will not engage in controversy, nor promote opinions, for in doing so it will lose the trust of one or more stakeholders. Uniquely important in the current situation, the infrastructure will need to patiently display ethical standards in actions and processes if the current uncertain atmosphere is to yield to clarity and unity of purpose. By processes (for example, blind round robins) it will determine (and provide the support to determine) facts, and report them to the scientific community, and stakeholders. Its greatest strength is that these factual results (from well-defined studies) even if their implications remain open to interpretation will be trusted by all. The infrastructure will be global in perspective, and implementation. As noted above, some of the challenges do not lie in Europe alone, nor can they be resolved there. Existing warm relations in the United States, Asia and Latin America (and beyond) will be further developed within the framework.

Many issues need to be addressed, short term, and longer term. A scientific culture must be built, and full acceptance of the challenges and difficulties of working in scientific excellence in such a new arena must be argued, and won, step by step. At this period in history, resolving even simple issues, such as the creation and provision of (nanoparticulate) biological end-point positive (and negative) controls, will have profound effects on the way that the User community performs its' work.

QualityNano is founded on a belief in the potential of nanoscience and nanotechnology. It will therefore look to the future, beyond the legacy of the current debates, and find creative approaches to organising and thinking, implementing new ways to deliver the promise nanotechnology to benefit mankind - safely. The union of nanoscience and living organisms is indissoluble. They have a long way to travel together in the future, as outlined in the vision statement of the European Technology Platform for nanomedicine. Well-conceived infrastructures to support that journey will give lasting value to European society, and far beyond.

Practically speaking, QualityNano will be **an accessible integrated European resource for research, regulatory, and industry (both small and large) developers in nanoscience and nanotechnology**. It will harness and integrate existing research expertise and facilities from across the EU member states into a cohesive interdisciplinary entity strongly focussed on scientific excellence and quality of execution in all aspects of nanomaterials processing and characterisation for assessment of their biological and environmental impacts.

It will offer a distributed set of transnationally accessible facilities, centrally managed, as with any infrastructure program, *but also offer a range of added-value services to users and stakeholders*.

These will include high quality ('approved') Nanomaterials, Training (and certification) in advanced characterisation methodologies and round robin validated protocols for biological assays, as well as Industry-oriented support, using flexibly configured distributed 'hubs' via which different constituencies can interact. Crucially, these hubs will embed existing (and emerging) core constituencies (via suitable program partners), promoting the concept of infrastructure as a 'learning' organization. Thus, whilst the vision of excellence and quality will be fixed, the means to achieve that end will evolve responsively.

QualityNano is primarily an analytical infrastructure whose purpose is to drive high quality research and testing practices. Physiochemical and other analytical characterization in the biological and safety contexts is quite different from analysis of nanomaterials for other applications. Some of the important (relevant) physiochemical characteristics are not yet fully understood. Even implementation of known science is not always evident, but in a new industry making its reputation, it is crucial. The fact that engineered structures have access to biological machinery, combined with their unique (for example high-surface area) properties, means that material quality and reproducibility are important, not just for this program, but long term, in industry in general. Details such as the tendency of nanomaterials to secrete difficult-to-remove relatively immobile impurities into an organism, or to sequester contaminants from the environment and transport them into living organisms, for example, can have profound consequences for predicting fate and behaviour in different cell types, tissues, complex matrices, organisms, and all require detailed characterisation to be interpreted correctly. Such aspects are believed to underlie some early negative toxicity reports, leading (in these specific cases) to unwarranted and widely publicised fears. There is a critical need to separate issues of quality from the durable questions of intrinsic nanomaterials safety. The potential for these issues to have negative impact on trust in global trade (where good practices are not universally accepted) are incalculable.

By fostering a new quality-based research and application consensus that values both the durability, and reproducibility of new findings, QualityNano will qualitatively affect the outcomes in this domain. It cannot address all the challenges, but it will provide the basis for those challenges faced, at what is certainly the most pivotal period in the adoption of nanoscience and nanotechnology in society.

The activities of QualityNano are summarised as follows:

Networking Activities:

- **NA1** - Management and coordination
- **NA2** - Nanomaterials Hub: an instrument for Quality Assurance testing of nanomaterials via Round-Robin trials, and their provision to the wider User community.
- **NA3** - Training Hub covering all aspects of best practice in nanomaterials for biological testing.
- **NA4** – Supporting the Nanosafety Cluster activities.

Transnational Access: Provision of transnational access to the nanomaterials processing, characterisation and exposure assessment facilities of the 15 TA Participants via a single application and evaluation process and 6-monthly calls for applications on the QualityNano website.

Joint Research Activities:

- **JRA1** - Development of strategies to eliminate and/or reduce variability in nanomaterials batch-to-batch reproducibility and to determine acceptable variability levels for biological applications.
- **JRA2** – Optimisation of traceability of nanoparticles by development of reliable labelling (radioactive, stable isotope and fluorescent).
- **JRA 3** - Development and validation of characterisation tools for nanoparticles *in situ* in biological, environmental or consumer milieu.
- **JRA 4** – Development of optimal modes of presentation of nanoparticles to cells, tissues, organisms and whole animals for quantitative reproducibility.
- **JRA 5** – Towards development of priority alternative *in vitro* tests to replace animal testing.

4 Key Challenges being addressed by QualityNano

Irreproducibility in nanomaterials leads to irreproducible biological impacts.

There remain genuine scientific challenges in making reproducible nanomaterials using early manufacturing processes. This is not a trivial issue, and it will take some years yet before it is resolved. However, it must be noted in the current context. Thus, because of the enormous surface-to-volume ratio presented by nanomaterials, it is not uncommon for 1 millilitre of dispersed nanomaterials (1wt%, 70nm) to present over $8m^2$ of surface area to the endogenous machinery of biological organisms. The level of care taken by the medical device industry to understand the role, and maintain the quality and reproducibility of medical device implants, with much smaller exposed surface areas, is barely conceivable in nanomaterials preparation. Yet, this is the standard we have to work towards and progress on urgently. Beneath several hundreds of nanometers, the immune clearance system is less effective, and nanomaterial surfaces may be in prolonged contact with biological systems. Thus, irreproducibility in surface quality or properties (more perhaps than variations in absolute surface area) inherent in current, poorly controlled batch nanomaterials synthesis methods can be amplified far beyond that expected based on their usual applications, which is not necessarily all surface-related. Not all variations are expected to be biologically significant. Some known factors include surface charge and crystallinity, but no systematic studies of the biological impacts from batch-to-batch variability have been attempted, in part because of the large variations in the methods themselves.

Paradoxically, even where such variations of surface quality do not present a real hazard, they can lead to a troubling irreproducibility in biological or toxicological assessments that in itself leads to controversy and a general lack of confidence in the capacity to do good science in this field. Attempts to suppress these effects (for

example, OECD, IANH, and other large national programs) have been made, choosing one representative batch that is maintained throughout the particular program, with the usual problems of such approaches. With nanomaterials, however, the problems can be more serious. Batch aging, especially in dispersion, is quite serious, and for many materials requires disposal of a given batch after three months, even if the storage conditions are optimal, an organizational issue that is itself challenging, and fraught with unforeseen difficulties. Additionally, chemical purity and surface modifications can introduce further variability in biological responses.

Unscientific lack of nanomaterial positive and negative controls for biological assays.

Amongst the most basic requirements of any well defined experiment is the need to have positive and negative controls to demonstrate that the assay is working but is not triggered non-mechanistically, and to present the biological or toxicological outcomes of these in any report. This is part of the basic social contract formed between scientists in all fields, for over a century. Much current nanosafety research is largely carried out in the absence of any such controls, or using non-nanomaterial positive controls (e.g. molecules), simply because there are few (if any) agreed positive control nanomaterials for the various biological end-points (e.g. apoptosis, cell cycle disruption, genotoxicity etc.). In those cases where chemical controls have been used, they generally have a different site of biological action (not using the same endogenous mechanisms) and are therefore of dubious value. This single scientific difficulty has, perhaps, lead to the most striking damage to the scientific reputation of the field, in the sight of the broader scientific community, and has deep cultural impacts for the nanosafety and nanobiology communities, limiting aspirations for the level of potential publications, and thereby damaging the careers of young researchers engaged in the field.

Unknown or poorly chosen dispersants for base nanomaterials.

Even the most basic issues, such as how to prepare nanoparticulate dispersion in biological media where they would be studied, are not always well understood. Naturally chemists and physicists have for years studied the dispersibility of nanomaterials for a variety of applications, but most dispersants are at least biologically disruptive, if not downright cytotoxic, at the levels required for good dispersion. In many cases, lack of common training, culture and understanding between nanomaterials scientists, biologists and toxicologists lead to the latter using directly a dispersed material without appreciating that some of the added components could lead to significant biological impacts themselves. Such issues were compounded by the fact that dispersants for specific materials are sometimes commercially valuable information, and in some cases nanomaterials purchased from companies were studied without knowledge of the added components. In such cases there was no opportunity to control the dose of the added dispersant or other additives, and even those that are quite safe under normal application conditions (for example after preparation in paints etc.) could lead to undesirable toxicological outcomes if studied at inappropriate concentrations. Such issues have proliferated to the point where, in the literature, it has become difficult to separate the biological role of nanomaterials themselves, from a multitude of other preparative details, often not clearly known, or reported.

Limited application of characterisation methods (in some cases limited capacity to characterise) to nanomaterials at any stage of their processing and analysis.

The framing of the call text of this particular program (Analytical Facilities) highlights this particular aspect of the challenges facing the nanosafety community, and thereby correctly cuts to the heart of one of the most critical issues of the field. This is an issue at every stage of nanomaterials system preparation, and impinges at every level, from the most fundamental science, to the most practical issues of regulatory outputs.

There are basic challenges that are a legacy from the inter-disciplinary origins of the field. For example, many biological and toxicological laboratories are only now acquiring basic fixed light scattering and zeta potential devices and many are not yet fully integrated into the laboratories. Many of advanced characterisation technologies will remain outside of the reach, or indeed reasonable interest, of the User community of biologists and toxicologists, and this must be acknowledged, and addressed.

Nanomaterials tracking, localization and characterisation in living organisms and the environment is relatively unknown as yet, and such limited information as exists has few cross checks and is of unknown reliability.

It is hard to believe that nanotechnology can have arrived at this phase of its development with such a lack of good quality, labelled nanomaterials suitable for biological applications and relevant to the scientific and safety issues at stake. This constitutes a serious bottleneck to progression of the field, and confidence in regulatory decisions.

There is limited access to even existing labelled materials (for example radio-, or isotope labelled materials) which tend to be available only to specific collaborators. Furthermore, the design of labelling strategy is often poorly aligned with the User community needs, which wishes to study nanomaterials of high economic relevance and high usage. Labelling strategies that significantly affect the surface can lead to quite different biological outcomes, and are therefore of more academic interest, and labels of the wrong intensity or misaligned in, for example, wavelength for typical biological instrumentation is also a serious practical problem, in part derived from the fact that labelling is often driven by chemists and physicists only in limited contact with biologists.

Lack of in situ characterisation of the nanomaterial-biomolecule complexes.

There are other serious issues for the field resulting from the lack of *in situ* characterisation of nanomaterials during biological and environmental studies. A key point, often missed in the immediacies of the nanosafety question, is that the future of the field as a true science requires *in situ* characterisation of the nanomaterial-biological complexes. It is now clear that in many biological fluids, nanomaterials (unless specifically designed not to) are coated by a very long lived biomolecular shell ('hard corona') that is sufficiently durable and thick as to determine the

early outcomes of translocation, localization in living organisms. Similar issues (although that arena is in an even earlier phase of development) pertain in the environmental context where the nanomaterial surface may be coated by a variety of naturally occurring biomolecules such as polysaccharides from organic matter. Thus, whilst fundamental for the discipline and basics of nanomaterial production and supply, the well known nanomaterial characterisation methods give parameters that may merely be proxies for the 'real' biological identity, that is, what living organisms really 'see'. This is a critical issue for the development of the field. There are practical issues also, for the nature of the plasma or serum used may lead to different outcomes.

Thus, the dispersion of nanomaterials in even the simplest biofluids such as blood plasma or in environmental fluids such as river water requires care, and understanding in practice. Furthermore, there are as yet great unknowns in the structure and evolution of such dispersions, and ongoing nanomaterial-biomolecule aggregation can affect the bioavailability of the nanomaterials. One cannot in this field expect the scientifically idealized outcome of perfectly stable dispersed materials, but one can at least insist that nominally identical dispersions used by different groups of scientists are indeed identical. Therefore, uncertainties in this arena may impact on the framing of poor, or poorly defined, dispersion protocols in which insufficient parameters are fixed to ensure reproducible dispersion and dispersion kinetics. In all these cases the lack of application of known characterisation methods, and the limited manner in which these have so far been translated for use in this field are currently limiting factors in the onward development and the implementation of regulation. There is also an overarching challenge regarding dissemination of this need for *in situ* characterisation techniques into the User community.

Poorly understood, poorly characterized, without agreed standards or experimental formats for presenting nanomaterials in biological, toxicological, environmental and occupational exposure studies means that dose, and dose rates are poorly understood, rarely uniform, and can lead to widely different 'actual' doses.

The problem of how to present the nanomaterials in a meaningful, reproducible, and bioavailable manner is challenging. Without specific measures, and when combined with issues of poorly controlled aggregation may lead the intracellular concentration for nominally identical nanomaterial concentrations and biological materials to differ by several orders of magnitude. Though less well understood, similar issues are believed to be relevant *in vivo* and in the environment, where different modes of preparation and delivery combine to lead to different 'presentation' of the nanomaterials. Occupational exposure scenarios are no different in the challenges presented, and (for example) implications for different modes of delivery, and measurement, of carbon nanotubes (CNTs) are poorly understood, and lack any agreed approach.

Poorly structured and poorly supported by infrastructures.

This challenge ranges from the lack of common set of laboratory practices and facilities from which the most expert can support those (often highly expert) biologists and toxicologists that lack expertise in system preparation and characterisation. The challenge is, however, deeper. In the absence of infrastructure, the community is fragmented, and is only slowly forming a vision of what it wishes to be.

5 QualityNano activities

QualityNano is founded on three functionally distinct elements to promote high quality and reproducible research on nanomaterials in contact with biological and environmental systems, and build the knowledge on nanosafety. Each of the (three) functional elements is essential, as are the linkages (both in process, and in people) that have been designed into them. The three elements are closely interlinked from an operational and management point of view, in addition to the close scientific linkages. The functional elements are as follows:

Networking Activity (NA): To ensure appropriate dissemination of the best practice in nanomaterials synthesis and dispersion in reproducible manners, characterisation of nanomaterials *in situ*, methods of presentation of nanoparticles to living systems, and alternative testing methods (i.e. the JRA topics), QualityNano has a strong focus on networking activities, such as training of young researchers (through the Knowledge Hub), provision of high quality nanomaterials (through the NanoMaterials Hub), contributing to research road-mapping and priority setting for the field, and supporting the development of internationally agreed archiving and databasing protocols for data generated within EU projects.

Transnational Access (TA): Physical access to 14 of the major nanomaterials processing and characterisation for health, safety and environmental application sites in Europe. Collectively, these sites enable Users to access small to medium scale equipment and facilities(with the appropriate knowledge to apply them in this context) through to some of the most highly equipped nano-characterization centres in Europe.

Joint Research Activity (JRA): 28 partners (including 14 of the TA partners), have been selected based on their unique contributions in research, where it pertains directly to new or improved methods that contribute to the infrastructure of the field. Several of these are outstanding scientists in particularly relevant research functions.

Networking Activities:

Annual Integrating Conference

The 2nd QNano Integrating Conference, "Quality in nanosafety assessment – driving best practice and innovation" was held from 27th February – 1st March 2013, IMG Conference Centre, Prague, Czech Republic.

- The need for high quality data and best practice in experimental design and reporting cannot be overstated, and is among the most pressing topics in the nanosafety arena at present. Coupled with this is the need to move beyond simple cytotoxicity testing, towards advanced models that are more representative of the *in vivo* environment and hypothesis driven and mechanism-based studies to understand subtle impacts potentially resulting from low-dose and /or longer term exposure to nanomaterials, all of which requires innovation. The aims of the conference were:To facilitate best practice and innovation in nanosafety assessment by providing a focal

point for the European and international research communities;
- To showcase some of the best scientific advances, both experimental and computational, in the field internationally;
- To promote the need for detailed characterisation of nanomaterials under the exposure conditions and *in situ* during and after exposure to living systems, including via provision of Transnational Access to some of Europe's leading nanomaterials characterisation facilities.

The conference was attended by 224 participants from 28 different countries from Europe, America and Asia. Approximately 25% of the participants were from central and eastern Europe. In addition, the largest cohort of delegates in attendance was from the host country, the Czech Republic (21%). In contrast, at the 2012 meeting no attendees from the Czech Republic were recorded. Delegates from Germany (14%), Ireland (8%) the UK (7%) represented the next largest cohorts of attendees.

All oral presentations were recorded and are available for viewing via the QualityNano Knowledge Hub. We are currently working to make this element of the site easier tonavigate and more user-friendly, although with the size of the files this is proving somewhat challenging.

An important element of the 2013 QualityNano conference was the showcase of the efforts and outputs of the first round of QualityNano Transnational Access Users, via the Transnational Access session, which had a central spot on day 2 of the conference, to ensure maximal participation. 5 QualityNano TA Users will gave short presentations on their QualityNano-funded work at JRC, UCD, KIT, UU and FUNDP.

Knowledge Hub

The QualityNano Knowledge Hub is intended to be a centralised resource to address the training and outreach needs in the area of processing, analysis and characterisation of nanomaterials for use in biological applications, focussing initially on the priority needs of young researchers, regulators and industry researchers and safety managers.

A centralised unit coordinates all aspects of training and good practice (guidance, hands-on training, industry training etc.). As part of the Knowledge Hub, QualityNano has set up up a system that can be accessed and utilised by all and current and future Users[1]. A key feature of the Knowledge Hub is the provision of on-line and e-accessible course material, initially from QualityNano events, but agreements will also be sought with organisers of other events to add their materials (with appropriate acknowledgments and terms of use) to the Knowledge Hub.

At the mid-term review in April 2013 it was decided to re-focus the Knowledge Hub WP on training events only, and to include training aligned to Round-robins as a key feature of this WP, with UoB taking over leadership of the WP. Some progress has been made since September 2013, although the amendment is not yet formalised, including budget and effort, making significant incurrence of costs by the new WP leader difficult / risky. The first environmentally-focussed training event is planned for 4-7th March 2014 and an ecotoxicology training is planned for May 2014. A round-robin focussed training is at an advanced stage of planning.

Since the re-focussing of the tasks to address training for RR activities, UoB has liaised with UCD, VITO and BfR regarding the layout, format and content of the first RR training event. The initial plan to train >50 researchers has been scaled back due to logistical challenges of hosting that many researchers with such high-end laboratory and equipment needs (i.e. 4 DLS machines, 4 flow cytometers etc.).

Nanomaterials Hub

QualityNano's aims in terms of the *Nanomaterials Hub* and its process of evaluation of nanomaterials via Round Robins are to:

- create a neutral space for nanosafety evaluations shaped by best practice and mutually recognised methodology evaluated by neutral processes such as round robin studies;
- increase the overall competency in Europe for quantitative nanosafety assessment through training and pre-validation of assays that ensure that researchers in Europe can:
- characterise the dispersion state of their nanoparticles under their exposure conditions;
- quantify the dose of nanoparticles actually taken up (by cells initially, but extending also to organisms and animals); and
- based on the dose (and localisation) information, assess the biological impacts of their nanoparticles towards a series of end-points compared to positive and negative control nanoparticles.

This will be achieved by an iterative process of refining assay protocols, pre-validating test nanomaterials, and ensuring competence in the performance of the assays. From this baseline, the community can then assess the applicability of the assays to a wide range of different nanomaterials, and undertake studies to assess the impact of deviations from the protocol on the outcome, as well as utilising the protocols to pre-validate candidate positive and negative control nanomaterials.

Within QualityNano, Round Robins are planned with three levels / rounds for each assay:

1. Initial benchmarking of the community .No protocol supplied, nanoparticles only supplied along with instructions to perform the specific assay as per their in-house method or the manufacturer's guidelines. *Intended to demonstrate the need for harmonisation of protocols and implementation of best practice. Intended to demonstrate the need for harmonisation of protocols and implementation of best practice.*
2. First evaluation of protocol. Protocol that has been assessed for its reproducibility and repeatability in intra-laboratory tests by the protocol sponsoring laboratory is then supplied to all partners, along with the test nanoparticles and the template for reporting back data. *Intended to assess protocol capacity and identify sources of variance across laboratories.*

[1]Users in the strictest sense means Users of the Transnational Access (TA) element of the QualityNano Research Infrastructure, who will also be provided with training in best practice as part of their TA visits, but in this case it means more generally all potential participants in the Training events (QualityNano and externally offered) and all those looking for training materials that they can utilise for their students.

3. Repeat performance of the (revised) protocol Finalised protocol (including clarifications on points of variation in previous attempt) distributed, along with test materials and reporting template. All participants should now get results within the accepted tolerance.

Intended as an informal / pre-validation of the protocol / assay for use with nanomaterials. Protocol can then be handed over to ISO / OECD etc. All partners and several additional partners are now actively engaged in a number of on-going Round Robin studies assessing a range of different physico-chemical and biological impacts. For each study, an initial benchmarking is performed to assess the overall competency of the participating laboratories at the basic assay. Following this the first round robin study with the agreed protocol is performed, and following an evaluation and discussion of the data, the protocol is adjusted and clarified as required, before the final round robin study is performed to demonstrate the robustness of the protocol and the proficiency of the laboratory at performing the assay.

For benchmarking of partner laboratory's performance in size determination and assessment of dispersion protocols, an initial RR study involving all TA partners, all WP2 and WP5 partners and any other partners or additional partners that wanted to participate. The sizes of three candidate nanoparticles (amine-modified polystyrene (PS-NH_2), carboxyl-modified PS (PS-COOH), and IRMM silica nanoparticles) were measured by all partners using as many of the techniques as they had available from Dynamic Light Scattering (DLS), Transmission Electron Microscopy (TEM), NanoSight, and disc centrifuge (DCS). For this baseline study particles were shipped to partners with no protocols provided and partners were instructed to use their in-house protocol or follow the instrument providers' guidelines.

In summary, the most significant progress in the Nanomaterials Hub to date has been in terms of the progress with respect to the Round Robins, both physico-chemical and biological, and in terms of the development of positive control Nanoparticles for apoptosis (with those related to genotoxicity and cell cycle arrest in development). The first multi-partner publication on the NanoSight Nanoparticle Tracking Analysis has been published (Hole et al. 2013; *J. Nanopart. Res.* 15: 2101) and the positive (and negative) control nanomaterials for apoptosis, namely amine (and carboxyl) modified polystyrene nanoparticles. Important insights regarding reproducibility of biological assays have also been made, specifically related to the inherent variability of the assays in the presence of nanoparticles, which themselves are outside the acceptable range for validation of test. As part of the mid-term review, it has been agreed to ensure a closer coupling of Round-robins with training in the Standard Operating Procedures (SOPs). Progress in terms of understanding approaches to storage and curation of nanomaterials has also been made, with the curation task now suspended (as this task is beyond the scope of the project and better handled by organisations with durability such as the JRC) and has been replaced post mid-term with a task assessing the impact of storage conditions on nanoparticles physic-chemical properties and development of optimal storage conditions for retention of nanomaterials quality over time.

Transnational Access

- QualityNano aims to provide approximately 400 Users with access to the state of the art characterisation equipment across the 15 Transnational Access Facilities shown in Figure 2, and to promote the need for characterisation of nanomaterials in situ in the medium in which they will be exposed to living systems.[7]
- Funding for approved applicants will cover the costs of International travel, accommodation, living costs for the researcher, and the cost of provision of the access for the host transnational access facility. Average visits 5-10 working days.
- Visits are fully supported with the technical expertise in the institute of equipment being accessed, and with protocols and nanomaterials as needed.

The User visits from the 3rd (33 User visits-; 408 User days-granted) and 4th calls (40 User visits-; 522 User days-granted) for QualityNano TA have now been completed. , In addition, several of the partners have now fully completed all of their TA visits. The 5th call for TA opened on the 16th September 2013, with a closing date of the 21st January 2014, applications for this call are currently being process. The 6th TA call will be open from 14th March 2014 until 18th April 2014.

Transnational Access is provided according to four thematic categories: (A) nanomaterial synthesis, (B) nanomaterial labelling and pre-processing, (C) nanomaterial characterisation *in situ* / *ex situ*, (D) nanomaterial exposure assessment. The 14 transnational access facilities, as detailed on the map in Figure 1, offer access under one or more of these categories. Details of the application process can be found on the QualityNano website: http://www.qnano-ri.eu/access.html.

Briefly, after contacting the technology expert at the host institution of interest the applicant (User) submits a project, which was previously agreed with the technology expert, through the online application system. The application undergoes an eligibility check and is afterwards evaluated by an external unbiased panel of experts, the User Selection Panel (USP). The USP provides feedback on the application through the online application system. Successful applicants are notified by the QualityNano project office, and have 1 year in which to complete their visit. Exact timing of the visit must be agreed between the User and the relevant technology expert at the host institution. A User Handbook and Frequently Asked Questions sheet are available on the QualityNano website, as well as the contact details for each transnational access facility.

Joint Research Activities

All of the research WPs are underway, with WP meetings scheduled, round robin activities in progress, and first batches of well-characterised nanomaterials available.

- JRA1 - strategies to eliminate and/or reduce nanomaterials batch-to-batch variability: Assessment of sources of variability in widely used synthesis methods is under way and strategies are being developed to eliminate or reduce such sources of variability. Different batches of Silica nanoparticles made via the Stöber synthesis route are being produced to verify the extent of variability among batches and identify the source of such variability. Strategies to reduce this variability will be developed.

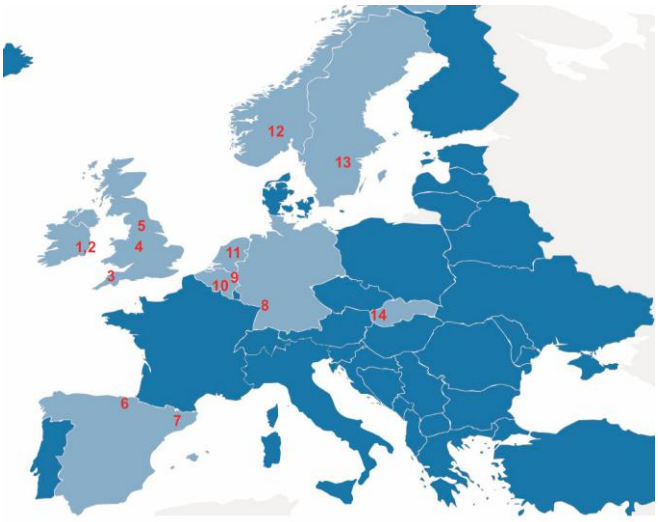


Figure 1: Map showing the locations of the institutes offering QualityNano-funded Transnational Access to state of the art characterisation facilities for nanomaterials in the contact with living systems. Note that a potential User cannot request to visit a facility in the country in which they work.

1 - University College Dublin - Biointeractions of nanomaterials *in situ.* 2 - Trinity College Dublin - High resolution characterisation of nanomaterials. 3 - University of Exeter - Environmental exposure and characterisation of nanomaterials. 4 - University of Birmingham - Stable-isotope labelling of NPs / *in situ* characterisation of NPs in the environment – ecocorona. 5 - University of Leeds - Scale up synthesis / characterisation of nanomaterials. 6 - CIC biomaGUNE - Radiolabelling / *in vivo* biodistribution of nanomaterials. 7 - The Catalan Institute of Nanoscience and Nanotechnology - Synthesis / characterisation of nanomaterials. 8 - Karlsruhe Institute of Technology - Characterisation of nanomaterials/ Aerosol exposure to nanomaterials. 9 – VITO - Biological characterisation of nanomaterials under GLP. 10 - Universite de Namur - Quantitative analysis of nanomaterials in complex matrices and *in vitro* exposure. 11 - Wageningen UR - Quantitative analysis of nanomaterials *in situ.* 12 - Norwegian Institute for Air Research - *In vitro* exposure and genotoxicity of nanomaterials under GLP. 13 - Uppsala Universitet - Cryo-TEM *in situ* characterisation of nanomaterials. 14 - Slovak Medical University - *In vivo* / *in vitro* exposure to nanomaterials (GLP).

- JRA2 - development of reliable labelling: A reliable strategy has been developed to isotopically label ZnO nanoparticles to allow for detection in *in vivo* experimental models; Fluorescently labelled nanoparticles are currently being synthesised and characterised.
- JRA 3 - Development and validation of characterisation tools for nanoparticles *in situ:* a protocol has been developed and refined for single particle Inductively coupled mass spectrometry (SP-ICP-MS); Training in the application of this protocol will be provided to JRA3 partners (and external researchers), and the protocol will be used to perform a round robin study to quantitatively assess the presence and composition of nanoparticles in complex organic mixtures.
- JRA 4 - Development of optimal modes of presentation of nanoparticles for quantitative reproducibility: A literature survey has been undertaken to identify the most widely used methods to present nanoparticles to *in vitro* cell systems; the most promising are being chosen as candidates for round robin testing in order to determine the method that produces the most reproducible and homogenous dose of nanoparticles across all cells in the culture. Effect of cell division / cell cycle on dose and uptake of nanoparticles is also being assessed.
- JRA 5 - Towards development of alternative *in vitro* tests: This WP is scheduled to commence research activities in February 2012. QualityNanoJRA 5 secured a session at the ESOF 2012 (European Science Open Forum conference (11-15 July, 2012, Dublin) http://www.dublinscience2012.ie/. The session was entitled '*What realistic alternatives are there to animal testing to ensure safe introduction of new technologies*?' and hosted a panel discussion targeted at scientists, policy makers and industry. The session is part of ESOF 2012 Theme 5. Science: Reshaping the frontiers of knowledge.

The vision of QualityNano is thus a unified and continuous flow of knowledge and information, from discovery to implementation and dissemination, enhancing the overall access, and service available to the research community (the Users), and raising the quality of the research outputs from the whole field.

6 Expected final results and their potential impacts and use

Exploitable results from QualityNano are defined as *knowledge having a potential for impact on standardisation or policy making* since the focus of QualityNano is on supporting quality driven research and the development of best practice and standardisation in the field.

High quality test nanomaterials, well characterised in situ in complex milieu

An ensuring output from QualityNano will be an improvement in the quality of nanomaterials themselves, and researchers' awareness of the effects or poor nanomaterials characterisation on their data and its interpretation. A key challenge being addressed within QNano is identification of the sources of batch-to-batch variability of nanomaterials, so that the field can move beyond the current approach of securing a single large batch of a nanomaterial and generating large amounts of data on that batch, and instead focus on ensuring that batches fit within defined parameters of "sameness" that reflect both assay sensitivity and acceptable tolerance from production.

Another key challenge being addressed within QualityNano is that of ensuring that labelled variants of nanomaterials are representative of the unlabelled variants, and that they are suitable for use from a regulatory point of view, that is having the same biological fate and behaviour, persistence etc.

Both of these approaches will have significant impact in terms of how regulatory testing in the future is defined and specified, and what levels of "sameness" testing will be required to demonstrate

that the batch tested is representative of the material / production process generally.

Protecting the current knowledge economy – legacy nanomaterials / products

An immediate priority is to stabilise the scientific environment in which the existing (legacy) nano-containing products and current developments are emerging. Unwarranted loss of products and processes that have had considerable European public and private investment is undesirable. Whilst QualityNano cannot (and should not) affect outcomes for any such materials, it is promoting stable scientific evidence-based dialogue, which, where justified will protect such investments. By promoting the need for detailed nanomaterials description and characterisation under the exposure conditions utilised, QualityNano will drive the improvement of data quality in the literature, and facilitate cross-comparability of results, thereby enabling current knowledge gaps to be filled quickly and effectively, and reducing the uncertainty that pervades this topic currently.

Securing the scientific and technical base & the *role of Science as a reliable 'Honest Broker'*

To scientists, regulators, and ultimately industry, Non-Governmental Organisations (NGOs) and governments, there are few more catastrophic outcomes than a general loss of faith in the veracity, neutrality, and potential of science to find answers. Whilst trust is preserved, there remains, irrespective of immediate difficulties, a nucleus for progress. In its absence there is no process or organization in modern society to act as 'honest broker' in issues such as these. QualityNano's drive and dedication to quality, reproducibility and the processes to support them will have an impact, far beyond the scientific facts, by establishing a core of trust with all stakeholders, with deep impacts from science, to regulation, to political structures and will foster a culture of innovation and commercialisation.

Securing the International Co-operation Domain

Trade and commerce in a global economy needs global accommodations and understandings. While it is rare for a science and technology activity to have deep and immediate impacts on trade and commerce, where regulation is concerned there is a strong drive towards harmonisation and global solutions. Indeed, the largest volume of manufactured engineered nanomaterials now derives from regions outside Europe, and access to the internet means that even products not approved for sale in Europe can be easily obtained. An important contribution from QualityNano will be to support the development of internationally agreed standards, protocols and approaches for nanosafety assessment. QUalityNano partners are actively involved in the new EU-US Communities of Research (CoR), with QualityNano providing the administrative support for the Ontology and Databases CoR.

Developing the knowledge economy of the future - a new value chain

The process of ensuring safety of nanomaterials in applications will, in time, emerge as a business opportunity in itself. Safety culture, equipment, trained specialists and practices will ensure a new set of industry services, consultancies and so forth to service this arena, and explicit connections are being made to these industries. QNano's ultimate aim will be to move towards innovation and economic development, ensuring safe and smooth implementation, in partnership with industry. Such a longer term view of the safety issue requires a new approach to organization of the R&D value chain in which 'safety by design' becomes an integral part of the process. This shift of culture and dynamic will require much scientific maturation. Certainly its basis is quality and reproducibility, for that provides secure and durable knowledge, and QualityNano will continue to drive this view.

A new scientific domain with immense positive potential for human health

The endogenous signalling and processing mechanisms of living matter are on the nanometer scale. Consequently, nanoscience will likely make some of its most enduring positive contributions in nanodiagnostics and nanomedicine, underpinned by the emerging scientific field of bionano interactions. Thus, the progressive growth of reliable, reproducible and ultimately quantitative mechanistic knowledge of how the nanoscale interacts with living organisms will have a durable value to society at large. This kind of knowledge is not well retained in small projects, or activities with short term priorities, and will be best captured in a more durable structure, for which QualityNano could reasonably be the seed.

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8 Directory

Table 1 Directory of people involved in the QualityNano project as beneficiaries.*

First Name	Last Name	Affiliation	Address	e-mail
Olivier	Aguerre-Chariol	Institut National de L'Environnement et des Risques Ineris	Parc Technologique Alata Verneuil en Halatte 60550 France	olivier.aguerre.chariol@ineris.fr
Rob	Aitken	Institute of Occupational Medicine	Research Park North / Riccarton UK-EH14 4AP Edinburgh United Kingdom	rob.aitken@iom-world.org
Sergio	Anguissola	National University of Ireland, Dublin / University College Dublin	Belfield IE-4 Dublin Ireland	sergio.anguissola@cbni.ucd.ie
Markus	Berges	Institut für Arbeitsschutz der Deutschen Gesetzlichen Unfallversicherung	Alte Heerstrasse 111 DE-53757 Sankt Augustin Germany	Markus.Berges@dguv.de
John	Boland	The Provost Fellows & Scholars of the College of the Holy and Undivided Trinity of Queen Elizabeth neat Dublin [Trinity College Dublin]	CRANN Trinity College Dublin Dublin 2 Ireland	jboland@tcd.ie
Flemming	Cassee	Rijiksinstituut voor Volksgezondheid en Milieu	RIVM PO Box 1 3720 BA Bilthoven The Netherlands	Flemming.cassee@rivm.nl
Kenneth	Dawson	National University of Ireland, Dublin / University College Dublin	Belfield IE-4 Dublin Ireland	kenneth.a.dawson@cbni.ucd.ie
Maria	Dusinska	Norsk institutt for luftforskning [Norwegian Institute for Air Research]	P.O.Box 100, Instituttveien 18 NO-2027 Kjeller Norway	maria.dusinska@nilu.no
Teresa	Fernandes	Heriot WattsUniversity	Heriot-Watt University Edinburgh Campus Edinburgh EH14 4AS United Kingdom	t.fernandes@hw.ac.uk
Tamara	Galloway	University of Exeter	Prince of Wales Road Exeter EX4 4PS United Kingdom	t.s.galloway@exeter.ac.uk
Karen	Griffin	National University of Ireland, Dublin / University College Dublin	Belfield, Dublin 4 Ireland	Karen.Griffin@cbni.eu
Frans	Kampers	Wageningen University	Dreijenplein 8 Wageningen 6703 HB Netherlands	frans.kampers@wur.nl

First Name	Last Name	Affiliation	Address	e-mail
Rafi	Korenstein	Tel Aviv University	Ramat Aviv Tel Aviv 69978 Israel	korens@post.tau.ac.il
Wolfgang	Kreyling	Helmholz Zentrum Muenchen [Helmholtz Centre Munich]	Ingolstaedter Landstrasse 1 Neuerberg 85764 Germany	kreyling@helmholtz-muenchen.de
Dominique	Langevin	Centre National de la Recherche Scientique	Campus Université Paris Sud, Bâtiment 510 Orsay 91405 Cx France	langevin@lps.u-psud.fr
Iseult	Lynch	University of Birmingham	Edgbaston Birmingham B15 2TT United Kingdom	i.Lynch@bham.ac.uk
Hans J.P.	Marvin	Wageningen University and Research Centre / Stichting DLO-RIKILT	Bornsesteeg 45 NL-6708 PD Wageningen The Netherlands	hans.marvin@wur.nl
Marco	Monopoli	National University of Ireland, Dublin / University College Dublin	Belfield, Dublin 4, Ireland	Marco.monopoli@cbni.ucd.ie
Sergio	Moya	Asociacion Centro de Investigacion cooperative en biomaterials	Paseo Miramon Parque Technologico de San Sebastian Edifico Empresarial C 182, San Sebastian 20009, Spain	smoya@cicbiomagune.es
Inge	Nelissen	Vlaamse Instelling Voor Technologish Ondersoek N.V.	Boeretang 200 Mol 2400 Belgium	inge.nelissen@vito.be
Stefan	Nygren	Uppsala University	St Olofsgatan 10B Uppsala 75312 Sweden	Stefan.Nygren@angstrom.uu.se
Vanessa	Pike	Natural History Museum	Research and Consulting Office The Natural History Museum, Cromwell Road London SW7 5BD United Kingdom	v.pike@nhm.ac.uk
Victor	Puntes	Catalan Institute of Nanotechnology	Campus de la UAB Edifici CM7 Barcelona 08193 Spain	Victor.Puntes@uab.cat
Joachim	Rädler	Ludwig-Maximilians Universität München	Geschwister-Scholl-Platz 1 Munich 80539 Germany	joachim.raedler@lmu.de
Michael	Riediker	Institut universitaire romand de Santé au Travail [Institute for Work and Health]	Rue du Bugnon 21 CH-1011 Lausanne Switzerland	michael.riediker@hospvd.ch

First Name	Last Name	Affiliation	Address	e-mail
Kai	Savolainen	Finnish Institute of Occupational Health	Finnish Institute for Occupational Health Helsinki Finland	Kai.Savolainen@ttl.fi
Olivier	Toussaint	Facultes Universitaires Notre-Dame de la Paix de Namur	Rue de Bruxelles 61 B-5000 Namur Belgium	olivier.toussaint@fundp.ac.be
Lang	Tran	Institute of Occupational Medicine	Research Park North / Riccarton UK-EH14 4AP Edinburgh United Kingdom	lang.tran@iom-world.org
Jana	Tulinska	Slovenska Zdravotnicka Univerzita V Bratislave	Limbova 12 Bratislava 83303 Slovakia	jana.tulinska@szu.sk
Eugenia	Valsami-Jones	University of Birmingham	Edgbaston Birmingham B15 2TT United Kingdom	e.valsami-jones@bham.ac.uk
Terry	Wilkins	University of Leeds	Houldsworth Building Clarendon Road Leeds LS2 9JT United Kingdom	t.a.wilkins@leeds.ac.uk

* Note there are also Additional partners who are non-funded who are not included here.

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SANOWORK

Safe Nano Worker Exposure Scenarios

Contract Agreement: NMP4-SL-2012-280716 Website: http://www.sanowork.eu
Coordinator: Anna Luisa Costa, ISTEC-CNR, Faenza, Italy

Table 1 Consortium List.

No.	Beneficiary name	Short name	Country
1	Institute of Science and Technology for Ceramics-Consiglio Nazionale delle Ricerche	ISTEC	Italy
2	Institute of Occupational Medicine	IOM	United Kingdom
3	Plasmachem Productions und Handel GmbH	PCHEM	Germany
4	Elmarco Sro	ELM	Czech Republic
5	GEA Process Engineering AS	NIRO	Denmark
6	Colorobbia Italia SPA	COL	Italy
7	Bayer Technology Services GmbH	BAYER	Germany
8	Institut National de l'Environnement Industriel et des Risques INERIS	INERIS	France
9	University of Lymerick	UL	Ireland
10	Università degli Studi di Parma	UNIPR	Italy
11	Università degli Studi di Pisa	UNIPI	Italy
12	Acondicionamento Tarrasense Associacion	LEITAT	Spain
13	Istituto Nazionale Assicurazione Infortuni sul Lavoro INAIL	INAIL	Italy

Contents

1. Summary

Project Duration: 1 March 2012 – 28 Feb 2015

Project Funding: 3.5 Mio. EUR

Sanowork project proposes a paradigm shift, addressing occupational risk management of nanomaterials. **Traditional approaches,** in fact, put in place engineering, administrative and PPE measurements **similar to those used** to manage **the risk of hazardous chemicals,** nevertheless they are limited by the still existing gap of knowledge about potential risk that can aris**e** by nanomaterials manufacturing processes. To overcome such limits, Sanowork project develops safety by materials design control measures able to design out risks rather than address them when they occur. At this purpose the following activities are carried out: 1. Development of different design option based risk remediation strategies (RRS), containing hazard and worker exposure potential; 2. Implementation of a rigorous exposure assessment in order to evaluate the effectiveness of existing and proposed exposure **reduction** strategies; 3. Hazard assessment by intelligent, iterative, hazard identification and in vitro testing strategy, aimed to ensure a rapid and effective evaluation, in real time, of the health impact mitigation strategies 4. perform risk analysis off line and on site in order to identify substance product properties and operational condition that ensure a safer worker exposure scenario. 4. Assess COST/ EFFICIENCY of the proposed strategies on the basis of risk analysis results, materials/properties efficiency, risk transfer to insurance underwriter community. The following "representative" pool of NMs and nanoproducts have been selected: ZrO_2 (chemical or ceramic raw material); TiO_2 and Ag nanoparticles (ceramic or textile photocatalytic/antibacterial coatings); CNTs (polymeric nanocomposites); polyamide nanofibers (nanostructured membranes for water depuration system), TiO_2 nanofibers (photovoltaic cells coatings). The strategy is addressed to mitigate risk by decreasing adverse health

hazard and emission potential of nanomaterials, setting back processes of transport to the point of entry. A sound balance between exposure and health hazards data, before and after the introduction of existing and proposed risk remediation strategies, allows to evaluate the effectiveness of existing and proposed exposure reduction strategies. The cooperation with industrial key partners such as Plasmachem, Elmarco, GEA Niro, Colorobbia,

Strong proponents of nanotechnology, such as Lux Research, anticipate that "nanotechnology applications will affect nearly every type of manufactured good over the next ten years." Nevertheless the promise by nanotechnology of a significant contribution in boosting the economy, living standards and improving the quality of life may be outweighed by the perceived occupational, environmental health and safety risks that it poses.

Due to the lack of quantitative risk assessment, underwriting such risk is particularly difficult and may compel the underwriter community to refuse to insure nanotechnology industry in the fear of potential bankruptcy. Small and Medium Enterprises (SMEs), which are in the driving seat of nanotechnology innovation, are particularly vulnerable to such conditions as they lack resources to put expensive preventive measures in place to safeguard their workers' health and safety.

Owing to increasing knowledge in the nanotechnological development and Occupational Health and Safety issues, there is a new awareness that safety options should also include "smart" nanoparticle (NP) design (i.e. safe by design) to ensure effectiveness of preventive measure.

Given the limited amount of information about the health risks associated with occupational exposure to NMs, the precautionary principle has suggested to take measures to minimize worker exposures. Research Institutions and Governmental Agencies have addressed specific efforts to clarify the nature and the extent of potential hazard raised by handling NMs and to provide a solid platform for nanotechnology occupational safety and health. Thus, a hierarchy of control measures as applied to inhalation and dermal risks, including elimination, substitution, process enclosure, engineering controls, procedural control, personal protective equipment (PPE) has been identified.

All these measures have been implemented on a voluntary basis by some industries worldwide, but the majority of companies does not foresee unintentional release of NMs throughout the life cycle.

Only recently, "Prevention through Design" (PtD) has been envisaged as a proactive tool to prevent possible exposure and risk. PtD is an approach (and in the U.S., a national initiative) to design out hazards rather than address them when there are exposures. Such an approach is particularly applicable to NMs at the molecular and process scales. At the molecular scale, there is potential for modification of molecules to retain commercial and scientific functionality while reducing toxicity. At the process scale, companies can look to the pharmaceutical industry for engineering controls that could be adopted for potentially hazardous NMs.

What is Sanowork

The SANOWORK project is built around the promotion, development and implementation of "Elimination/Substitution" control strategies and proposes to fill the gaps that already delay their diffusion. In order to address nanomanufacturing industries needs, SANOWORK project propose sustainable Risk Remediation

Bayer guarantees an accurate exposure assessment in the workplace and a realistic COST estimation of proposed RRS.

2 Background

Strategies with a balanced approach between design for manufacturing and design for safety.

The evaluation of proposed Risk Remediation Strategies will pass through a globally harmonized analysis and reporting of process/NMs performances, hazard data, emission/exposure collection, in relation to operational conditions and NMs physico-chemical properties. The process/NMs performances and risk specific evaluation will be performed off line (NMs as delivered, provided by companies or surface engineered during the project, exposure scenarios at lab scale level) and on-site (NMs collected on-site, exposure scenarios at pilot scale level). The process and NMs performances, as well as exposure and hazard profiles, will be assessed BEFORE and AFTER the introduction of risk control extra-steps.

We will develop and integrate NMs design options strategies within target processing lines as extra-steps for improving the efficiency of the process while preserving and/or increasing NMs performances. Health risk depending on the intrinsic hazard and exposure frequency/concentration level, will be evaluated according to NMs physico-chemical properties.

Five strategies will be proposed based on NMs surface engineering so that exposure can be reduced but if exposure has accidentally occurred the health hazard would still be decreased. Based on the knowledge of NMs dispersion behaviour, a combination of self-assembled monolayer coating and tailored aggregation processes will be developed in order to decrease the hazard and/or emission potential of target NMs (strategies: I, II, III, V). The described strategies will be accompanied by a process of immobilization of NMs with an expected exposure potential (strategies: IV). The SANOWORK proposed strategies are industry-driven and will therefore comply with the following criteria:

satisfying the production requirements;

cost-effectiveness and suitability for large-scale production;

easy processing-line implementation for manufacturing nano-structured components;

decreasing exposure potential and/or health impacts, while preserving nano-scale properties.

Manufacturing processes, relevant for different industrial sectors, were identified and the proposed 'primary prevention' of risk will be integrated within six processing lines and implemented at pilot scale level by companies involved in SANOWORK.

The final goal is to develop and demonstrate the efficacy of "design options" based Risk Remediation Strategies, providing practical tools for:

- developing potentially useful safe design features;
- preventing NMs related worker injuries;

- reducing the need of expensive risk management measures;
- implement safe manufacturing processes.

3 Scientific and technological challenges

Sanowork's key strengths are underlined below.

KEY CONCEPT

- Industrial driven strategies applied on-site to target processing lines.
- Product design options for safer nanotechnology.
- Decreased emission potential and/or human hazard, preserving the nano-scale properties.
- Practical risk assessment of NMs with a reasonable balance between health hazards and exposure data.

KEY BENEFITS

- Cost-effective tools for safer manufacturing processes.
- Primary prevention of potential risks that can occur during worker manufacturing or customer use.

WORK PLAN DESCRIPTION

The overall work plan is designed for 42 operational months and comprises 7 Work Packages (WPs). A schematic representation of the work plan is reported in Figure 1.

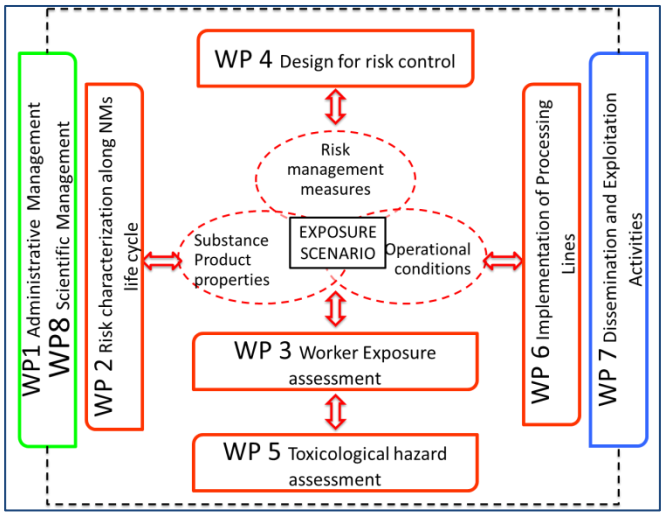

Figure 1: *Sanowork WPs description (Pert Chart)*

An exposure scenario as requested by REACH deals with the collection of substance/product properties, operational conditions, risk management measures to take the exposure of workers to NMs under hazardous level. The SANOWORK S/T methodology is driven by the REACH definition of exposure scenario and establishes a synergy between WPs 2-6, in order to take as much indications as possible for the development of a safe worker exposure scenario.

WP1 and WP8 are the Administrative and Scientific management workpackages, respectively. In this WP, we will implement a rigorous administrative management process to ensure the timely achievement of the project deliverables and the scientific excellence of each workpackage task.

WP2 is related to the assessment and analysis of the risks caused by the occupational exposure to SANOWORK target NMs, in different stages of their manufacturing life cycle. LEITAT will develop databases which will be versatile to include new data from other sources. The essential information on NMs characteristics, exposure potential and toxicity to be used for the risk assessment will be included from literature sources as well as from data generated in the project by WP3 and WP5 worker exposure and toxicological hazard characterization, respectively. The risk analysis will provide the inputs to: 1) evaluate the effectiveness of existing or proposed risk remediation strategies; 2) assist UL in transferring information to insurance companies.

An occupational exposure assessment strategy (WP3), will be developed by INERIS. INERIS will collect exposure data 1) off-line, by semi-quantitative approach on as-delivered NMs and on chronic or accidental scenarios, at the lab scale (data useful for the so called RISK 1 assessment); 2) on-site, by a quantitative approach on processing lines (data useful for the so called RISK 2 assessment). It will also apply a control banding approach to risk assessment and management, providing data useful for a comparison between existing and proposed risk remediation strategies.

The toxicological hazards (WP5) will be assessed by IOM, UNIPR and UNIPI. ISTEC will develop "Design Options" based Risk Remediation Strategies and evaluate NMs functional properties and their performances in relation to specific steps of the process. UL will assist ISTEC, INERIS, UNIPR, UNIPI and IOM providing a physico-chemical characterization of NMs.

PCHEM, NIRO, ELM, COL and LEITAT will develop industrial demonstration platforms to prove the integration of proposed risk control extra steps in different pilot lines (WP6), and make the industrial scenarios available for exposure assessment on-site. ISTEC will supervise the implementation of target processing lines and will assist UL for COST/BENEFIT evaluation. The results of the risk analysis (WP2) merged with COST/BENEFIT evaluation (WP6) of the proposed strategies will represent the basis for the definition of the safe worker exposure scenarios, tailored for each of the target processing line.

In WP7 the results and other outputs of the project will be disseminated internally and externally, after appropriate intellectual property protections, as and when needed. The results of risk analysis and COST/BENEFIT evaluation will be disseminated and used to inform the industrial partners about the strategies to implement in their processes, in order to create a safer workplace environment. Sanowork is structured and organized around 4 technical Work Packages (WP2-6), beside project management and dissemination/exploitation of results (WP1, WP8 and WP7).

4 Objectives

The Sanowork project develops safe by material design concepts, addressing three main goals:

1) TO DEVELOP "DESIGN OPTION BASED" RISK REMEDIATION STRATEGIES
2) TO INTEGRATE THEM IN MANUFACTURING PROCESSING LINES
3) TO EVALUATE THEM:
   5 by implementing EXPOSURE assessment methodologies in the WORKPLACES
   6 by performing a RISK analysis BEFORE and AFTER the application of the proposed strategies
   7 by analyzing COST / BENEFIT on the basis of the risk analysis results, NMs performances & extra steps cost evaluation.

Figure 2 shows a schematic of the target nano-objects investigated and the risk determinant factor towards whom RRS have been addressed.

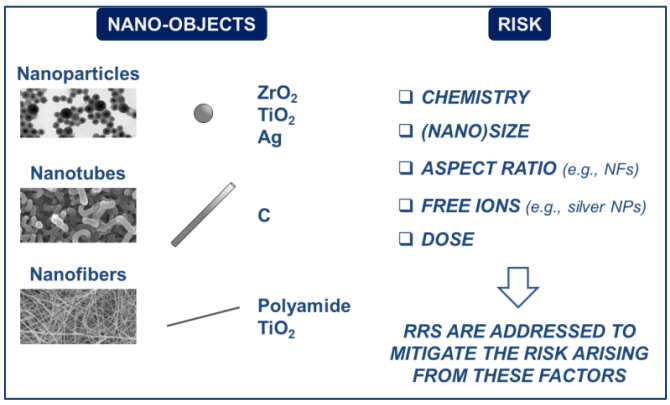

Figure 2: *Sanowork target nano-ojects and risk determinant factor considered to develop RRS*

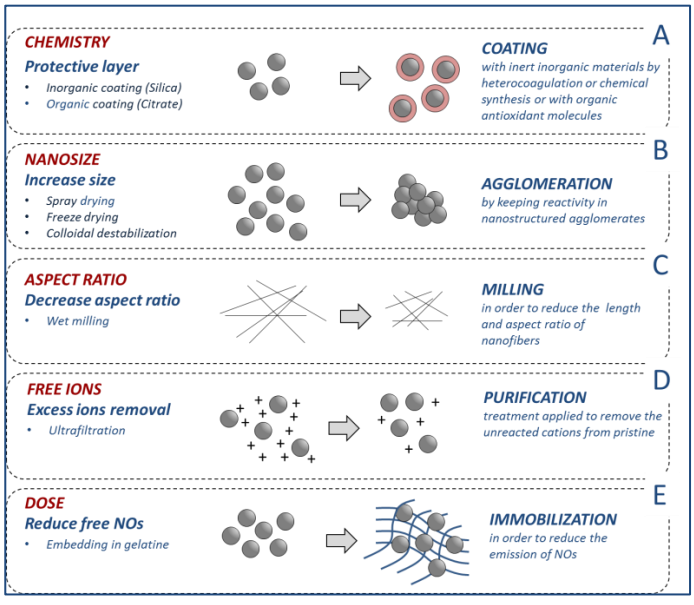

Figure 3: *Sanowork RRS integrated into 6 processing lines*

The safety by molecular design RRS developed and integrated into six manufacturing processing lines are schematized in Figure 3.

They are all based on surface engineering modification aimed to decrease health impact and/or exposure potential in occupational industrial scenarios.

PL	Risk Analysis	Exposure scenario	Data source	Associated experimental exposure data	Data source
1	Environmental Risk Assesment	Washing / Disposal / Recycling of ZrO_2 NPs Production Process	Environmental Exposure Scenario to be identified	Quantitative data of the generated nanowaste	Plasmachem
2	Off-line	Application of ZrO_2 NPs as pigments and in ceramics (Industrial scale)	MARINA Worker Exposure Scenario (already sent)	Off-line: dustiness	Deliverable 3.1 and INERIS on going data
4	Off-line	Application of TiO_2 NF in the production of dye-sensitized solar cell (Industrial scale)	MARINA Worker Exposure Scenario (already sent)	Off-line: dustiness	Deliverable 3.1 and INERIS on going data
5	Based on literature data and Sanowork Hazard data	Realistic exposure industrial scenario	Colorobbia (Deliverable 3.1)	Data available from literature or estimated	Literature /Estimation
6	On site	Feeding of the extruder (Pilot scale)	MARINA Worker Exposure Scenario (already sent)	CPC & TEM –Area-Background & Activity (Basic campaign) CPC & PAS & FMM & Areo Track & TEM— Area-Worker-Background & Activity with/without nano (Intensive campaign)	INERIS CD
6	Off-line (for comparison purposes)	Feeding of the extruder (Pilot scale)	MARINA Worker Exposure Scenario (already sent)	Dustiness	Deliverable 3.1
6	Off-line	Feeding of the extruder (Industrial scale)	MARINA Worker Exposure Scenario (already sent)	Dustiness	Deliverable 3.1

Table 1 *Risk analysis levels considered on the base of available exposure scenarios*

5 Progress and Outcomes to date

The project reaches the 24 months term at 1st of March 2014.

The work performed by each WP and the main activities performed until now are summarized below.

WP2 The progress of WP2 in the first 24 months is according to the original workplan.

The most notable results so far were:

- *The hotspots of each processing line/NM were identified and all the information was compiled, evaluated and reported.*
- *The evaluation of the risks and benefits of the processes has been used to make a first evaluation of risk management strategies.*
- *The definition and creation of the databases is another important achievement. After consortium agreement, the physicochemical an toxicological databases were uploaded in the website, which will allow to maintain up to date and available for all the partners all the information obtained during the execution of the project.*
- *So far, most of the necessary information available about the NM selected as representative ("reference NM") existing on literature has been collected and summarized for its use in the risk characterization approach.*
- *The UL team has worked closely with the insurance industry and have strengthened links with the Lloyds insurance market. An underwriting model for nano-materials and the first iteration of this model along with an in depth discussion of the methodologies underpinning the approach developed was published in April 2013 in the prestigious journal Nature Nanotechnology.*

During the Mid Term Meeting it was made an overview of the data available or generable in the next months, to perform Risk Analysis. It was established different levels of risk analysis and agreed which processing lines prioritize and which characterization complete in order to get sound and useful data to perform the evaluation of risk remediation strategies (RRS), MS6 expected for Month 30, see Table 1, where exposure scenarios and data collected at the present are reported.

Deliverables/Milestones achieved

- *D2.1: General description of the NMs and their life cycle stages (Month 3)*
- *MS4: Selection of Risk Remediation Strategies based on NMs risk off-line and performances analysis has been achieved at M18*

WP3 The progress of WP3 in the first 24 months is according to the original work plan.

The following significant results were obtained:

- *Qualitative and semi quantitative exposure results of the 6 process lines.*
- *Evidence the presence of inhalable nanostructure materials in the work places.*
- *The exposure scenarios identified with a ranking of the exposure risks*
- *Offline tests validation of the risk remediation strategies applied to the process lines*
- *Semi-quantitative risk assessment carried out on the 6 process lines through different control banding (CB) approach.*

Deliverables/Milestones achieved

- *MS1 "Worker Exposure to Nanomaterials: Handbook on the Strategy and Methods for Offline Characterizations and On-site Measurements" (Month 6)*
- *D3.1 Report on the development and the application of a qualitative worker exposure methodology.*
- *D3.2 Results of the risk assessment methodology via a control banding approach*

WP4 The progress of WP4 in the 24 months is according to the original workplan.

The following activities summarized by tasks are summarized below:

Task 4.1 *Risk remediation strategies applied to Processing Line 1*

Organic coating was not applied because ZrO2 powder was well water dispersible and stable even without the aid of any dispersant. Moreover the addition of dispersing agent can represent an extra cost and negatively affect the following sedimentation and recycle steps. Anyway samples with organic coatings were prepared in order to verify any effect from the toxicological point of view. The citrate coating was preferred with respect to PEG because it is a short and negatively charged molecule which could present some advantages from a toxicological point of view. Inorganic coating with silica was not foreseen but it was added in order to have an inorganic counterpart useful to be compared with the organic coating and with the other nanoparticles coated with silica (PL5).

Task 4.2 *Risk remediation strategies applied to Processing Line 2*

RRS of coating was not applied considering that both silica sol and citrate salt could negatively affect the final sintering of ceramics.

RRSs of organic/inorganic coating were proposed in order to improve the powder dispersion and the subsequent granulation. However considering that the pristine material is easily dispersible without any additives, the organic/inorganic coatings have been avoided. In addition, both silica sol and citrate salt could negatively affect the sintering step, so the investigation has been focused on uncoated ZrO2, and for this processing line the risk remediation strategy of coating has not been applied. Freeze drying was added as alternative method to spray-drying, typical used for the powder treatment of ceramic process. The performance evaluation of ZrO2 as additive for the preparation of ZrSiO4:Pr was introduced in order to better identify the advantages obtained from spray drying unfeasible by using the traditional ceramic process.

Task 4.3 *Risk remediation strategies applied to Processing Line 3*

The organic film coating was prepared applying gelatin instead of PEG and alginate. With respect to PEG, gelatin is more biocompatible while with respect to alginate it is cheaper. Both aspects are fundamental for accomplishing the objectives.

Task 4.4 *Risk remediation strategies applied to Processing Line 4*

Surface coating was avoided and only the size control strategy was applied, because it was supposed to be the less detrimental for the final materials performance.

Task 4.5 *Risk remediation strategies applied to Processing Line 5*

Due to the not foreseen presence of a great amount of unreacted Ag+ two remediations strategies at the aim to purify the pristine sample were added: purification and immobilization.

The evaluation of the antibacterial activity of pristine and modified Ag samples was performed at sol state and provided useful data to predict the antibacterial activity of the sols applied as coating on

ceramic tiles, whilst the photocatalytic performance of TiO2 modified samples were evaluate in probe reaction by using a typical dye.

***Task 4.6** Risk remediation strategies applied to Processing Line 6*

No significant effects in the modified samples (spray-drying) in comparison with pristine ones were found.

Deliverables/Milestones achieved

- *D4.2 "Report on Physiochemical and Functional properties of Pristine and Modified Nanomaterials" (Month 24)*

WP5The progress of WP5 in the first reporting period is according to the original workplan.

The most notable results are summarized below:

A series of viability experiments to identify the hazardous properties of pristine NMs and to assess in vitro relevant cell responses, were carried out. Viability was assessed with the resazurin method in a range of doses from 2.5 (0.3125 for silver) to 80 μg/cm2 of monolayer surface at three experimental times (24, 48 and 72h) in two cell lines (A549 alveolar carcinoma cells and Raw264.7 macrophages).

-
- *According to discussions during periodic meeting at steering board level, it was decided to not perform toxicological hazard assessment of ZrO2 (Processing Line 2, PL2) and of Polyamide fibers (PL3), given the lack of relevant exposure scenarios.*
- *All the materials, included in the remaining four PLs (PL1, 4,5,6), have been tested both in their pristine (BEFORE) and remediated (AFTER) forms, towards macrophages and alveolar epithelial cells, as an in vitro model of the gas exchange region of the lung. The toxicity profile of several TiO_2 compounds on two different PLs was also tested during the last working period, in particular upon receipt the effects of TiO_2 wet-milled nanofibres were compared to those of pristine TiO_2 nanofibres. Results were gathered with UICC crocidolite and Aeroxide P25 as benchmark control materials, in order to provide data on well-studied materials, and validate efforts in risk assessment of the test materials.*
- *For one nanoparticle (Ag PL5) the testing strategy has already yielded data sufficient to implement a model for a hazard characterization strategy, to develop in collaboration with the SME.*
- *Details of the progress are given below for each material/processing line.*

Deliverables/Milestones achieved

- *MS2 "Definition of a panel of toxicological tests to assess hazardous properties" (Month 6)*
- *D5.1 "Report on hazard potential identification" (Month 15)*

WP6 The progress of WP6 in the 24 months is according to the original workplan.

As shown in Figure 4, on the base of exposure scenarios available, the six processing lines were evaluated at different technology readiness level: for the lack of a real industrial scenarios and due to the low exposure potential as revealed by INERIS preliminary campaigns, for PL 2, 3 and 4 the implementation was performed and studied at lab-scale level, for PL 6 and 1 at pilot-scale level, whilst only for the PL 5 within a real industrial setting*It was further finalized exposure scenarios BEFORE and AFTER and industrial/lab scale facilities available for their characterization.*

Deliverables/Milestones achieved

- *D6.1 "Implementation of Processing Lines 1-6 (BEFORE)" (Month 6)*
- MS5 "*Implementation of Processing Lines" (Month 18)*
- *D6.2 "Implementation of Processing Lines 1-6 (AFTER)" (Month 24)*

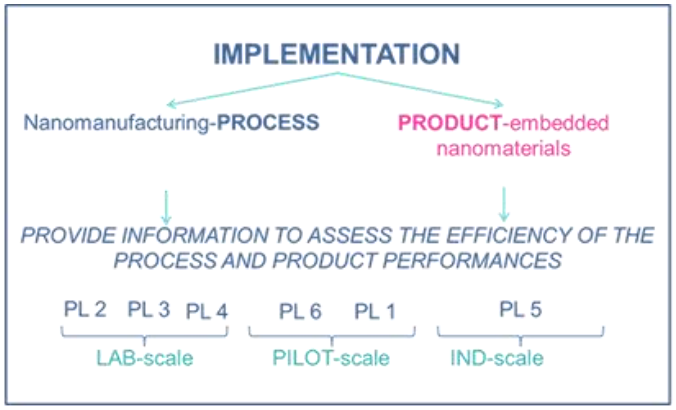

Figure 4: TRLs for the implementation of processing lines

WP7 The progress of WP7 in the first 24 Months is according to the original workplan.

The most recent and notable results are:

- *It was organized the 1° Workshop of projects funded under the same topic: SANOWORK, NANOMICEX and SCAFFOLD, at IT 2014 (Athens, 11 April, 2014).*
- *It was presented as a contribution to the following conferences:*

NanotechItaly 2013, Venice 27-29 November 2013

Authors: Anna Luisa Costa

Title: Safety by nano-design strategies applied to real industrial case studies (ORAL)

11° ICEM 2013 - 3-8 November 2013, Foz do Iguassu, Brasil

Authors: Chiara Uboldi, Sebastiano Di Bucchianico, Camilla Delpivo, Magda Blosi, Anna Costa, Fabio Coppedè, Lucia Migliore

Title: The importance of surface coatings in determiningthe toxic potential of nanoparticles (POSTER)

20° Convegno di Igiene Industriale "Le Giornate di Corvara" – 26 - 28 March 2014

Authors: Giuseppe Castellet y Ballarà, INAIL

Title: Progettazione "sicura" di nanomateriali

NANOTOX 2014, 7th International Nanotoxicology Congress" to be held on April 23rd-26th, 2014

Authors: Craig A. Poland, Julia Varet , Ovidio Bussolati, Gordon Fern, Lucia Migliore, Sebastiano Di Bucchianico, Steve Hankin, Enrico Bergamaschi ***Title:*** *Use of physicochemical characterisation information for the rapid and cost-effective identification of potential hazards during nanomaterial and nano-product development*
Authors: G. Janer, J. Cabellos, C. Poland, E. Bergamaschi, L. Migliore, A.L. Costa, S. Vazquez-Campos ***Title:*** *Using in vitro toxicity data for human risk assessment of nanomaterials. Assessment of feasibility.*

Authors: M.G. Bianchi, M. Allegri, O. Bussolati, M. Blosi, S. Ortelli, E. Bergamaschi Title: Titanium dioxide nanoparticles enhance LPS-dependent activation of murine macrophages via the Toll-like Receptor 4 (TLR4) pathway

Authors: M. Allegri, M.G. Bianchi, O.Bussolati, A. Costa, M. Blosi, C. Delpivo, S. Ortelli, C.A. Poland, J. Varet, E. Bergamaschi ***Title:*** *In vitro toxicity of high aspect ratio titanium dioxide nanofibers*

4th International Colloids Conference: "Surface Design and Engineering," June 15 - 18, 2014, Madrid, Spain

Authors: *S. Ortelli, M. Blosi, C. Delpivo, I. Gualandi, D. Tonelli, I. Fenoglio, P. Matteucci, C. Poland, A.L. Costa* ***Title:*** *Safety by design" approach to manage nanotitania surface photoreactivity*

The 8th International Conference on the Fundamental Science of Graphene and Applications of Graphene-Based Devices, June 23-27, 2014, in Gothenburg, Sweden

Authors: *Craig A. Poland, Julia Varet, Gordon Fern, Ovidio Bussolati, Lucia Migliore, Sebastiano Di Bucchianico, Lang Tran, Enrico Bergamaschi, Steve Hankin* ***Title:*** *Potential Hazards of Graphene: Physicochemical properties of nanomaterials that promote toxicity and the use of targeted characterisation in the rapid and cost-effective screening of potential hazards during R&D*

US-EU Workshop, Bridging NanoEHS Research Efforts, [Arlington, December 2-3, 2013]

Authors: *Enrico Bergamaschi_Member attendee - on behalf of SanoWork Project*

Industrial Technology 2014, 9-11 April 2014, Athens

Authors: *Davide Gardini on behalf of Sanowork Consortium* ***Title:*** *Integration of safer by design nanoproducts into real industrial case studies*

- *the following scientific papers were prepared*

P Published

1) A.L. Costa, "A Rational Approach for the Safe Design of Nanomaterials", Nanotoxicology: Progress toward Nanomedicine, Second Edition, Editor(s):Nancy A. Monteiro-Riviere, C. Lang Tran (2014)

2) NIRO, "Free nanoparticles in work places can be bad for your health" and "It's better to have more safety than too little", article istributed to Danish companies, universities, schools and work places in general

In *Submission*

1) Hester, K., Mullins, M., Murphy, F. and S. A. M. Tofail (2014). "Common Law and Nanotechnology: The issue of toxicity in tort litigation" Nanotechnology Perceptions

2) Baublyte, L., Mullins, M. and S. A. M. Tofail (2014). " Insurance Market's Perception of Nanotechnology and Nano materials Risks "

3) McAlea, E.M, Mullins, M., Murphy, F. and S. A. M. Tofail (2014). " Engineered Nano materials: Risk Perception, Regulation and Insurance " Journal of Risk Research

4) A. Costa, M. Blosi, C. Delpivo, S. Ortelli, Giovanni Baldi, Arianna Signorinio, M. Allegri, M.G. Bianchi, O.Bussolati, E. Bergamaschi "Nanosilver: an innovative paradigm to promote its safe and active use", ACS Nano

5) A.L. Costa, S. Ortelli, M. Blosi, C. Delpivo, D. Gardini, I. Gualandi, D. Tonelli, I. Fenoglio, V. Aina, Abbasi Ghandi, Tofail Syed, M. Dondi, "An integrated approach to assess photocatalytic activity of TiO2 in self-cleaning textiles", Journal of colloids and Interface Science

6) Chiara Uboldi, Sebastiano Di Bucchianico, Camilla Del Pivo, Fabio Coppedè, Magda Blosi, Anna Costa and Lucia Migliore, "Remediated titania nanoparticles to reduce lung exposure at work places: an in vitro study" Int. J. Mol. Sci. 2014, 15, 1-x manuscripts; doi:10.3390/ijms150x000x

Nearing Submission

1) McAlea, E.M, Murphy, F. Mullins, M. and S. A. M. Tofail (2014). " QSAR Methodology for Correlating Noisy Hazard and Physicochemical Measurements of Nano Materials within a Bayesian Framework"

3)M.G. Bianchi, M. Allegri, M. Blosi, S.Ortelli, A.Costa, C. Delpivo, O. Bussolati, E. Bergamaschi "Titanium dioxide nanoparticles enhance LPS-dependent activation of murine macrophages via the Toll-like Receptor 4 (TLR4) pathway", TOXICOLOGY LETTERS

3) Authors (INERIS, ISTEC) "Dustiness testing, a support to nanosafety-by-design

WP8 The scientific coordination of the project is documented by minutes of meetings and by Mid Term Scientific Report (submitted 29/10/2013) and Interim Progress Report (D8.2, submitted 16/04/2014)

Deliverables/Milestone

- *D8.2 "Interim Progress Reports" (Month 24)*

6 Expected Impact

The Sanowork expected impacts are discussed in relation to objectives addressed

To develop practical and cost effective strategies for reduction of worker exposure to NMs during all stages of NMs production, use and disposal

NMs design strategies developed by SANOWORK project will contribute effectively to reduce health hazard and exposure potential. In particular it will develop: 1) aggregation control strategy, especially addressed to decrease emission potential during production and use, also including accidental spills; 2) inorganic/organic coating especially addressed to decrease health impact and exposure during use and disposal inside and outside the workplace; 3) techniques for disintegration of NPs during washing, especially addressed to decrease emission potential of disposal treatment.

To establish synergy with QualityNano: the European Union-funded infrastructure for NM safety testing

A proposal for improving physicochemical characterization of modified samples and to allow a sound correlation with health impact assessment results have been submitted to QualityNano platform, on April 2014. Electron microscopy facilities of University Trinity College have been identified to carry out the project.

To contribute to the advancement of the EU NanoSafety Cluster goals and agenda by:

- providing data on toxicological effects of target NPs (pristine and modified ZrO2 NPs, polyamide and TiO2 nanofibers, TiO2 and Ag NPs and CNTs) to facilitate the formation of 'a consensus on nanotoxicology in Europe';

- Creating Life Cycle Database on physico-chemical, and toxicological properties of these materials based on FP6 and FP7 research. This database will be made available to NanoSafety Cluster 'to avoid duplicating work and improve efficiency';

- Identifying worker exposure scenarios and implementing the prevention/intervention measures to minimise Risk;

- Participating in meetings and workshops organised by NanoSafety Cluster for 'discussion, problem solving and planning R&D activities in Europe';

- Preparing and implementing a communication strategy to inform the identified stakeholders: industries, insurance companies, general public on risk remediation tools that prevent risks during production, use and disposal of NMs.

To facilitate research cohesion and integration in this area

The cohesion and integration of the results generated by SANOWORK will be facilitated by the active participation of the project members in the NanoSafety Cluster meetings and by dissemination activities of the project. More specifically, the SANOWORK partners will integrate with the activities within the NanoSafety Cluster through activities in the following areas:

- pristine and modified ZrO2 NPs, polyamide and TiO2 nanofibers, TiO2 and Ag NPs and CNTs and their physicochemical (chemistry, crystal structure, size, shape, coating, surface charge etc.);

- results on sound toxicological endpoints: 1) cell viability, including apoptosis, and mitochondrial activity; 2) induction/inhibition of pro-inflammatory cytokines and chemokines; 3) oxidative stress; 4) effects on unique cell function or cell –specific endpoints (e.g. immune function, barrier properties, etc…); 5) particle uptake and translocation; 6) fibrogenicity; 7) genotoxicity and cell transformation;

- results of worker exposure assessment plan;

- COST/BENEFIT evaluation of proposed risk remediation tools;

- insurance risk quantification of identified worker exposure scenarios.

Exploitation Strategy

An exploitation strategy seminar was organized by ISTEC on 29th May 2013, in Faenza (Italy). A consultant (Mr. Andrea Di Anselmo), META Group) was appointed to deliver the seminar. Before the agreed date of the seminar, the consortium was asked by the consultant to identify exploitable results, level of innovativeness and selling point, market size, possible competitors, cost, etc. After compiling all contributions, the consulted drafted a "first synthesis report" where three key exploitable results were identified each involving several partners. The main exploitable results able to increase Sanowork proposal impact were:

i) Services including surface engineering of NMs and practices and guidelines on Toxicity, exposure and risk assessment (UNIPR leading)

ii) Identification of new insurable product (UL leading)

iii) Patents on safety by design surface treatments (ISTEC leading)

The consortium was split into 3 groups according to each exploitable result. Each group discussed what the potential for exploitation and risks associated and how the partners can insure the success in exploiting it.

A set of recommendation specific and general were provided to guide the partner to plan for the successful dissemination, exploitation and IPR management of the project.

7 Directory

Table 1 Directory of people involved in this project.

First Name	Last Name	Affiliation	Address	e-mail
Anna Luisa	Costa	ISTEC-CNR (ISTEC)	Via Granarolo, 64, 48018 Faenza, Italy	anna.costa@istec.cnr.it
Lang	Tran	INSTITUTE OF OCCUPATIONAL MEDICINE (IOM)	Research Avenue North, Riccarton, 45, EH14 4AP, EDINBURGH, United Kingdom	lang.tran@iom-world.org
Alexei	Antipov	PLASMACHEM PRODUKTIONS- UND HANDEL GMBH (PCHEM)	RUDOWER CHAUSSEE, 29, 12489, BERLIN, Germany	antipov@plasmachem.com
Denisa	Stránská	ELMARCO SRO (ELMARCO)	Svárovská, 621, 46010, Liberec 10, Czech Republic	denisa.stranska@elmarco.com
Karin	Nordström Dyvelkov	GEA PROCESS ENGINEERING AS (NIRO)	GLADSAXEVEJ, 305, 2860, SOBORG, Denmark	karin.dyvelkov@geagroup.com
Giovanni	Baldi	COLOROBBIA ITALIA SPA (COL)	Via Pietramarina, 123, 50059, Sovigliana Vinci, Italy	baldig@colorobbia.it
Matthias	Voetz	BAYER TECHNOLOGY SERVICES GMBH (BAYER)	Build. E41, Kaiser-Wilhelm-Allee, 51368, LEVERKUSEN, Germany	matthias.voetz@bayer.com
Thangavelu	Jayabalan	INSTITUT NATIONAL DE L'ENVIRONNEMENT ET DES RISQUES INERIS (INERIS)	Parc Technologique Alata, 60550, VERNEUIL EN HALATTE, France	Thangavelu.JAYABALAN@ineris.fr
Tofail	Syed	UNIVERSITY OF LIMERICK (UL)	NATIONAL TECHNOLOGICAL PARK, PLASSEY, LIMERICK, Ireland	Tofail.Syed@ul.ie
Enrico	Bergamaschi	UNIVERSITA' DEGLI STUDI DI PARMA (UNIPR)	Via Antonio Gramsci, 14, 43100, Parma, Italy	enrico.bergamaschi@unipr.it
Lucia	Migliore	UNIVERSITA' DEGLI STUDI DI PISA (UNIPI)	Via S. Giuseppe, 22, 56126, Pisa, Italy	l.migliore@geog.unipi.it
Socorro	Vázquez-Campos	ACONDICIONAMIENTO TARRASENSE ASSOCIACION (LEITAT)	PASSEIG 22 DE JULIOL, 218, 08221, TERRASSA, Spain	svazquez@leitat.org
Giuseppe	Castellet y Ballarà	ISTITUTO NAZIONALE ASSICURAZIONE INFORTUNI SUL LAVORO (INAIL)	Via Roberto Ferruzzi, 38/40, 00143, ROMA, Italy	g.castellet@inail.it

8 Copyright

Scaffold

Innovative strategies, methods and tools for occupational risks management of manufactured nanomaterials (MNMs) in the construction industry

Contract Agreement: NMP4-SL- 2012-280535 Website: http://scaffold.eu-vri.eu/
Coordinator: Jesús M. López de Ipiña, Tecnalia Research and Innovation, Miñano-Alava, Spain

No.	Beneficiary name	Short name	Country
1	Fundación TECNALIA Research and Innovation	TECNALIA	Spain
2	Commissariat à l'Énergie Atomique et aux Énergies Alternatives	CEA	France
3	National Centre for Scientific Research "DEMOKRITOS"	DEMOKRITOS	Greece
4	Centralny Instytut Ochrony Pracy - Państwowy Instytut Badawczy	CIOP-PIB	Poland
5	Acciona Infraestructuras S.A.	ACCIONA	Spain
6	Asociación Española de Normalización y Certificación	AENOR	Spain
7	Mostostal Warszawa S.A.	MOSTOSTAL	Poland
8	ROSSAL SRL	ROSSAL	Romania
9	Tecnología Navarra de Nanoproductos S. L.	TECNAN	Spain
10	NETCOMPOSITES Limited	NETCOMPOSITES	UK
11	Institutul de Cercetari Pentru Echipamente si Tehnologii in Constructii	ICECON	Romania
12	European Virtual Institute for Integrated Risk Management	EU-VRI	Germany
13	Tyoeterveyslaitos	FIOH	Finland
14	Regents of University of Minnesota	UMN-PTL	United States

Contents

1 Summary

Project Duration: Three years

Project Funding: 2,5 Mio. EUR

Manufacturated nanomaterials (MNMs) and nanocomposites are being considered for various uses in the construction industry and related infrastructure industries, not only to enhance material properties and functions but also in the context of energy conservation. Despite the current relatively high cost of nano-enabled products, their use in construction materials is likely to increase because of highly valuable properties imparted at relatively low additive ratios, rapid development of new applications and decreasing cost base MNMs as they are produced in larger quantities.

Thus the use of nano-products in the construction industry is a reality and can be expected to grow in the near future. Consequently, there is a general uncertainty with respect to health

and safety risks and how to properly manage them in order to protect workers and be in compliance with OHS legislation.

SCAFFOLD is an industrial oriented idea specifically addressed to provide practical, robust, easy-to-use and cost effective solutions for the European construction industry, regarding current uncertainties about occupational exposure to MNMs. This will be achieved by introducing a new paradigm to improve workers protection against MNMs in construction, based on a novel holistic Risk Management approach (RMM).

The aim of SCAFFOLD is to develop, test, validate and disseminate a new holistic, consistent and cost effective Risk Management Model (RMM) to manage occupational exposure to MNMs in the construction sector. This will be done by integrating of a set of innovative strategies, methods and tools developed within the project into consistent state-of-the-art safety management systems (OHSAS 18001 + ISO31000).

2 Background

Manufactured nanomaterials and nanocomposites are being considered for various uses in the construction industry and related infrastructure industries, not only for enhancing material properties and functions but also in the context of energy conservation (Figure 1, 2). Despite the current relatively high cost of nano-enabled products, their use in construction materials is likely to increase because of highly valuable properties imparted at relatively low additive ratios, rapid development of new applications and decreasing cost base MNMs as they are produced in larger quantities.

Recent studies suggest that workers handling nano-products have mostly worked with cement or concrete products, coatings or insulation materials. Other types of products, including road-pavement products, flame retardant materials or textiles, were only indicated by some. However, a survey developed by FIEC and EFBWW (2009) shows that the majority of workers and their employers in the construction sector (~75%) are not aware that they work with nano-products.

Occupational exposure to these emerging risks may be accidentally or incidentally produced at different stages of the construction industry life cycle (Figure 3). Due to the novelty, these same nano-products might pose new health and safety risks to the worker on-site, which scientists are just starting to understand. Detailed information about the product composition and their possible nano-specific health and safety issues though, is generally lacking and the information available for the raw material manufacturer is often lost while stepping down the user chain. As a consequence, for the average construction company it is very difficult to conduct a proper risk assessment and organize a safe workplace for its employees.

Despite the potential risks, the use of nano-products in the construction industry is a reality and can be expected to grow in the near future. Consequently, there is a general uncertainty with respect to health and safety risks and how to properly manage them to protect workers and be in compliance with OHS legislation. This calls for a new approach for dealing with uncertainties, providing construction companies with new strategies, methods and tools to appropriately manage these emerging risks.

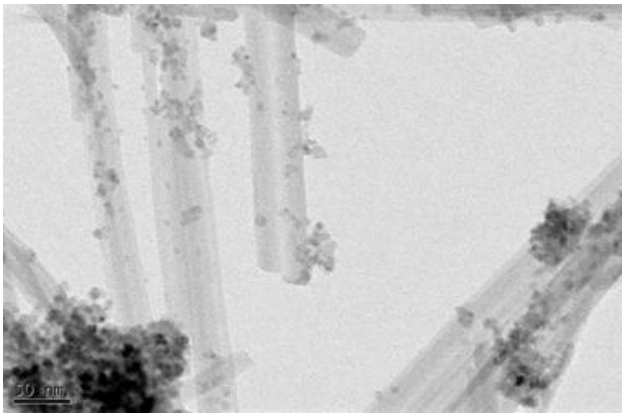

Figure 1. *TEM image of TiO2 supported on modified sepiolite used as a decontaminant additive for construction materials.*

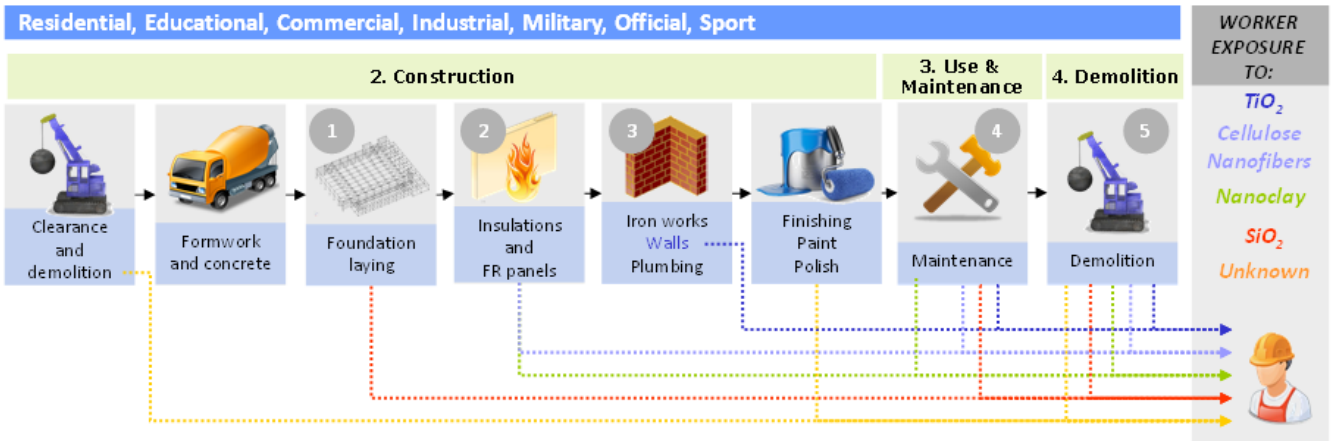

Figure 2. Exposure scenarios identified in the building process (in the framework of WP1-Scaffold)

3 Scientific and technical challenges

SCAFFOLD is an industry-oriented idea specifically focussed on providing practical, robust, easy-to-use and cost effective solutions to the European construction industry, regarding current uncertainties about occupational exposure to MNMs. This will be achieved by introducing a new paradigm to improve workers protection against MNMs in construction, based on a novel holistic Risk Management approach (RMM).

The core of the project is the integration of three basic elements into the new paradigm (Figure 4):

- Existing relevant strategies and methods for risk management coming from ongoing or relevant research that has concluded (e.g. FP7 - EU NanoSafety Cluster projects such as NANOHOUSE, NANOSAFE, NANEX, …; projects from the EU-OSHA and other relevant projects).
- Innovative strategies, methods and tools produced from focused research undertaken by SCAFFOLD to cover selected gaps.
- Consistent state-of-the-art OHS management models (OHSAS 18001 + ISO 31000)

The integration of these three inputs will allow the construction of a novel, consistent and cost-effective SCAFFOLD RMM, particularly addressed to SMEs. The RMM will be able to incorporate any relevant future input produced by new research, assuring RMM sustainability. In addition, compatibility of the RMM with other management systems (e.g. quality - ISO 9001, EFQM, environmental - ISO 14001, EMAS), will facilitate its integration by organizations, should they wish to do so.

4 Objectives

The aim of the SCAFFOLD project is to develop, test, validate in real conditions and disseminate a new holistic, consistent and cost effective Risk Management Model (RMM) to manage occupational exposure to MNMs in the construction sector. This will be done by integration of a set of innovative strategies, methods and tools developed by the project into consistent state-of-the-art safety management systems.

The SCAFFOLD project has the following specific S&T objectives:

1. To assess effectiveness of existing risk reduction strategies, methods and equipments (confinement of processes, PPEs, filtration, etc) in construction scenarios.
2. To develop novel methods leading to the formation of less risk-posing MNMs: safer concentrated dispersions of metal oxide nanoparticles for concrete manufacturing.
3. To propose safer process alternatives for nanocomposite / coatings production jointly with safer nanocomposites and coatings formulations (minimising emissions in machining / spraying tasks or in case of fire).
4. To produce novel strategies and methods for exposure assessment (inhalation and dermal) and modelling adapted to the real sector scenarios, jointly with exposure data in the sector and a decision making strategy for risk assessment.
5. To develop novel risk protection strategies for the sector, including a proposed method for ISO standardization and a decision making strategy for PPEs selection.
6. To adapt the Control Banding approach to the sector, and to test it.
7. To construct a robust and cost-effective model (RMM) for risk management of occupational exposure to MNMs along the life cycle. This will include a set of innovative tools (Toolkit) to support implementation and customized applications for SMEs of the construction sector.
8. To test and validate the RMM and associated tools in construction industry (Industrial Use Cases in Large companies and SMEs).
9. To deploy a strategy to promote implementation of the SCAFFOLD approach in the European construction industry.
10. To coordinate dissemination actions with the *European Nanosafety cluster* to maximize the project's impact.
11. To strengthen synergies among MNMs research groups in Europe, Canada and the USA.
12. To produce pre-standardization and pre-regulation documentation addressed to standardization Technical Committees and regulators regarding construction nano-products, PPEs and OHS management issues.

Figure 3. Some working scenarios in construction, involving exposure to MNMs

SCAFFOLD (Figure 4) will collect, review and analyse relevant quantitative and qualitative information and data on current strategies, methods and tools for workers protection against MNMs (WP1), in order to identify needs and gaps for proper risk management. Four main topics will be analysed: *Risk prevention* (MNMs safe design, safe design of manufacturing processes, etc), *Risk assessment* (occupational exposure and toxicology, measurement equipment and procedures, exposure limit values, etc), *Risk protection and control* (filtration, PPEs, etc) and finally *Risk management* (safety management models, tools, implementation level, work procedures, "good practices", *risk communication*, etc).

A complementary and focused research in the above mentioned four fields (WP2 to WP5) will be developed to fill the identified gaps selected and provide innovative input on strategies, methods and tools to construct an integrated RMM and a set of advanced software tools (Toolkit). In order to assure RMM & Toolkit robustness, soundness and cost-effectiveness, testing and validation activities will be carried out in real conditions in a sample of large companies and SMEs involved in the project Consortium (WP6). As a key factor for the success of the project, relevant dissemination and exploitation activities will be carried out to promote implementation of SCAFFOLD approach in the European Construction industry, particularly in SMEs (WP7).

To guarantee a feasible approach and a cost effective project, an initial project roadmap has been developed by the Consortium and the following priorities selected (A review of all of them will be done in WP1):

- Six applications of MNMs in construction: 1) Depollutant mortars, 2) Self-compacting concretes, 3) Stabilised, Bituminous road-surface, 4) Self-cleaning external coatings, 5) Fire-resistant panels and 6) Insulations.
- Five MNMs: TiO2, SiO2, Cellulose Nanofibres, Carbon Nanofibres and Nanoclays.
- Six categories of exposure scenarios (integrating 26 individual exposure scenarios): 1) Manufacturing MNMs, 2) Manufacturing products containing MNMs, 3) Preparation, mixing, and application on site, 4) Assembly and machining, 5) Demolition and disposal and 6) Accidental fires (Combustion of MNMs).
- Five European Industrial Use Cases covering life cycle steps of MNMs,

Within the SCAFFOLD Consortium five industrial partners will carry out the demonstration activities through real-life Industrial Use Cases (IUC). IUC will focus on three stages of the MNMs Life Cycle

in the construction sector: 1) MNMs manufacturing (Raw MNMs and construction products containing MNMs); 2) Use in construction sites (Building construction and Civil works, including potential maintenance activities) and 3) Disposal in demolition field.

Table 1 Work packages (WP) of Scaffold

WP	Title	Topic
1	*Profiling the European construction industry that face MNMs occupational exposure*	WP1 will aim to develop a profile of the European construction industry, in order to face the occupational exposure to *MNMs* and to provide relevant available information - strategies, methods and tools - to construct the RMM & Toolkit (WP5).
2	*MNMs Risk Prevention*	WP2 will aim to develop innovative strategies and methods for Risk Prevention of the occupational exposure to MNMs by safe product design.
3	*MNMs Risk Assessment*	WP3 will aim to develop innovative strategies and methods for Risk Assessment of the occupational exposure to MNMs in scenarios of the construction sector.
4	*MNMs Risk Protection and control*	WP4 will aim to design and develop innovative strategies and methods for Risk Protection and control against the occupational exposure to MNMs in construction.
5	*MNMs Risk Management: Integration of solutions*	WP5 will aim to integrate relevant available results from WP1 and innovative results produced in WP2to WP4 to construct a Risk Management Model (RMM) - ISO 18001 & ISO 31000 based – and a set of innovative tools (Toolkit - Software) to effectively manage the occupational exposure to MNMs in construction.
6	*Testing and validating the RMM & Toolkit in construction industry*	WP6 will aim to test and validate the RMM & Toolkit in real conditions - Five Industrial Use Cases (IUC).
7	*Dissemination and exploitation*	WP7 will deal with raising public awareness, disseminating project results and defining & managing dissemination and exploitation plans.
8	*Project management*	WP8 will deal with coordinating and managing the project by covering technical, administrative, legal and financial issues of the project and the relation with the EC.

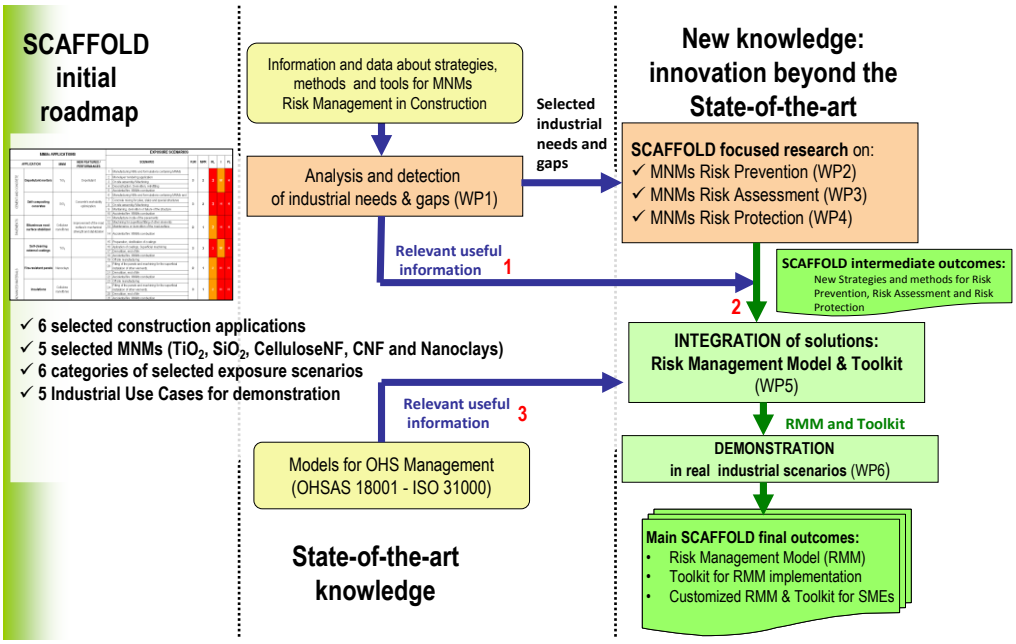

Figure 4 The SCAFFOLD approach

5 Progress and outcomes to date

Scaffold begins in May 2014 the last year of work. The project has produced to date about twenty deliverables (Figures 5, 6,7,8), but the main outcomes will occur precisely in the latter period, until April 2015.

In this last stage of the project, results generated by the main research work packages (Risk prevention, Risk assessment and Risk protection) will be integrated to produce practical risk management solutions (Risk management model and Toolkit). Such solutions will be tested and validated in five construction scenarios (industrial case studies), in Spain, Poland and Romania.

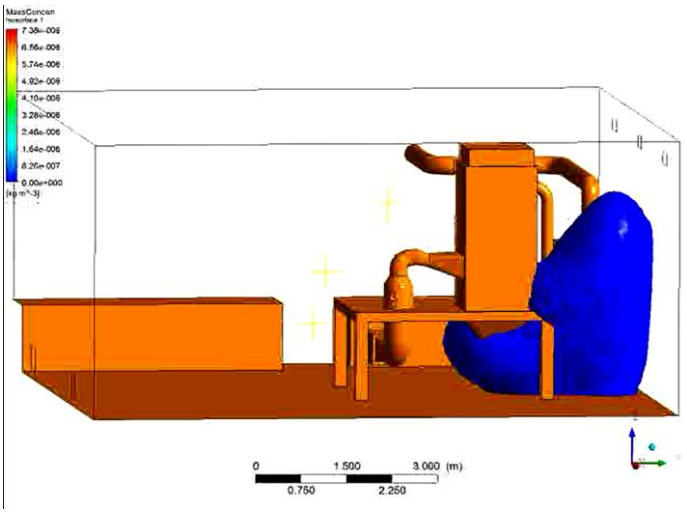

Figure 5. Modeling accidental releases of MNMs

The expected exploitable results, essentially in the field of new products and advanced services, will be:

1. Safer products for construction: new dispersions, formulations for fire-retardant panels, concretes, bituminous pavements, coatings, insulations.
2. Library of guidelines, containing strategies, methods and tools for risk prevention, risk assessment, risk protection and risk management in construction.
3. Scaffold – Toolkit, an advanced software containing databases, procedures, models, tools for training, etc; designed to facilitate the initial review, implementation, monitoring and audit of a risk management model for managing occupational nano-risks in construction (OHSAS 18001 / ISO 31000 based).
4. MNMs trapping device for air monitoring
5. Standardization: CEN Workshop Agreement and new ISO work item proposal for testing PPEs.
6. Proposal for a European OHS strategy on the occupational exposure to MNMs in construction
7. Roadmap for OHS in construction (MNMs in construction)

To guide the exploitation of results, the consortium organized in April 2014, in Athens - with the support of the European Commission - an Exploitation Strategy Seminar. In the same vein, trying to maximize the project impact, SCAFFOLD is coordinating actions with the European construction industry - through the European Construction Industry Federation (FIEC) - and with other interested parties, such as prevention societies, the European Agency for Safety and Health at work (EU-OSHA), CEN, PEROSH, the EU-nanosafety cluster and the FP7 projects sharing topic with Scaffold (SANOWORK, NANOMICEX).

Figure 6. Particle characterization in simulated fire scenarios.

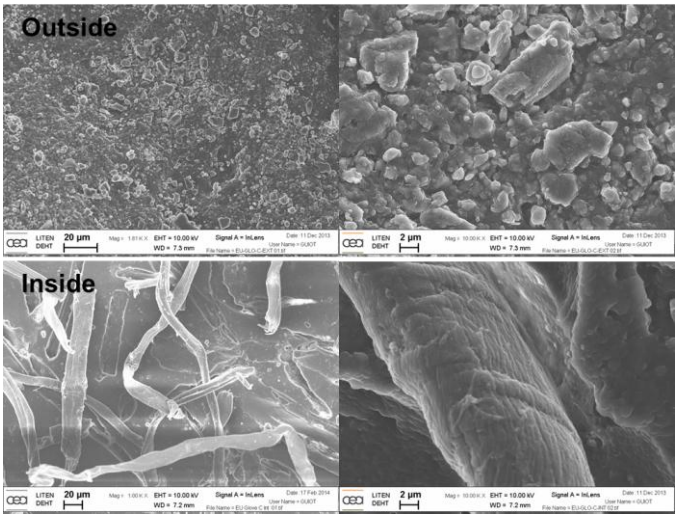

Figure 7. Observation by SEM and EDX measurements on gloves, masks and clothing involved in real scenarios

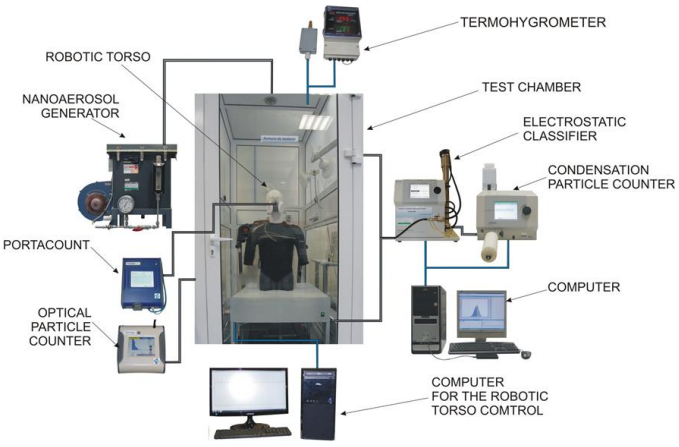

Figure 8. Test stand for testing total inward leakage of RPD against nanoaerosols

6 Expected impact

European Industry and Society

Employment in nanotechnology will grow to reach a predicted 10 million jobs worldwide in 2014. This will account for 11% of the employment in the manufacturing sector. If the population and the occupational structure in the EU remain unchanged, it would mean that almost six million people will be working in Europe's nanotechnology sector by 2014. As it has been already mentioned, the construction industry is the biggest industrial employer in EU27 (3 million enterprises and 14,9 million people employed). In parallel, the construction industry has one of the worst occupational health and safety records in Europe. The presence of SMEs is a highly relevant issue in the construction industry, representing 99.9% of all enterprises in the sector. In total, there are 13.1 million people employed in SMEs of the construction industry (88%). SMEs are more vulnerable to occupational risks and in particular those companies working in dangerous sectors like construction. SMEs account for 82% of all occupational injuries and about 90% of fatal accidents. In total, SMEs account for around 80% of all occupational diseases caused by chemical agents. In addition, SMEs in the construction industry are generally present *through a subcontractor,* which represents a relatively high share (45%) compared to other sectors.

In this context, the implementation of SCAFFOLD's practical and cost effective strategies, methods and tools for reduction of worker exposure to MNMs will produce a very important impact on companies and workers, in terms of OHS improvement. A proper management of MNMs based on the SCAFFOLD approach will preventively contribute to avoiding potential chemical accidents and diseases at work. This preventive approach will produce parallel benefits by reducing non-safety bills both in construction companies and in the whole European industry.

The implementation of SCAFFOLD framework in SMEs will have a very relevant impact on the European construction industry (SCAFFOLD will produce a specific approach for SMEs customizing both the RMM and the Toolkit). A Spanish study shows that a relatively large, and increasing percentage of SME subcontractors (23 %) recognise the obligation to fulfil a safety management standard (e.g. OHSAS 18001) imposed by their clients. Increasing awareness on the part of the contractors about occupational risks of MNMs will bring new requirements for SMEs. The implementation of SCAFFOLD approach will provide SMEs with a competitive advantage.

Market

By 2025 it is expected that nanocomposites will be a 6.5 billion € market, with volumes approaching 2.2 million tonnes. Construction will emerge as a significant market. Related sources suggest that 360 million pounds of nano-additives will be required by 2020 at a value of 1.4 billion €, over half of which will be used to purchase CNTs. Regarding nano-oxides, the global market was 166 million Euros in 2006, and is estimated to reach 760 million Euro by 2011.

A critical issue regarding the success of any method for MNM production is its cost effectiveness. But a second market requirement is to guarantee safety of the product during the complete life cycle. In this sense, SCAFFOLD results are very promising since they allow the use of reasonably low cost raw materials and, furthermore, this production method results in important increase of product safety and savings in energy and

cost. Therefore, it is imperative that the HSE issues (safe product design, safe manufacturing, OHS risk management, compliance with OHS regulations, etc) surrounding these materials and sectors in particular are adequately solved and risk prevention strategies put in place rapidly. SCAFFOLD may play an important role in this sense.

Regarding the market of safety management systems, the OSHAS 18001 is the worldwide reference in occupational health and safety management. The number of certified companies has increased dramatically since 2003. In three years (from 2007 to 2009) the number increased by 73% in the world. A recent report shows how the penetration of this standard is higher amongst SMEs; in fact, the study shows that 81% of the companies were SMEs, 52% between 50 and 250 workers and 29% with less than 50 employees. Concerning the construction sector, it is leading the implementation of OHSAS (37%), which shows the commitment of the construction companies, especially SMEs, to the improvement of labour safety in Europe. Certification market for OSHAS 18001 will be twice its current size in two years (in 2012 more than 100.000 companies will be certified).If the tendency continues, 80% of those companies will be SMEs. It is easy, from a very conservative perspective, to estimate that market will be doubled again by 2020, which would easily represent a 2,000 M€ market only for OSHAS 18001. Considering the current evolution of nanoparticles and nanomaterials, and also considering the expected evolution of OHSAS 18001, the creation and commercialization of the RMM model could have a great impact in the market, especially for SMEs.

European policies, regulations and standards

The Community Strategy on Health and Safety at work for the period of 2007 – 2012 included nanotechnology as an important topic to be developed in the context of the identification of new and emerging risks. Consequently, It is expected that SCAFFOLD will tangibly impact the new European strategy, currently under development.

SCAFFOLD will contribute positively to European regulations by providing new information to 1) elaborate better regulations about issues related to safety of MNMs and OHS issues and 2) to support compliance with current legislation requirements.

Standardisation is a highly relevant issue in construction, covering around 3,000 work items on product standards and test methods (CEN). Of these, about 500 standards will be harmonised under the Construction Products Directive (89/106/EEC). Pre-standardization activities of the SCAFFOLD project will positively contribute to developing European standards that aim to reduce potential barriers that might cause an increase of the time to place in the market of new construction nano-products (nanocomposites, nano-coatings, etc) and PPEs. In addition, the SCAFFOLD project will also provide innovative inputs for new/improved OHS standards in the field of OHS management and workers protection against MNMs.

7 Directory

Table 2 Directory of people involved in this project.

First Name	Last Name	Affiliation	Address	e-mail
Jesús M.	López de Ipiña	Fundación TECNALIA Research and Innovation	Parque Tecnológico de Alava Leonardo Da Vinci 11 E-01510 Miñano-Alava Spain	jesus.lopezdeipina@tecnalia.com
FranÇois	Tardif	Commissariat à l'Énergie Atomique et aux Énergies Alternatives	Rue des Martyrs 17 FR-38054 Grenoble France	ftardif@cea.fr
Neofytou	Panagiotis	National Centre for Scientific Research "DEMOKRITOS"	Patriarchou Gregoriou Str. GR-15310 Aghia Paraskevi Greece	fonta@gel.demokritos.gr
Elzbieta	Jankowska	Centralny Instytut Ochrony Pracy - Państwowy Instytut Badawczy	Czerniakowska 16 PL-00701 Warszawa Poland	eljan@ciop.pl
Jaime José	Cubillo	Acciona Infraestructuras S.A.	Valportillo II ES-28018 Alcobendas - Madrid Spain	JCubillo@acciona.es
Javier	García	Asociación Española de Normalización y Certificación	Génova 6 ES-28004 Madrid Spain	jgarcia@aenor.es
Pawel	Poneta	Mostostal Warszawa S.A.	Ul Konstruktorska 11A PL-02673 Warszawa Poland	P.Poneta@mostostal.waw.pl
Anca	Pintea	ROSSAL SRL	Bogdan Dragos 119 RO-61001 Roman Romania	anca62pintea@yahoo.es

First Name	Last Name	Affiliation	Address	e-mail
Idoia	Sancet	Tecnología Navarra de Nanoproductos S. L.	Area Industrial Perguita ES-31210 Los Arcos - Navarra Spain	idoia.sancet@tecnan-nanomat.es
Ben	Hargreaves	NETCOMPOSITES Limited	4A Broom Business Park, Bridge Way GB-S41 9QG United Kingdom	Ben.hargreaves@netcomposites.com
Adrian	Tabrea	Institutul de Cercetari Pentru Echipamente si Tehnologii in Constructii	Sos Pantelimon 266 Sector RO-021652 Bucaresti Romania	certicon@icecon.ro
Olivier	Salvi	European Virtual Institute for Integrated Risk Management	Haus der WirtschaftWilli-Bleicher-Strasse DE-70174 Stuttgart Germany	salvi@eu-vri.eu
Helene	Stockmann-Juvala	Tyoeterveyslaitos	Topeliuksenkatu 41 a A FI – 00250 Helsinki Finland	Helene.stockmann-juvala@ttl.fi
David	Pui	Regents of University of Minnesota	Oal Street SE 200 US-55455 2070 Minneapolis United States	dyhpui@umn.edu

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SIINN

Safe Implementation of Innovative Nanoscience and Nanotechnology

Contract Agreement: NMP4-2010-265799 Website: http://www.siinn.eu
Coordinator: Rainer Hagenbeck, Forschungszentrum Jülich GmbH, Germany

No.	Beneficiary name	Short name	Country
1	Forschungszentrum Jülich GmbH	JÜLICH	Germany
2	TEMAS AG	TEMAS	Switzerland
3	Bundesministerium für Verkehr, Innovation und Technologie	BMVIT	Austria
	Third Party linked to BMVIT according to Special Clause 10: Austrian Institute of Technology	*AIT*	*Austria*
4	Executive Agency for Higher Education, Research, Development and Innovation Funding	UEFISCDI	Romania
5	Service Public de Wallonie	SPW–DGO6	Belgium
6	Fundacion madri+d para el Conocimiento	madri+d	Spain
7	National Funding Agency for Research	ANR	France
8	National Hellenic Research Foundation	NHRF	Greece
9	Technology Strategy Board	TSB	United Kingdom
10	Federal Ministry of Education and Research	BMBF	Germany
11	Ministerio de Economía y Competitividad	MINECO	Spain
12	Veneto Region	RVE	Italy
13	Chief Scientist Office, Ministry of Health	CSO-MOH	Israel
14	Science Foundation Ireland	SFI	Ireland
15	Eidgenössisches Department des Inneren	EDI	Switzerland
16	Stichting voor de Technische Wetenschappen	STW	The Netherlands
17	Veneto Nanotech	VN	Italy
18	Commissariat à l' énergie atomique et aux énergies alternatives	CEA	France
19	Fundação para a Ciência e a Tecnologia	FCT	Portugal

Contents

1 Summary

Project Duration: 1 August 2011 – 31 July 2014

Project Funding: 1.5 Mio. EUR

The aim of the ERA-NET SIINN is to help create an optimum environment within Europe with which to promote the safe and rapid transfer of innovative nanoscience and nanotechnology (N&N) research and development into industrial application.

Starting in 2011, the ongoing pooling together of appropriate national and regional resources in order to create a sustainable, coordinated, transnational programme of N&N-related RTD work across Europe is utilising the synergies of the national or regional programmes and the genuine desire of their owners to cooperate. Thus, in addition to strengthening the European Research Area itself, SIINN has created an effective network of ministries, funding agencies and academic and industrial institutions active in the N&N fields, which, together with further stakeholders such as industry, other N&N networks and organisations, standardisation bodies, etc., are actively creating Europe's first sustainable transnational programme of applied N&N research.

The commercial application of products containing nanomaterials (NMs) is increasing rapidly, but one important question, the potential risks of NMs for environment, human health and safety (EHS), remains a substantial barrier to their wide innovative use.

Therefore as a first priority, SIINN has put its initial focus on developing a consolidated framework with which to address and manage nanosafety issues i.e. nano-related EHS risks. A first draft of the consolidated framework has been circulated to European experts and members of the NanoSafety Clusters in 2013. Comments by experts will be taken into account for an updated version of the document which will be available in 2014.

National activities in Europe in N&N EHS remain largely uncoordinated and fragmented, resulting in the sub-optimal use of available resources, such as human resources, research funding and research infrastructures. Furthermore, the data used for EHS assessments world-wide is often based on toxicological studies of nanomaterials which are scant, unreliable or even contradictory; the data is often gathered for nano-systems which are either ill-defined or not clearly defined at all.

The SIINN project thus focuses on ways of remedying this unsatisfactory situation and concentrates on obtaining sound toxicological data for NMs for nanosafety and EHS risk assessment and management.

SIINN's activities are undertaken in growing cooperation with various national and international networks, organisations and groupings, including the NanoSafety Cluster, the QualityNano infrastructure, the OECD Working Party on Nanomaterials (WPNM), and the NANOfutures ETIP.

Two transnational calls have already been published in 2012 and 2013 allowing scientists from participating countries and regions to perform joint research activities. A third joint call is planned for 2014. The intention is to provide a basis for long term joint activities of funding organizations coordinating their programmes in the area of nanosafety and nanotoxicology in close contact with the research community and the industry.

2 Background and Goals

Nanosciences and Nanotechnologies (N&N) are two of the fastest growing research areas of the last decade and more than 1000 nano-enabled products are currently available on the market in more than 20 different countries, whereby the total global market for nanoproducts is expected to exceed € 3000 billion by the year 2015.

However, the most serious handicaps for nanomaterials to enter the market are EHS-related issues or to be more precise, the lack of accurate and reliable data on which to base a detailed assessment of the EHS behaviour of such man-made or "engineered" nanomaterials.

The primary aim of the SIINN ERA-NET is therefore to create an optimal, sustainable environment within Europe in order to promote the safe, rapid transfer of innovative N&N research and development into industrial application.

The uncertainties associated with the safe use of engineered nanomaterials presently hinder the creation of a new, globally highly competitive, nano-based industry within the EU. Therefore as a first priority, SIINN focuses on developing a consolidated framework with which to address and manage nano-related EHS risks. This includes the development of a joint, transnational R&D programme looking not only into EHS risk assessment but also of necessity the toxicological behaviour of nanomaterials. The framework is being developed based on existing, verified information and knowledge, complemented with calls for actual research projects to close identified data gaps. By utilising this framework, the foundation will be laid for the rapid market uptake of safe, nano-based technologies and products and this will strengthen the development of high added-value products as the basis of a new, globally competitive industry in Europe.

Other parameters such as production engineering, quality control or the protection of intellectual property rights are also very relevant for stimulating technology transfer in the nanomaterials sector. However, along with the EU Commission and industry itself, the governmental bodies (or their representatives) united in the SIINN consortium are convinced that at this stage it is nanosafety and nanotoxicology which should be the immediate focus of their resources within the first three-year phase of this ERA-NET.

Responding to the apparent increasing knowledge gap between the development of N&N and our understanding of how nanomaterials interact with the environment and the human body, many research and technological development studies now also address nano-specific aspects of product safety. Because of the complexities of nanomaterial-containing systems, however, where the physical and biological impacts of these nanomaterials are highly dependent upon the system themselves, the problem of the reliability of current physical and biological data for nanomaterials is both real and large. The large number of studies regarding engineered nanomaterials also poses problems in terms of data management and reliability, especially as data are often shown to be even contradictory.

Thus, studies focusing on the behaviour of nanoparticles systems in biological settings are being carried out in many areas of the world but their results are not always transferable or directly applicable.

The SIINN ERA-NET has therefore been devised in order to also overcome this problem by setting the conditions through joint, transnational cooperation at governmental or regional level within Europe which will enable science and society to be provided with reliable data which can be implemented for the safe use of engineered nanomaterials.

A common database platform which will allow entry and searching from a unique starting point in the various existing nanosafety data sources (verified by SIINN) is therefore under development and will be implemented as a tool to aid programme owners and implementers in deciding on future research themes. This tool will, as a spin-off of the SIINN ERA-NET, be made available to all interested stakeholders (government, industry, education, research, standardisation bodies) via the SIINN website.

Europe maintains a strong nanotechnology research base, heavily supported by public funding in nanotechnology research at both European Union and national levels. SIINN will launch at least two joint transnational research calls in the field of nanosafety, nanotoxicology and risk assessment during its initial three-year life. This joint effort could ultimately lead to joint RTD programmes being developed between the EU Member States and States associated to FP7 or HORIZON 2020. In the mid-to-long term, joint

activities with key countries outside of Europe (e.g. the USA or Japan) are also feasible. Currently, concrete discussions with representatives of the U.S. National Science Foundation (NSF) and the U.S. Environmental Protection Agency (EPA) concerning their participation in the third transnational SIINN call are ongoing.

At the end of the project's initial three years, SIINN will have established a coordinated, transnational programme of nanosafety and nanotoxicology-related activities across Europe which will address the potential environmental, health and safety implications of nanoscale materials, and which will include the development of standards for environmental and toxicological studies of nanoparticles and a metrology infrastructure supporting these standards. Joint activities going beyond 2014 are also under discussion in the SIINN consortium.

3 Current Status of SIINN ERA-NET

The SIINN ERA-NET started its work on August 1, 2011. The kick-off-meeting in September 2011 established the structures and confirmed the readiness of the partners to cooperate on establishment of joint research activities. The meeting of the SIINN Steering Committee in December 2011 made decisions leading to the preparation for the first joint SIINN call which was published in March 2012. The meetings of the Steering Committee in November 2012 and September 2013 reviewed the progress and discussed the next steps.

At the end of 2013, the six workpackages (WP) have reached the following major results:

Within WP1, the important criteria and terms in the area of nanomaterial toxicology were defined and the health and safety relevant information which is currently available from, and to, Europe were examined. The corresponding report is available on the SIINN web page. Based on the analysis of this information, WP1 identified important knowledge gaps with respect to the occurrence and toxicity of manufactured nanomaterials and these were the basis for formulation of the topics of the first and second transnational SIINN call.

Within WP2, an inventory of directed liaisons, initiatives and actions with respect to the national activities of the SIINN partner countries and regions was accomplished. Contacts to the major international organizations active in the field N&N safety were established. The common SIINN database platform will make use of the NANOhub data base of the Commission's Joint Research Centre in Ispra. It will also be applied for the inclusion of data stemming from the research projects funded by SIINN. The first draft of the Consolidated Framework for EHS was distributed to international experts and members of the NanoSafety Cluster in 2013. The first official version will be published in 2014 taking into account recommendations by the addressed experts. A roadmap towards the first consolidated RTD programme is available as a draft version in the protected part of the SIINN website.

WP3 has prepared a first inventory of existing characterisation methods. A data collection with respect to the identification of gaps in evidence-based EHS risk assessment for human health and environmental safety is in progress.

WP4 has established the parameters, procedures and documents for the first and second joint call for proposals. The first call was launched in March 2012. Funding organisations from nine countries and regions have participated in the call. At the submission deadline in June 2012, 15 eligible proposals were submitted. After an international evaluation, three of the proposals were selected for funding, with a funding volume of 2.3 mio. Euro in total. The projects have started in June 2013. A joint kick-off meeting of the selected projects with participation of involved funding agencies took place in Berlin in June 2013. Further details about the selected projects are available on the SIINN web page (www.siinn.eu). The second call was launched in June 2013. Funding organisations from six countries and regions have participated in the call. At the submission deadline in November 2013, 28 eligible proposals were submitted. The funding decision will be taken in April 2014. It is expected that projects selected for funding will start in November 2014. The third call is now under preparation and is expected to be published in September 2014. The implementation of the third call requires an extension of the ERA-NET SIINN by one year (project end in July 2015).

WP5 is concerned with communication and dissemination. The SIINN web page provides information for the wider public and, through its password-protected internal part, also serves as a means of communication between the SIINN partners and as a repository for documents. The PR and communication plan is a jointly agreed basis for disseminating information about SIINN and its results.

WP6 has established the management structures and bodies and is assuring their smooth functioning. The contractual arrangements have been adapted to the present actual status.

4 Summary of SIINN's Key Expected Impacts

- Strengthening of the European Research Area in nanoscience and nanotechnology;
- Decrease in RTD fragmentation and improvement in the coordination and exploitation of synergies between the owners of national funding programmes, other authorities related to N&N and nanosafety, the corresponding research community and industry, including an enhanced interaction with the EU Framework Programme and the generation of a programme of transnational RTD; Generation of a carefully examined set of data which will allow reliable guidelines for the development of legal frameworks (e.g. precautionary measures and steps towards regulations) to be developed to increase safety and reduce risks through all stages of a product's life-cycle, from R&D to disposal and recycling;
- The efficient identification of knowledge gaps from this data set, helping to clearly and efficiently specify goals for current and future transnational research programmes;
- Efficient use and leverage of resources (such as knowledge, capital and investment at European level) through common calls, thereby avoiding duplicity in projects (unless specifically required) and enhancing the common use of knowledge, capital and investment at European level;

- The possibility of the rapid assessment and management of potential risks is a crucial success factor for industry to enable the more rapid adoption of N&N for the development of safe products;
- A higher standard of safety and confidence for the population and the environment which will help promote acceptance for applications of nanotechnology.

5 Organisation of SIINN

The project is organized into six workpackages:

Workpackage 1 *Identification of sources and inventory of available information*

Workpackage 2 *Liaison with European and global initiatives; networking and information management and exchange; roadmapping*

Workpackage 3 *Validation of existing characterisation and EHS assessment methods (including life-cycle validation) and identification of knowledge deficiencies*

Workpackage 4 *Contractual framework and implementation of joint calls*

Workpackage 5 *Dissemination, exploitation and sustainability*

Workpackage 6 *SIINN coordination and management*

The overall strategic concept of SIINN is to first catalogue which information is available to researchers and what state is it in. This is the central task of workpackage 1 (WP1).

Parallel to this, workpackage 2 (WP2) establishes close liaisons with organisations working in the EHS risk assessment of nanomaterials in order to form a close network and to exchange information. This cooperation also includes various strategically important tasks such as the identification of best practices, synergy potentials and the elaboration of recommendations for future collaborations on the strategic and operational level addressing nanomaterial EHS including precautionary measures, pre-normative work, steps towards regulations, common actions and projects. Together with this information, WP2 will also develop a roadmap to describe these future activities necessary in the risk assessment of nanomaterials.

WP3 looks closely at the EHS risk assessment of nanomaterials and (following on from WP1) aims at establishing the reliability of the available data. Noting any irregularities or deficiencies, this WP sets down a list of research objectives which is used as input to WP4, the WP charged with establishing the joint transnational research programme and carrying out tenders for R&D projects to assess these deficiencies.

For the first time in the nanomaterials sector in Europe, joint transnational calls have been carried out to overcome identified deficiencies in current nanosafety knowledge for assessing the risks of nanomaterials and nanomaterial-containing products. The experiences from the first and second call will be used as a starting point for the third call, which probably will be published in September 2014.

WP5 is responsible for the dissemination of information both within the project itself as well as to external recipients and stakeholders such as government bodies, industry, research organisations, standardisation bodies and importantly, the public at large.

Finally, WP6 performs the management of the SIINN project and ensures that the tasks and deliverables are undertaken according to timetable and within the scopes required for the success of the project. WP6 covers the technical and administrative management of the project and includes any horizontal issues such as quality management.

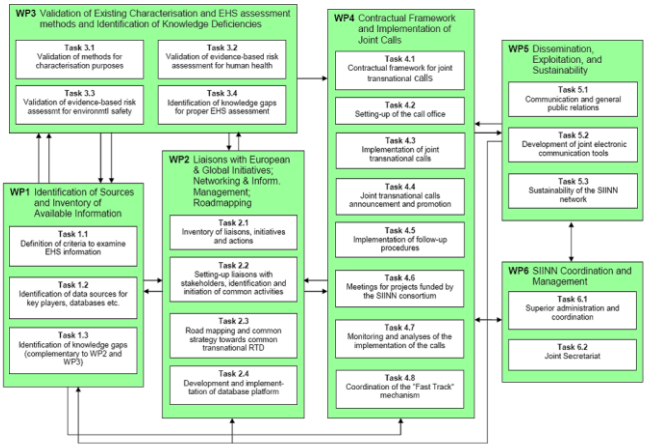

Figure 1 Interplay of SIINN Workpackages (WPs)

The SIINN management structure has been defined to allow for clear responsibilities and rapid decision making whilst still maintaining the flexibility required with respect to its membership structure and to participation in the transnational calls and other planned activities. This structure is based on experience gathered in similar large international projects and in other ERA-NETs.

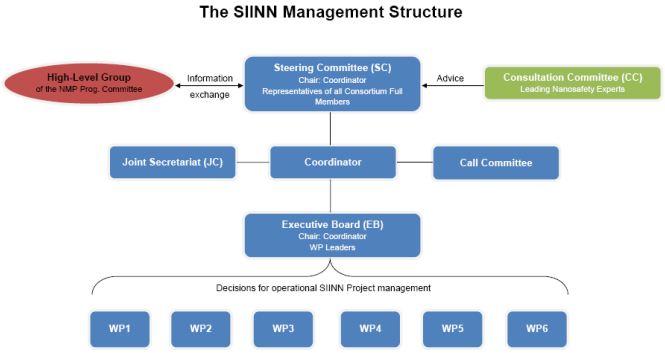

Figure 2 Management Structure of the SIINN Project

Although the full consortium is responsible for the overall policy of the SIINN network through the network's most senior body, the Steering Committee, the operational management has been delegated to a formally constituted executive body, the Executive Board, which is composed of the coordinator and workpackage leaders. Past experience has demonstrated that such a body is paramount if strategy is to be speedily and efficiently implemented. The Executive Board prepares all recommendations on policy and strategy issues which are required to be addressed and decided upon by the Steering Committee.

In addition, a nanomaterials technology and innovation advisory group, the Consultation Committee, has been formed, whose members assist with the strategic and operational needs of the SIINN ERA-NET. One member of the COM's NMP Programme Committee serves in the Consultation Committee. Since 2012, the SIINN Coordinator is a member of the High Level Group of EU Member States and FP7 Associated States on Nanosciences and Nanotechnologies. This assures a close link to related activities of the EU.

6 Directory

Table 1 Directory of people involved in this project.

First Name	Last Name	Affiliation	Address	e-mail
Carlos	Almeida Pereira	Fundação para a Ciência e Tecnologia	Fundação para a Ciência e Tecnologia Avenida Dom Carlos I, 126 1249-074 Lisboa, Portugal	carlos.pereira@fct.pt
Carles	Cané	Director CNM-IMB (CSIC) Ministry of Economy and Competitiveness MINECO	Campus UAB 08193 Bellaterra Spain www.imb-cnm.csic.es	carles.cane@imb-cnm.csic.es
Estrella	Fernández	Ministry of Economy and Competitiveness (MINECO) Dirección General de Investigación Científica y Técnica Subdirección General de Proyectos Internacionales	C/ Albacete 5 5ª Planta Sur 28071 Madrid Spain	estrella.fernandez@mineco.es
Mariana	Lassalle	Department Health and Biology	Agence Nationale de la Recherche Rue Watt 7 75013 Paris, France	mariana.lassalle@agencerecherche.fr
Antonio	Bonaldo	Veneto Region Section Research and Innovation	Regione del Veneto Fondamenta Santa Lucia, Cannaregio 23 30121 Venezia, Italy	ricercainnovazione@regione.veneto.it
Bertrand	Fillon	Commissariat à l' énergie atomique et aux énergies alternatives CEA	CEA/LITEN 17 rue des Martyrs 38054 Grenoble Cedex 9, France	bertrand.fillon@cea.fr
Anthony R.	Flambard	Project Management Jülich PtJ	Projektträger Jülich PtJ Zimmerstr. 26-27 D-10969 Berlin, Germany	a.flambard@fz-juelich.de
Rainer	Hagenbeck	Project Management Jülich PtJ	Projektträger Jülich PtJ Forschungszentrum Jülich GmbH D-52425 Jülich, Germany	r.hagenbeck@fz-juelich.de
Karl	Hoehener	TEMAS AG	TEMAS AG Egnacherstraße 69 CH-9320 Arbon, Switzerland	karl.hoehener@temas.ch
Graham	Mobbs	The Technology Strategy Board Innovation Programmes Advanced Materials & Nanotechnologies	North Star House, North Star Avenue B1 UK- SN2 1JF Swindon United Kingdom	Graham.Mobbs@tsb.gov.uk
Mats-Olof	Mattsson	AIT Austrian Institute of Technology GmbH Department Health & Environment	AIT Austrian Institute of Technology GmbH Donau-City-Strasse 1 A-1220 Wien, Austria	Mats-Olof.Mattsson@ait.ac.at
Arturo	Menéndez	Fundacion madri+d para el Conocimiento	Velazquez, bajo izquierda 76 28001 Madrid, Spain	amenendez@madrimasd.org
Giorgia	Merlin	Veneto Nanotech	Headquarters - Veneto Nanotech S.C.p.A. Via S. Crispino 106 I - 35129 Padova, Italy	giorgia.merlin@venetonanotech.it

First Name	Last Name	Affiliation	Address	e-mail
Floor	Paauw	Stichting voor de Technische Wetenschappen	Stichting voor de Technische Wetenschappen Van Vollenhovenlaan 661 3527 JP Utrecht The Netherlands	f.paauw@stw.nl
Alexander	Pogány	Information and Industrial Technologies, Space	Bundesministerium für Verkehr, Innovation und Technologie Renngasse 5 A-1010 Wien, Austria	alexander.pogany@bmvit.gv.at
Avi	Israeli	Ministry of Health	Ministry of Health 2 Ben Tabai Street 93591 Jerusalem, Israel	avii@MOH.HEALTH.GOV.IL
Michael	Ryan	Science Foundation Ireland SFI	Science Foundation Ireland Wilton Park House Wilton Place Dublin 2, Ireland	michael.ryan@sfi.ie
Evangelia	Sarantopoulou	National Hellenic Research Foundation	Ethniko Idryma Erevnon Vassileos Constantinou Avenue 48 11635 Athens, Greece	esarant@eie.gr
Christoph	Studer	Federal Office of Public Health Division of Chemical Products	Federal Office of Public Health Bollwerk 27 CH-3011 Bern, Switzerland	christoph.studer@bag.admin.ch
Speranta	Tanasescu	Executive Agency for Higher Education, Research, Development and Innovation Funding	Mendeleev Street, no. 21-25 district 1 Bucharest Romania	speranta.tanasesc@uefiscdi.ro
Cédric	Volcke	General Directorate - Economy, Employment and Research (DGOEER)	Service Public de Wallonie Place de la Wallonie 1 5100 Jambes, Belgium	cedric.volcke@spw.wallonie.be
Frank	Wolf	Federal Ministry of Education and Research Division 511 (New Materials, Nanotechnology)	Bundesministerium für Bildung und Forschung, BMBF Referat 511 Heinemannstrasse 2 D- 53175 Bonn, Germany	Frank.Wolf@bmbf.bund.de

7 Copyright

SIRENA

Simulation of the release of nanomaterials from consumer products for environmental exposure assessment

Contract Agreement: LIFE11 ENV/ES/596 Website: www.life-sirena.com
Coordinator: Idoia Unzueta, RTD Manager at INKOA SISTEMAS, Bilbao, Spain

Table 1 Consortium List.

No.	Beneficiary name	Short name	Country
1	INKOA SISTEMAS	INKOA	SPAIN
2	CRANFIELD UNIVERSITY	CRANFIELD	UNITED KINGDOM
3	FUNDACIÓN TECNALIA RESEARCH AND INNOVATION	TECNALIA	SPAIN
4	ROBERT GORDON UNIVERSITY	RGU	UNITED KINGDOM

Contents

1 Summary

Project Duration: 02 January 2013 – 30 December 2015 (36M)

Project Funding: 1.140.942 € (Total budget)

Nanomaterials are increasingly being developed for a range of industrial uses. However, there is a lack of standardised methods for estimating the release of nanomaterials to the different environmental compartments - air, water, soil – during the various stages of any nanotechnology-enabled product's life cycle. Current legislation for chemicals and environmental protection was not originally formulated bearing nanomaterials in mind. Industry has currently to follow a precautionary principle to minimise potential exposure levels and risks. However, to enable the full economic potential of this exciting sector, whilst ensuring safety for the environment and human health, better understanding and knowledge of the associated risks is needed. This will enable a better regulatory framework in relation to the effects of nanomaterials. The implementation of systematic approaches for risk assessment of nanomaterials by industry and the ability of the sector to communicate this successfully to consumers is of paramount importance for the successful penetration into the market and sustainability of this innovative technology.

The 'SIRENA' project aims to improve understanding of risks associated with nanomaterials through the demonstration and testing of a methodology to simulate the unintended release of nanomaterials from consumer products. It will replicate different life cycle scenarios based on nanocomposites drilling and crashing to be adopted by a wide number of industrial sectors to get the necessary information for exposure assessment. 'SIRENA' aims to anticipate exposure scenarios from the proposed uses of nanomaterials. It will work with different sample specimens consisting of a variety of nanocomposites currently used in three different industrial sectors: aerospace, automotive and renewable energy. Examining these scenarios will help identify parameters for the exposure assessment required in risk assessment, data that should be available prior to commencing the detailed risk assessment of a nanomaterial. The project thus expects to extend the existing knowledge base in relation to risks associated with nanomaterials and contribute to decisions to minimise potential impacts throughout nanocomposites' life cycles. It intends to provide producers and manufacturers with suitable tools and procedures to mitigate risks by choosing products conveying minimum nanomaterial release. Ultimately the project expects to facilitate the adaptations needed for nanomaterials in the current regulatory framework and support implementation of EU

environmental policy and legislation in relation to chemical products (REACH) in particular.

2 Background

One of the main applications of nanotechnology in material science is the development of **polymer nanocomposites,** reinforced polymers with low quantities of nanosized organic or inorganic ingredients dispersed into a thermoplastic or thermoset polymer. The use of nanoparticles (NPs) in composites manufacturing offers enormous advantages over traditional macro- or microsized fillers and applications across a wide range of industrial sectors are currently on the market.

Information about the potential risks of an unintended release of ENMs (engineered nanomaterials) embedded into plastic composites along their life cycle and associated environmental exposure is curentlylacking, even if evidence has emerged in the literature suggesting that some ENMs may have negative impacts in environment. **The risk is function of both exposure and hazard, and those can be influenced by the product life cycle and the product design**.

SIRENA's main motivations include:

• Current legislation for chemicals and environmental protection was not originally formulated bearing ENMs in mind. Thus, there is an actual need to **adapt actual regulatory framework** in relation to impacts associated to ENMs.

• The **need of standardized methods for estimating the release of ENMs** to the different environmental compartments (air, water, soil) from the increasingly number of Nanotechnology-enabled products during the different stages of its life cycle is evident, so that the associated exposure levels are minimized in the frame of a precautionary principle until legislation is adapted to the new challenges associated to nanomaterials.

• **Industry must evaluate and, if feasible, quantify, the risk of embedded nanoparticles release to the environment** throughout the life cycle as an integral part of its innovation and design process for consumer products incorporating nanotechnology and make this information available to the relevant regulatory authorities and consumers.

• There is a need to increase existing knowledge for NMs risk Assessment (RA), mainly **data related to the exposure rates**, and it should be obtained by the use of standard methodologies developed under a holistic life cycle perspective so that research efforts are comparable and integrated. This is further supported by the Article 13 of REACH for the general requirements for generation of information on intrinsic properties of substances which specifies that ecotoxicological and toxicological tests and analyses may be omitted where justified by information on **exposure** and implemented risk management measures.

• The **safety perception of Nanotechnology by the public** conditions the successful penetration into market of this novel technology. The implementation of systematic approaches for ENMs Risk Assessment by industry and its communication to consumers is of paramount importance for the sustainability of Nanotechnology.

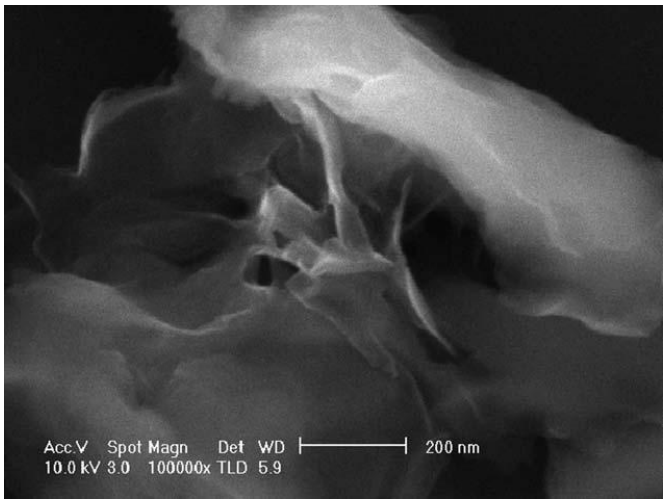

Figure 1: SEM microphotograph confirms that MMT is fully exfoliated in a Polyurethane (PU) matrix. Courtesy of Dr James Njuguna (RGU).

3 Objectives

The **main objective** of SIRENA is to **demonstrate** and **validate** a methodology to simulate the unintended release of nanomaterials from consumer products by replicating different life cycle scenarios. Developed methodologies could be adopted by a wide number of industrial sectors in order to get the necessary information for the exposure assessment.

Additionally, it is intended:

- To contribute to the effective implementation of European Commission's regulatory framework, mainly in relation to environmental protection and chemicals (REACH).
- To anticipate exposure scenarios from the proposed uses of nanocomposites. These exposure scenarios will contribute to decisions on the extent of the hazard characterization and will provide parameters for the exposure assessment required in risk assessment.
- To increase the existing knowledge in relation to risks associated to nanomaterials, enabling the safe handling of nanomaterials and minimizing potentially associated impacts throughout product life cycle.
- To demonstrate a methodology for a rapid premarket safety assessment analysis.
- To provide manufacturers with tools and methodologies to mitigate the resulting risk by choosing products conveying minimum nanomaterials release in a "premarket" safety analysis.
- To contribute to the eco-responsible design of nanomaterials and nanocomposites.

The suitability of proposed methodology will be demonstrated in composites and nanocomposites incorporated in emerging

applications of **automotive**, **aeronautics**, and **energy and construction sectors**.

4 Innovative and demonstrative nature of the project

Now 10 years ago, UK's Royal Society and Royal Academy of Engineering mentioned that one of the difficulties in determining potential future exposure of the environment and humans to manufactured nanoparticles is the lack of information about both **the extent to which they are used in products** and also **the likelihood of such particles being released from nanomaterials in a form or quantity that might cause harm to humans or the environment**[1].

The US Environmental Protection Agency (EPA) mentioned in 2010 that estimates for releases to water, air and soil from manufacturing, processing, formulations, industrial use, commercial use, consumer use, recycling and/or remanufacturing of ENMs (ie the complete life cycle of ENMs) are needed, therefore confirming the presently persistent lack of knowledge in ENMs release from consumer products. On the same document test recommendations are provided so that the rate at which ENMs embedded into consumer articles are released into water and air is determined under **conditions of product use**[2].

The actual situation is that still there are **many unknown data on the actual release of NPs –and associated exposure levels- from consumer products throughout their life cycle**[3] and that testing protocols are to be developed, optimized and demonstrated in terms of suitability for their use in different industrial applications.

In the last years, many research efforts are being focused towards the development of adequate testing strategies to evaluate the toxicology and ecotoxicology of different ENMs, but, studies focused on developing methodologies to evaluate the exposure towards NPs generated throughout the life cycle of man-made Nanotechnology based products are only starting to emerge: NEPHH Project – www.nephh-fp7.eu; NANOPOLYTOX Project - www.nanopolytox.eu; NANOFATE Project - http://www.nanofate.eu; Wohlleben, W. et al. 2011. *On the Lifecycle of Nanocomposites: comparing released fragments and their in-vivo hazards from three release mechanisms and four Nanocomposites…*

NEPHH FP7 founded Project has demonstrated that physical processing at manufacturing, use and recycling phases actually conveys NPs release that need to be evaluated in relation to their (eco)toxicological potential[4]. Within NEPHH, methods for evaluating, characterizing and quantifying the release of nanoparticles from reference composite matrixes -not including nanofillers in their formulation- and nanocomposites with different fillers have been developed.

In detail, the frame of NEPHH three different engineering matrixes (Polypropylene –PP; Polyamide 6 – PA6 and Polyurethane –PU) were selected and nanoreinforced with four different types of Silicon based nanomaterials at a fixed weight percentage. For comparison purposes, the same matrixes without any reinforcement were chosen as reference materials. Series of macro samples were produced at laboratory scale. Lightweight materials as those being gradually introduced in Aerospace and Automotive Industries were produced but the specific application was not defined.

In the execution of NEPHH some improvement potential became evident if industrial and commercial samples are of interest. The reasons are hereby detailed:

- For a given comparison, materials with the **same or similar functionalities** should be assessed. For example: if a non-reinforced PP matrix is chosen as a reference and then compared with the same matrix with a 5% wt montmorillonite, the resulting plastic will be radically different, since the addition of a given amount of nanoreinforcement turns the piece incomparable in terms of mechanical performance. In fact, at industrial scale the introduction of nanoreinforcements does not always imply the complete replacement of the traditional micro fillers (glass fibres, for instance) but mixed combinations of both, depending on the desirable properties of the intended piece.
- The **definitive composition of a given piece will very much vary depending on the specific piece and expected functionalities**. While PP is used in car bumpers and body panels, PA is applied in door handles and hubcaps but also the type and quantities of nanoreinforcement used will vary even if the same engineering matrix is used.
- The potential release will very much depend on the **specific application of interest** and simulation protocols should ideally be defined bearing this fact in mind. (A paint to be used outdoors will not undergo the same degradation process as a paint to be used indoors).

For the reasons above, the demonstration of the suitability of laboratory scaled developed approaches for the replication of the mechanical processing techniques also at **industrial scale** with pre

[1] 2004 – The Royal society and The Royal Academy of Engineering – Nanoscience and nanotechnologies

[2] Interim Technical Guidance for Assessing Screening Level Environmental Fate and Transport of, and General Population, Consumer and Environmental Exposure to NM. http://www.epa.gov/oppt/exposure/pubs/nanomaterial.pdf

[3] NanoSafe 2010 – Communication from F. Gottschalk, T.; Sonderer, R.W.; Scholz, Modeled Environmental concentrations of engineered nanomaterials (TiO2, ZnO, Ag, CNT, Fullerenes) for different regions

[4] S. Sachse, A. Irfan, H. Zhu, J. Njuguna, (2011) "Morphology studies of nanodust generated from polyurethane/nanoclay nanofoams following mechanical fracture" Journal of Nanostructured Polymers and Nanocomposites, 7;5-9

market materials for ENMs release assessment revealed necessary. Within SIRENA we intend to go two steps forward:

- By demonstrating that developed methods and protocols are replicable in a **wide range of industrial sectors** (automotive, aerospace, renewable energy) and that industry can incorporate developed methodologies and protocols as a standard testing protocol for the evaluation and characterization of the risk of NPs release throughout the life cycle of Nanotechnology enabled products. The approach developed within NEPHH based on one scenario can be adopted for assessing the level of NP release from different materials from a wide range of industrial sectors in different scenarios and the related environmental effect, therefore contributing to address actual knowledge gaps in risk assessment of ENMs from an integrated life cycle perspective.
- By replacing laboratory scale produced composites (originally targeted within NEPHH) by **materials in pre market stage**, in order to demonstrate methodologies' suitability.

The SIRENA LIFE Project intends to provide necessary output on the combination of evolving toxicological knowledge with life cycle concepts that could mitigate the uncertainty about the effects of ENMs on human health and the environment associated to the actual exposure rates and to integrate this information within the general approach for Risk Assessment for the effective implementation of EC policies aimed at the environmental protection, but, furthermore, outcomes of present project will also lead to increased human welfare protection.

5 Overall view of the Workplan

The SIRENA Life Project is structured into **Preparatory Actions** (materials manufacturing, methodologies evaluation and pilot experience) and **Implementation Actions** that include the effective assessment of ENMs release from nanocomposites by two different approaches (nanocomposites drilling and nanocomposites crashing) and **Best Practice Manuals development** upon results achieved. Apart from those, significant efforts are devoted to **Project Monitoring**, **Project Dissemination** and **Project Management**. All these actions are actively supported by a **Technological Surveillance (TSS)** action that monitors, captures and critically evaluates new information being made available on relevant internet based sources of information.

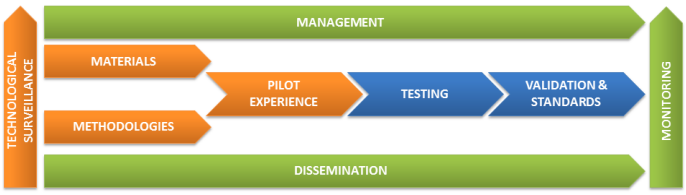

Figure 2: Representation of the inter-relations amongst project actions

6 Progress and Outcomes to date

In the first year of the implementation of SIRENA different preparatory actions have been developed:

A.1. Technological Surveillance and Benchmarking

A.2.Materials

A.2.1. Nanocomposites/ENMs selection for each of the industrial sectors of evaluation (Aerospace, Renewable Energy, Automotive).

A.2.2. Nanocomposites manufacturing.

A.3. Methodologies

A.3.1. Evaluation of the different environmental exposure scenarios of Nanotechnology enabled products throughout their Life cycle.

A.3.2. Evaluation of current technologies and protocols for environmental exposure assessment for consumer products incorporating nanoparticles/ENMs

In addition to those, monitoring, dissemination and management actions have been developed as described next.

6.1.1 A.1. Technological Surveillance and Benchmarking

The **Technological Surveillance System** (TSS) whose objective is to properly trace and asses relevant information in the areas of interest of SIRENA, has been designed and is currently being implemented with a total number of **123 inputs** included in the database on January 2014.

Different types of documents (scientific articles; proceedings from conferences, symposia, thesis, etc.) containing any of the terms in a list of related key words are searched using different search and metasearch engines and specific software tools. The items captured are classified into five major topics as described below:

1- **Nanomaterials used in the production of nanocomposites**

Information related to the benefits of adding nanofillers in the production of polymeric nanocomposites is captured. Nanocomposites based on other matrixes (ceramic/metallic, etc.) are not under the scope of this project.

2- **Real processes that cause release of nanomaterials. Physico-chemical characterization of generated samples in the release and associated (eco)toxicological profile. Methods to quantify the release**

Information about real processes that may cause environmental release (caused by intentional or incidental mechanisms) of ENMs from solid polymeric matrixes through an item´s life cycle are gathered under this topic. Additionally, information related to the physical and chemical characterization and quantification of particles released to the environment in these real processes is also compiled under this topic. Documents related to the toxicological evaluation of particles released in real processes from solid polymeric matrixes containing nanomaterials will also be classified under this topic.

Information about studies centred in the release minimization methods is also considered.

The intended release of particles from drug therapy applications and the unintended release of particles from composite debris from medical devices are not covered.

3- Nanomaterials release simulation technologies. Physico-chemical characterization of generated samples in the release and associated (eco)toxicological profile Methods to quantify the release

Protocols and methodologies used by the different research groups in order to simulate the release to the environment of Engineered Nanomaterials from solid polymeric matrixes throughout an item´s life cycle are compiled in this topic.

As in the previous topic, all the information related to the physical, chemical and toxicological characterization and quantification of particles released to the environment in these simulated processes will also be compiled under this topic.

The simulation processes of release of particles from drug therapy applications and from composite debris from medical devices are not covered.

4- Related regulatory and standardisation activities

Changes in the regulatory frame in relation to nanomaterials and nanotechnology occurred during the duration of the project are captured, including the publication of international standards.

5- Nanocomposites market

Information about consumption of polymeric nanocomposites (real and estimations), geographical distribution of the nanocomposite market, market share of the different nanofillers, etc. is taken into account under this topic.

The outcomes of the TSS have been made freely accessible in its specific section on the website.

6.1.2 A.2. Materials

In relation to the **materials** to be used for the manufacturing of the testing specimens, the reference formulations and the nanofillers have been defined. The different matrixes were defined during the proposal preparation as described next:

EPOXY RESIN was selected for the **aeronautical sector**. Some of the characteristics of this matrix include its exceptional adhesion, corrosion and chemical resistance and toughness. In the case of the **renewable energy sector**, **POLYESTER** was chosen. The reasons for this selection include material's cost effectiveness, high performance and environmental friendliness. Finally, **POLYPROPYLENE** was selected for the **automotive sector**. Possibly one of the most versatile plastics, Polypropylene contributes to fuel-efficient vehicles replacing heavier materials.

For each of the industrial sectors of relevance, ENMs have been selected in order to increase the performance of the non-nanoadditivated matrixes. The selection of ENMs and defined weight percentages is summarized in the next table. The reference formulations are also mentioned.

INDUSTRIAL SECTOR	REFERENCE FORMULATION	PROPERTIES TO BE IMPROVED	NANOREINFORCEMENTS	NANOCOMPOSITE FORMULATION
	Neat Epoxy	Electrical conductivity	Carbon nanotubes (CNT) Carbon nanofibres (CNFs)	Epoxy + 2% CNTs Epoxy + 2% CNFs
	Neat Polyester	Mechanical properties (Tensile, Flexural and Impact behaviour)	SiO_2 NPs Al_2O_3 NPs	Polyester + 5% SiO_2 Polyester + 5% Al_2O_3
	Polypropylene + 20% Talcum	Density Mechanical properties (Tensile, Flexural and Impact behaviour)	Wollastonite (WO) Montmorillonite (MMT)	PP + 5% WO PP + 5% MMT

Table 1: Selection of test materials

The selection of the processing techniques and necessary equipment for nanocomposites manufacturing has been performed according to the chemical nature of the selected matrices.

6.1.3 A.3. Methodologies

With regard to the methodologies, environmental exposure scenarios beyond manufacturing stages are being assessed by the assimilation of NMs to traditional plastic additives and their associated emission scenarios.

Additionally, a total of 32 relevant publications addressing the simulation of the release of embedded NMs from nanocomposites by physical mechanisms have been identified and are actually being evaluated for exposure assessment in nano-release scenarios.

6.1.4 C. Monitoring

The monitoring actions implemented so far include:

- C.1. Identification of the specific indicators and sources of verification,
- C.2. Assessment of the initial situation – Value of the main indicators, and
- C.3. Monitoring actions

The impact of the SIRENA LIFE project is being monitored by means of five specific indicators that were identified during proposal preparation. The number of research efforts dedicated to ENMs release simulation (Indicator N2) has significantly increased in comparison with the original value of present indicator (6 references VS 64 references listed in the last update of the outcomes of the TSS –January 2014-) but, in absence of a standard guideline, the heterogeneity of the approaches used and lack of details represent significant challenges to the comparison of the different approaches (Indicator N3). Additional areas of application of the outcomes of SIRENA identified in the implemented monitoring actions (Indicator N5) include bio-nanocomposites. Standard methodologies specifically addressing the simulation of different life cycle stages of nanocomposites and addressing the associated nano-release (Indicator N1) have not been traced. For what refers to Indicator N4, the last updated figure of the PEN Inventory of Nanotechnology based Consumer Products (October 2013) refers to 1628 products or product lines. An additional reference is that of the report *Nanosafety in Europe 2015-2025: Towards Safe and Sustainable Nanomaterials and Nanotechnology Innovations* by Savolainen, K. et al which estimates a $3,000 Billion

market size for products incorporating ENM by 2020 (Based on Roco, 2010[5]).

6.1.5 D. Dissemination

The dissemination actions to date are regularly updated on project's website. The first workshop of SIRENA is of major relevance: it will be held within the NANOSTRUCT Conference in Madrid, on the 21st May 2014 (http://www.nanostruc.info/)

7 Future actions planning

In the next year of execution of SIRENA, the preparatory actions will have finished and implementation actions (testing) will have started. Major milestones for the next period include:

- M.A.1 Establishment and management of a TSS
- M.A.2 Specimens for testing
- M.A.3 Environmental Exposure Scenarios and protocols for Environmental Exposure Assessment.
- M.A.4 Pilot Experience for protocols adequacy confirmation

Within tests planned two different approaches for the nano-release assessment in different use scenarios are considered: **drilling and crashing**. In this sense, adequate test chambers will be constructed and protocols defined.

Airborne particles will be collected and sampled. Larger Particles which deposit under the influence of gravity will be size fractioned and characterized by means of Scanning Electron Microscopy - SEM, Transmission Electron Microscopy - TEM, Dynamic light scattering –DL-S, Nanosight. By means of electron microscopy, micrographs of the particles will be used to identify particle shape and size. Dynamic light scattering and Nanosight are employed to detect particle size distributions, as well as number of particles in a solution. Additionally Energy dispersive X-Ray spectroscopy (EDX) will be used to identify the chemical composition of the materials.

The release rate of ENMs embeded into plastic nanocomposites in use scenarios is intended.

On the basis of the demonstration actions of the SIRENA LIFE project, best practice manuals for nano-release assessment in nanocomposites-based consumer products validated from an industrial perspective will be produced. These guidelines could be used as an starting point for standardized technical instructions on the TR/TS Nanocomposites - guidance on ageing / particle release[6].

8 Expected Impact

Expected results from SIRENA have been hereby listed:

- A **searchable database** including outcomes from the technological surveillance system.
- A state of the art report in relation to the **methods to simulate the release of nanomaterials** from consumer products in different life cycle stages.
- Evaluation of **exposure scenarios** of applications selected for testing.
- **Exposure data** to support risk-management decision-making and regulations development to protect human health and the environment.
- **Validated methodologies** and **prototypes** for Environmental Exposure Scenario (EES) replication in different stages of the life cycle to be used by industries placing into market a variety of consumer products incorporating nanomaterials.
- **Best practice manuals**
- **Novel approaches** in the R&D phase of **new products** generation.

The (eco)toxicological profile of the released particulate material, though relevant for risk assessment of nanocomposites is not addressed in the main objectives of SIRENA since only the release is covered. A battery of samples will be generated and made available to the nanosafety community for (eco)toxicological testing upon request.

9 About the LIFE Programme

The **LIFE Programme** is the European Union's funding instrument that supports environmental and nature conservation projects. The general objective of LIFE is to contribute to the implementation, updating and development of European Union environmental policy and legislation by co-financing pilot or demonstration projects with European added value.

LIFE began in 1992 and to date there have been three complete phases of the programme (LIFE I: 1992-1995, LIFE II: 1996-1999 and LIFE III: 2000-2006). During this period, LIFE has co-financed some 3104 projects across the EU, contributing approximately €2.2 billion to the protection of the environment.

[5] The long view of Nanotechnology development: The National Nanotechnology Initiative at ten years. Mihail C. Roco

[6] Draft report of the execution of mandate M/461 "Standardization activities regarding nanotechnologies and nanomaterials" Revised roadmap and timetable. Secretary of CEN/TC 352. Doc N 304 Supersedes N295

The programme has three components: LIFE+ Nature and Biodiversity, LIFE+ Environment Policy and Governance, and LIFE+ Information and Communication.

LIFE+ Nature and Biodiversity co-finances best practice or demonstration projects that contribute to the implementation of the Birds and Habitats Directives and the Natura 2000 network. In addition, it co-finances innovative or demonstration projects that contribute to the implementation of the objectives of Commission Communication (COM (2006) 216 final) on "Halting the loss of biodiversity by 2010 – and beyond".

LIFE+ Environment Policy and Governance co-finances innovative or pilot projects that contribute to the implementation of European environmental policy and the development of innovative policy ideas, technologies, methods and instruments.

LIFE+ Information & Communication co-finances projects relating to communication and awareness raising campaigns on environmental, nature protection or biodiversity conservation issues, as well as projects related to forest fire prevention (awareness raising, special training).

Additional information can be found on: http://ec.europa.eu/environment/life/.

10 Directory

Table 2 Directory of people involved in this project.

First Name	Last Name	Affiliation	Address	e-mail
Idoia	Unzueta	INKOA SISTEMAS	Ribera de Axpe 11, Edificio D1, Dpto. 208. 48950 Erandio	Idoia@inkoa.com
María	Blázquez	INKOA SISTEMAS	Ribera de Axpe 11, Edificio D1, Dpto. 208. 48950 Erandio	maria@inkoa.com
Maitane	Oviedo	INKOA SISTEMAS	Ribera de Axpe 11, Edificio D1, Dpto. 208. 48950 Erandio	moviedo@inkoa.com
James	Brighton	CRANFIELD UNIVERSITY	Building 61, Cranfield University, Cranfield, Bedfordshire MK43 0AL	j.l.brighton@cranfield.ac.uk
Verónica	Marchante	CRANFIELD UNIVERSITY	Building 61, Cranfield University, Cranfield, Bedfordshire MK43 0A	v.marchanterodriguez@cranfield.ac.uk
Laura	Gendre	CRANFIELD UNIVERSITY	Building 61, Cranfield University, Cranfield, Bedfordshire MK43 0A	l.gendre@cranfield.ac.uk
Ainhoa	Egizabal	FUNDACIÓN TECNALIA RESEARCH AND INNOVATION	Parque Tecnológico de San Sebastián, Mikeletegi Pasealekua, 2 E-20009 Donostia-San Sebastián (Spain)	ainhoa.egizabal@tecnalia.com
Cristina	Elizetxea	FUNDACIÓN TECNALIA RESEARCH AND INNOVATION	Parque Tecnológico de San Sebastián, Mikeletegi Pasealekua, 2 E-20009 Donostia-San Sebastián (Spain)	cristina.elizetxea@tecnalia.com
James	Njuguna	INSTITUTE FOR INNOVATION, DESIGN & SUSTAINABILITY ROBERT GORDON UNIVERSITY	Riverside East, Garthdee Road Aberdeen, AB10 7GJ United Kingdom	j.njuguna@rgu.ac.uk
Kristof	Starost	INSTITUTE FOR INNOVATION, DESIGN & SUSTAINABILITY ROBERT GORDON UNIVERSITY	Riverside East, Garthdee Road Aberdeen, AB10 7GJ United Kingdom	k.starost@rgu.ac.uk

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SUN

Sustainable Nanotechnologies

Contract Agreement: 604305 Website: http://www.sun-fp7.eu
Coordinators: Danail Hristozov, Antonio Marcomini

Table 1 Consortium List.

No.	Beneficiary name	Short name	Country
1	University Ca' Foscari Venice	UNIVE	Italy
2	European Research Services GmbH	ERS	Germany
3	BASF	BASF	Germany
4	Nanocyl	NCYL	Belgium
5	Colorobbia	COL	Italy
6	Plastic Components and Modules Automotive	PCMA	Italy
7	Veneto Nanotech	VN	Italy
8	MBN Nanomaterialia Spa	MBN	Italy
9	Die Innovationsgesellschaft mbH	INNO	Switzerland
10	Plasmachem GmbH	PCHEM	Germany
11	Malsch Techno Valuation	MTV	Portugal
12	The REACH Centre Ltd	TRC	UK
13	ETSS Gottschalk and Co	ETSS	Switzerland
14	Institute for Occupational Medicine	IOM	UK
15	Spanish National Institute for Agriculture and Food Research and Technology	INIA	Spain
16	Rijksinsituut voor Volksgezondheid en Milieu	RIVM	The Netherlands
17	Toegepast Natuurwetenschappelijk Onderzoek	TNO	The Netherlands
18	National Research Centre for the Working Environment	NRCWE	Denmark
19	Swiss Federal Laboratories for Materials Science and Technology	EMPA	Switzerland
20	Fraunhofer-Institut für Molekularbiologie und Angewandte Oekologie	IME	Germany
21	Centre Européen de Recherche et d'Enseignement	CEREGE	France
22	Consiglio Nazionale Delle Ricerche	CNR	Italy
23	RIKILT Institute of Food Safety	RIKILT	The Netherlands
24	Technical University of Denmark	DTU	Denmark
25	Heriot-Watt University	HWU	UK
26	Karolinska Institute	KI	Sweden
27	University of Aveiro	UAVR	Portugal
28	University of Plymouth	UoP	UK
29	Rheinisch-Westfaelische Technische Hochschule Aachen	RWTH	Germany
30	Aarhus University	AU	Denmark

31	University of Vienna	UNIVIE	Austria
32	Vrie Universitet Amsterdam	VUA	The Netherlands
33	University of Leeds	LEEDS	UK
34	University of Bremen	UniHB	Germany
35	University of Limerick	UL	IE

Contents

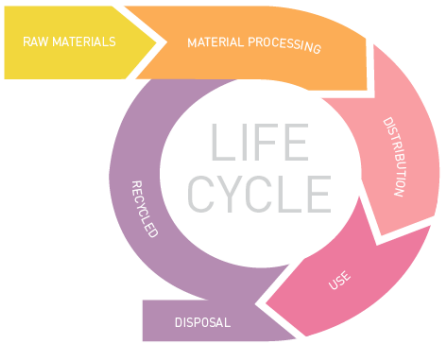
1 Summary

Project Duration: 42 months

Project Funding: 13 539 313 euro

Our understanding of the environmental, health and safety (EHS) risks from nanotechnologies is still limited, which may result in stagnation of nanoinnovation and economic growth. There have been other technologies (e.g. asbestos) that revealed unexpected ecological and health effects only several years after their broader market introduction. In the worst cases this caused tremendous costs for society and the enterprises in the form of lock-in effects, over-balancing regulations and demolished consumer confidence.

The Sustainable Nanotechnologies (SUN) project is based on the idea that the current knowledge on EHS risks of manufactured nanomaterials (MNs), while limited, can nevertheless guide nanomanufacturing to avoid future liabilities if an integrated approach addressing the complete product lifecycle is applied.



SUN was launched on 1 October 2013 and will continue for 42 months, bringing together 35 partners from 12 EU countries. With a total budget of about 13 539 313 euro, SUN is among the highest funded projects of the EU FP7 research programme.

Unlike other nano-EHS projects advancing the understanding of the properties, interactions, fate, impacts and risks of nanomaterials, SUN was envisioned to walk down the road from scientific implications to industrial applications while at the same time inform regulatory oversight.

The SUN research process integrates the bottom-up generation of nano-EHS data and methods with the top-down design of a Decision Support System (DSS) for practical use by industries and regulators.

The SUN industrial partners will test the DSS against supply chains of real products. This validation will culminate in guidelines for safe nanoscale product and process design. In addition, SUN will identify needs for future research and assign priorities for current regulation. We will work with major international stakeholders to implement the SUN results into practice and regulation.

2 Background

Several EU research projects (e.g. ENPRA, MARINA) have studied the short-term risks from production and use of MNs. However, most of them have used pristine MNs produced specifically for testing, which are not representative for real lifecycle scenarios. Pristine MNs undergo weathering and transformation reactions when incorporated into products and when released from them. However, the identity of MNs released from actual products used by occupational users and consumers is largely unknown. The NANORELEASE (Consumer Products) project identified the end-of-life (e.g. shredding, incineration, landfilling, recycling) as the lifecycle stage where significant release could occur, especially for product where the MNs are bound in a matrix, but only few projects (e.g. GUIDEnano) are investigating MNs end-of-life exposure scenarios.

Some new modelling studies have investigated consumer exposures to MNs, released from products, but no empirical data on consumer release and exposure measurements are available. In contrast, data are slowly emerging on workplace emission, exposure characteristics and source strengths from different release scenarios in the production stage.

MNs released from industrial processes and consumer products will ultimately end up in the environment. Several studies have shown that MNs accumulate to a significant extent in the sludge of Sewage Treatment Plants. Sewage sludge is used as fertilizer in

many countries. Therefore it is a relevant source of MNs entering soil affecting terrestrial organisms and potentially humans. In addition, the waste water can be a dominant source of MNs in the aquatic compartment. Since many MNs react when they come into contact with natural media such as freshwater or seawater, typically by agglomeration, it is expected that they will preferentially partition to sediments. Many NMs are (designed to be) persistent, which may lead to the long-term exposure of humans, terrestrial and aquatic organisms, thus posing long-term health and ecological risks and disrupting vital ecosystem services.

Toxicity data for MNs have been produced in various EU projects. As a result, a coherent profile of NMs health hazards begin to emerge. However, our understanding of the long term effects of MNs is at an early stage, especially for MNs released from real products and for environmental species. Moreover, risk analyses still face considerable remaining knowledge-gaps concerning biological uptake and toxicity action modes as well as environmental behaviour, fate and exposure to MNs.

Until now, the risks and the environmental impacts of only few nanoproducts have been studied from lifecycle perspective, and a handful of generic LCA studies exist, mainly focusing on material synthesis and product formulation. This is mainly due to the lack of reliable Lifecycle Inventory data for the use and the end-of-life stages.

Risk management measures currently applied to MNs (e.g. engineering controls, administrative controls, personal protective equipment) do not significantly depart from conventional safety practices for handling chemicals. These procedures are based upon the properties of the bulk form or the solvent carrier and not on nano-specific characteristics. The same is valid for the current waste management practices (e.g. composting, landfilling, incineration and recycling). Only recently, Safety by Design has become a national initiative in the US. This concept is material-specific and aims to retain the functionality of materials and products, while reducing their health and environmental risks. In EU the SANOWORK, GUIDEnano and NANoREG projects are developing Safety by Design and waste management strategies for nanoscale TiO_2, CuO and Ag.

3 Scientific and technological challenges

As with any new technology, large-scale production and commercialization of nanotechnologies require an understanding of their ESH impacts, and must develop strategies for their safe production, use and disposal. Today we face a challenge to achieve reproducibility in industrial/product performance; and to understand and mitigate the potential risks emerging from innovation in nanotechnology. The fundamental issue is that nanomaterials undergo complex transformations during their lifecycles, which affect not only their environmental and health effects, but also their industrial applications.

4 Objectives

In order to address the gaps summarised in section 2 and the challenges described in section 3, The SUN consortium identified a set of objectives within the following 3 central themes of sustainable nanoinnovation.

THEME 1 : MATERIALS, PRODUCTS AND PROCESSES

• Perform a data gap analysis with regard to the SUN case studies in order to prioritise data production in the project

• Map hot spots release of nanomaterials during different stages of the value chains in order to guide cost-effective strategies for release and exposure estimation

• Assess the environmental impacts arising from each lifecycle stage of MNs and compare the results to conventional products with similar uses and functionality

• Develop and validate criteria and guiding principles for green nanomanufacturing (low energy consumption, eco-friendly materials) and for setting environmental quality targets

THEME 2 : RISK ASSESSMENT

• Collect and characterize MNs released from real products in different lifecycle stages for use in (eco) toxicological and behaviour/fate studies

• Model the behaviour/fate of MNs and assess their exposure concentrations in the environment (i.e. air, water, sediment and soil compartments)

• Develop and validate methods (incl. high-throughput and content tools) for prediction of long-term effects of MNs in humans and on ecosystem services in environments subjected to multiple stressors

• Develop and validate a three-step tiered approach for qualitative to quantitative assessment of inhalation and dermal to gastro-intestinal occupational and consumer exposure to MNs, based on high-quality collated and project-generated emission rates, exposure measurements and contextual information

• Use the exposure and effects data acquired from other projects and the data newly produced in SUN for quantitative lifecycle-oriented ecological and human health Risk Assessment

THEME 3 : SAFE PRODUCT AND PROCESS DESIGN

• Describe best available technologies/practices for reduction of exposure and effects of MNs in different lifecycle stages

• Develop the following innovative risk reduction methods and practices and include them in guidelines for safe nanoscale product and process design:

- safety by design elimination/substitution and waste isolation practices to reduce the release of nanomaterials from products/composites or to induce their accelerated alteration/degradation in order to reduce their environmental persistence and bioaccumulation

- methods to analyse the evolution of the product quality parameters, process conditions and interactions, in real-time, to subsequently exercise control over them, increasing both product safety and quality

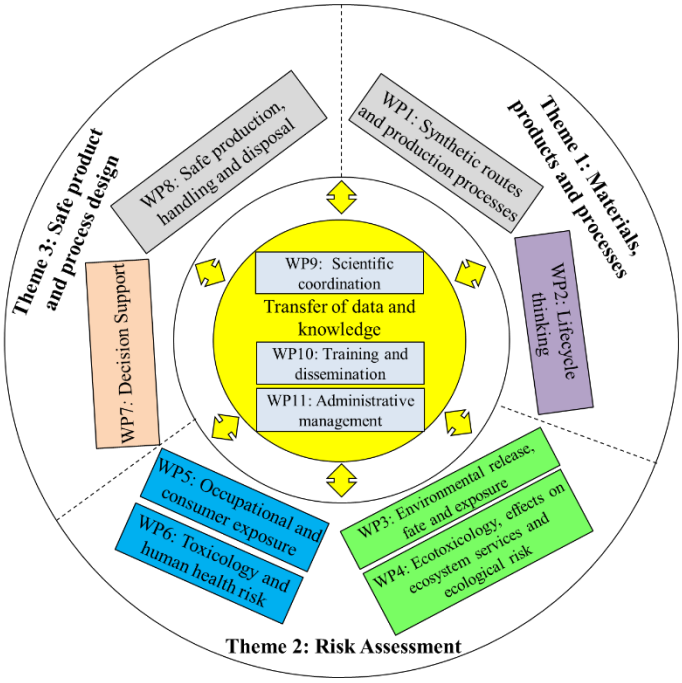
- best practices to minimise release and exposure of MNs during handling of waste flows containing MNs

• Develop/validate the user-friendly software-based Decision Support System for estimating MNs risks for different targets (e.g. workers, consumers, ecosystems) in each lifecycle stage and

evaluating to which extent the available technologies/practices could reduce this risk (incl. cost-effectiveness analysis)

5 Organisation

SUN consists of 8 scientific Work Packages (WP) distributed among three main themes.



WP1 is both the start point and end point of SUN: real products and processes throughout all stages of the lifecycle set the stage for all activities in WP 3-6. The outcomes, both direct feedback from WP3-6 as well as the results of WP7 and WP8, namely the Decision Support System and guidelines, feed into the current practice of the industry partners.

WP3-6 will produce data on physico-chemical characteristics, hazard, exposure, risk and environmental impact in a number of 'real-life' case studies (cf. page 6) where such data does not already exist in sufficient quality. WP3-6 benefit from the excellent network of the partners; most major European national projects will provide data, which SUN does not have to duplicate. WP7 will develop practices, methods and tools to facilitate safe production, handling and disposal of MNs and incorporate them into case-specific guidelines for safe product and process design. Finally, the tools (and data, where needed) produced in WP1-7 will be integrated into the SUNDS in WP8.

In addition we foresee three horizontal Work Packages. Sharing results and taking decisions in a consortium of thirty-five partners is a challenge, but the partners have a long history of collaboration; so, we are confident that the size of SUN will not affect its efficiency. WP9 will deal with the scientific coordination, which comprises scientific steering to keep the work plan up to date and to avoid 'mission creep'. The non-scientific, administrative management of the project is done in WP11, shared between the Coordinator and a dedicated project management company (ERS). WP10 is dedicated to dissemination activities. This does include conventional publications, conference-contributions and website, and a significant commitment to collaborate with other initiatives (e.g. in the Nanosafety Cluster), but as well workshops and trainings for the 'end users' of SUN's results, namely industry and regulators.

6 Expected Impact

The overall impact of SUN is to provide nanomanufacturers and regulators with data and tools to address the above scientific and technological challenges. The project aims to give clear answers to questions from regulatory authorities, and open new possibilities for innovators to design greener nanotechnologies. This will be achieved through development and application of new methods and tools for prediction of exposure and effects on humans and ecosystems and implementable practices for risk prevention and management covering the complete lifecycles of nanomaterials. This approach aims to protect innovation by providing industries with data and prospective tools to streamline effective decision making about safer products and processes.

To test and validate the proposed tools and to maximize the impact of the project, we carefully scoped the data generation and analysis to address the most important concerns that regulators and manufacturers currently face. The markets covered by the SUN case studies TiO_2, SiO_2 and Irgazin are large: plastics: 235,000,000 tons worldwide, thereof EU sales worth € 295 billion and 1,450,000 jobs in EU; pigments: 317,000 tons, worth €4 billion, and nano-fillers: 242,000 tons. Because the highest profit margins for material producers are in the formulation and synthesis of compounds we focus the SUN activities on these steps of the value chains. The sintering ceramic material WC was selected to ensure the applicability of the methods developed in the project also to the impressive portfolios of the cement/concrete and fillers industries. The rest of the materials were selected less for their commercial impact, but because of their very considerable consumer and environmental safety impact: CuO, Ag: fewer than 1,000 tons, but of ecotoxicity concerns; MWCNT: less than 300 tons, but of human toxicity concerns.

7 Directory

Table 1 Directory of people involved in this project.

First Name	Last Name	Affiliation	e-mail
Antonio	Marcomini	UNIVE	marcom@unive.it
Danail	Hristozov	UNIVE	danail.hristozov@unive.it
Andrea	Brunelli	UNIVE	andrea.brunelli@unive.it
Elena	Semenzin	UNIVE	semenzin@unive.it
Vrishali	Subramanian	UNIVE	vrishali.subramanian@unive.it
Alex	Zabeo	UNIVE	alex.zabeo@unive.it
Hildegard	Luhmann	ERS	hildegard.luhmann@european-research-services.eu
Nandine	Bresch	ERS	nadine.bresch@european-research-services.eu
Oliver	Panzer	ERS	oliver.panzer@european-research-services.eu
Sigrid	Schüller-Ravoo	ERS	sigrid.ravoo@european-research-services.eu
Wendel	Wohlleben	BASF	wendel.wohlleben@basf.com
Julie	Muller	NANOCYL	julie.muller@nanocyl.com
Giovanni	Baldi	COLOROBBIA	baldig@colorobbia.it
Enrico	Parola	PCMA	enrico.parola@magnetimarelli.com
Etienne	Valentin	PCMA	etienne.valentin@magnetimarelli.com
Pasquale	Iacobone	PCMA	pasquale.iacobone@magnetimarelli.com
Giorgia	Merlin	VN	giorgia.merlin@venetonanotech.it
Stefano	Pozzi Mucelli	VN	stefano.pozzimucelli@venetonanotech.it
Alvise	Bianchin	MBN	research@mbn.it
Paolo	Matteazzi	MBN	research@mbn.it
Cristoph	Meili	INNO	christoph.meili@unisg.ch
Helmut	Elbert	INNO	helmut.elbert@innovationsgesellschaft.ch
Tobias	Widler	INNO	tobias.widler@innovationsgesellschaft.ch
Alexei	Antipov	PLASMACHEM	antipov@plasmachem.com
Ineke	Malsch	PLASMACHEM	postbus@malsch.demon.nl
Matteo	Dalla Valle	MTV	m.dallavalle@thereachcentre.com
Fadri	Gottschalk	TRC	fadri.gottschalk@etss.ch
Lang	Tran	ETSS	lang.tran@iom-world.org
Martie	van Tongeren	IOM	Martie.VanTongeren@iom-world.org
Peter	Ritchie	IOM	Peter.ritchie@iom-world.org
José M	Navas	IOM	jmnavas@inia.es
María-Luisa	Fernández-Cruz	INIA	fcruz@inia.es
Flemming	Cassee	INIA	flemming.cassee@rivm.nl
Ilse	Gosens	RIVM	ilse.gosens@rivm.nl
Derk	Brouwer	RIVM	dick.brouwer@tno.nl
Wouter	Fransman	TNO	wouter.fransman@tno.nl
Keld Alstrup	Jensen	TNO	kaj@nrcwe.dk
Alejandro	Caballero	NRCWE	Alejandro.Caballero@empa.ch
Bernd	Nowack	EMPA	nowack@empa.ch
Karsten	Schlich	EMPA	karsten.schlich@ime.fraunhofer.de
Kerstin	Hund-Rinke	Fraunhofer IME	kerstin.hund-rinke@ime.fraunhofer.de
Jerome	Rose	Fraunhofer IME	rose@cerege.fr
Anna Luisa	Costa	CNR	anna.costa@istec.cnr.it
Magda	Blosi	CNR	magda.blosi@istec.cnr.it
Ruud	Peters	RIKILT	ruudj.peters@wur.nl

First Name	Last Name	Affiliation	e-mail
Stefan	Weigel	RIKILT	stefan.weigel@wur.nl
Steffen Foss	Hansen	DTU	sfha@env.dtu.dk
Teresa	Fernandes	HWU	T.Fernandes@hw.ac.uk
Vicki	Stone	HWU	v.stone@hw.ac.uk
Bengt	Fadeel	KI	Bengt.Fadeel@ki.se
Hanna	Karlsson	KI	Hanna.L.Karlsson@ki.se
Mónica João	Barros Amorim	UAVR	mjamorim@ua.pt
Richard	Handy	UOP	rhandy@plymouth.ac.uk
Andreas	Schaeffer	RWTH	andreas.schaeffer@bio5.rwth-aachen.de
Anne	Wyrwoll	RWTH	anne.schneider@bio5.rwth-aachen.de
Hanna	Maes	RWTH	hanna.maes@bio5.rwth-aachen.de
Michael	Hennig	RWTH	michael.hennig@rwth-aachen.de
Janeck	Scott-Fordsmand	AU	jsf@dmu.dk
Frank	von der Kammer	UNIVIE	frank.kammer@univie.ac.at
Dick	Roelofs	VU/VUMC	dick.roelofs@vu.nl
Xue Zhong	Wang	LEEDS	x.z.wang@leeds.ac.uk
Terry	Wilkins	LEEDS	t.a.wilkins@leeds.ac.uk
Arnim	von Gleich	UNIHB	gleich@uni-bremen.de
Henning	Wigger	UNIHB	hwigger@uni-bremen.de
Michael	Steinfeldt	UNIHB	mstein@uni-bremen.de
John	Mulcahy	UL	John.Mulcahy@ul.ie
Martin	Mullins	UL	martin.mullins@ul.ie

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Working Group Number: 1

Working Group Name: Materials

Website:http://www.nanosafetycluster.eu/working-groups/materials-wg.html
Chair: Sergio Moya Co-Chair: Rune Karlsson

Table 1: Working Group Contributing projects List

No.	Project Acronym	Role in Working Group	Representative
1	NanoSolutions	Safety classification	Sergio Moya
2	NanoValid	Methods development	Rune Karlsson
3	NanoDefine	Nanomaterial definitions	Rudolf Reuther
	See member directory (below)		

Contents

1 Summary

In recent years, intensive activity has taken place in developing methods, mostly *in vitro*, to evaluate the potential impact of nanomaterials (NMs) on biological systems and to distinguish between low and high toxicity materials. The planning of *in vitro* tests involves several aspects such as sample preparation, selection of cell type, dose ranges, dispersion methods etc. For some of these experimental parameters, standard operating protocols (SOPs) already exist or are being developed. In addition to SOPs, toxicologists also need reference materials with well-defined physical-chemical characteristics to validate their methods and deliver reliable results. Actual activities focus on the metrological traceability of single characteristics of ENPs, like size and composition, but may ignore critical parameters like agglomeration, stability, aging and formation of protein corona in testing media. Key outcomes of this Materials WG are the identification of appropriate reference materials (RM), establishing minimum physical-chemical characteristics requirements for toxicological testing, identification and validation of physical-chemical testing methods, and identification of new developments in material science and nanomaterials based product formulations that can pose challenges for their toxicological evaluation.

2 Scientific and technological challenges

Current resources or test methods are not likely to enable safety assessments of the numerous novel NMs and nanotechnology based products that are emerging at an ever increasing pace. The international dialogue and collaboration activities have helped to understand the complexity of EHS aspects, further highlighting the need for joint international efforts in developing protocols and test methods to assess the health and safety impact of NMs and to provide proper characterization methods. The Working Party for Manufactured Nanomaterial (WPMN) at OECD has been developing guidance on how to apply chemical test guidelines for nanomaterial assessment and evaluating the need for new test methods.

The necessity for improved characterisation of NMs (nanomaterials) has recently emerged as a crucial aspect for the reliable assessment of the risks associated with ENP-handling and potential emission of NMs from products and devices including nanomaterials in their formulations. However, to date there is no clear understanding about what minimum requirements are necessary to establish a reference material which can be used to validate and compare toxicology methods. Actual approaches to RMs for nanotoxicology are based on the certification of one parameter, either size or composition, and frequently ignore other important characteristics such as particle size distribution in real testing media, nanomaterial stability, aging, protein corona formation, etc. One-parametric RMs are probably insufficient for the realisation of reliable and comparable toxicological studies.

A critical need for harmonisation of terminology in nanotechnology has also been identified. Confusion exists because some terms are used interchangeably in many fields; at the same time, the same terms have specific and often opposite meanings in certain fields.

3 Objectives (short, medium & long term)

Short term: Worldwide, various groups e.g., governments, treaty-based organisations, standards development organisations, and research consortia, have more or less independently generated priority lists of NMs for the potential development of RMs, and created lists of characterisation requirements for the RMs to better understand the results of exposure and toxicity assessments. It is generally recognised that one of the principal obstacles to obtaining adequate characterization of NMs and their potential risk is the scarcity of reliable RMs (that is, RMs produced in a scientifically valid way) for the development and validation of exposure assessment tools (instruments, protocols, methods) and toxicological evaluations (materials, protocols).

RMs can provide researchers with suitable materials (including positive and negative controls) to develop harmonised protocols for in vitro and in vivo toxicity testing and elucidate mechanisms of toxicity resulting from nanoscale properties. Also, materials to verify instrument or method performance and operator or laboratory proficiency can be made available.

Medium term: There have been multiple efforts to define NMs, including a focus on defining them for regulatory purposes which enables products containing NMs to be identified and regulated, with limited success to date. Most of these definitions focus solely on size aspect at the nanoscale with some also including surface area and shape. There have also been several suggestions for approaches to classify and prioritise NMs for safety assessments, including the OECD Sponsorship Programme approach based on commercial importance and volume of production.

The emphasis in the EU Commission's definition on NMs is on external dimensions, which may result in the exclusion from the definition of materials with an internal structure (e.g. porous materials with a relatively large internal surface area) or materials with a surface structure at the nanoscale. Therefore, further information is necessary on the interpretation of information on NMs in products and the impact of porosity (internal surface area) on the hazard of NMs. Closely related to the problems associated with the definition of NMs is the choice of a proper metrics for NMs. Particularly complex is how to define the metrics to measure nanomaterial concentration for toxicological testing: particle number, surface area, element concentration, etc. The proper detection, quantification and characterization of NMs are critical pre-requisite tests for the safety assessment of the materials under analysis.

1. *Classification by dimensionality / shape / morphology:*
Shape-based classification is related to defining NMs, and has been synopsized in the ISO terminology.
2. *Classification by composition / chemistry:*
This approach groups NMs based on their chemical properties.
3. *Classification by complexity / functionality:*
Currently, the NMs that are in routine use in products are likely to be displaced by NMs designed to have multiple functionalities or resulting from hierarchical fabrication, displaying supramolecular organization, etc ; the so called 2nd-4th generation NMs.
4. *Classification by biointerface:*
There is also the hypothesis that NMs acquire a biological identity upon contact with biofluids and living entities.

Systems biology approaches will help identify the key impacts and nanoparticle interaction networks.

Multiple reports have identified sets of physical-chemical parameters that should be reported for NMs. However, not all properties are relevant for all NMs, and many are not easily measured on a routine basis. An additional challenge is the fact that many of the physical-chemical properties of NMs are context-dependent and, as such, will be subject to change, depending on the surroundings in which the ENM are presented. The distinction between the synthetic and biological identities of NMs is therefore suggested. The synthetic identity describes the chemical, structural and compositional nature of the nanoparticles, including any surface coatings, ligands or labelling molecules; the *biological identity* describes the bio molecules that absorb to the nanomaterials under specific conditions and the impact of these on their dispersion properties-Long term: A full understanding of the key descriptors for characterising ENM along with validated methods to identify and quantify ENMs in complex matrices is vital in order to identify crucial parameters relevant for risk assessment. This is also important for the measurement of the relevant ENM properties that correlate exposure with biological impacts. This will require agreed reference states for NM characterization, libraries of reference materials, and a framework for understanding later generation NMs.

The required research priorities to achieve this are to:
1. Develop systematic sets of ENMs with properties varied in a stepwise manner that will allow assessment of the significance of each property for toxicity.
2. Describe "reference" states and agreed media compositions to enable identification of significant biomarkers and enable a move towards a predictive toxicity assessment.
3. Understand the longer term fate of nanomaterials and nanomaterial-based products following their interaction with living systems.

4 Workplan for 12 month horizon

- Compile materials list that are under consideration/development as RMs from various groups;
- A systematic literature study of which pc properties are priority for risk assessment of ENPs;
- Compile a list of pc characterisation techniques that are under validation with the aim of being established as reference methods.

5 Progress and Outcomes to date

A teleconference took place on 15th January 2014. Points that were discussed included: WG1 objectives, WG1 membership, reproducible manufacturing, standardisation issues, research infrastructures, interaction with other WGs and dissemination/next meeting. Of particular importance is cooperation with WG2 on toxicology evaluation. Needs that were identified include evaluation of current toxicology methods in relation to material characteristics and evaluation of pc methods and corresponding protocols/SOPs that are under development for standardisation.

Two deliverables for the next 12-month period were formulated. The chair (Sergio Moya) and WG1 members agreed on a roadmap and deliverables to be presented in Antalya in follow up emails. Sergio Moya presented the WG1 at the NSC meeting at the NanoTox conference, Antalya, Turkey, April 2014. Objectives of high importance that were reported include the identification of new material developments of interest to the nanosafety community, categorisation of standard materials of importance for toxicological testing, identifying physical-chemical properties relevant for toxicological assessment, identifying and comparing methods/SOPs for physical-chemical characterisation/ dispersion/ labelling under development. Further, Sergio Moya announced a WG1 working session to take place at the next NSC meeting in Syracuse, Italy, October 2014.

6 Expected Impact

WG 1 Materials aims at contributing to a harmonised terminology regarding nanomaterials definition, metrics, etc, since at the moment this hinders accurate description of many nanomaterial properties. There is also a need to build consensus within the nanotechnology, and environmental, health and safety communities to prioritise RM needs and better define the required properties and physical-chemical forms of candidate materials. The WG also aims to further, discuss and clarify that where RMs are not available if "representative test materials" that lack reference or certified values may be useful for toxicology testing. Finally, it will work towards establishing validated methods (standardised protocols) that are critically needed for reproducible characterisation of nanomaterials as delivered in relevant media and as administered to toxicological models.

Some of the longer term required research priorities identified for material characteristics include: development of systematic sets of ENMs with physical-chemical properties varied in a stepwise manner allowing the assessment of the significance of each property for toxicity; descriptions of "reference" states and media compositions to enable the identification of significant biomarkers and facilitate a move towards a predictive toxicity assessment; development of reference analytical methods that enable the studying of the longer term fate of particles following their interaction with living systems, i.e. complex matrices and developing risk assessment procedures that include the changes of ENM during their life cycle in a targeted manner.

7 Directory

Table 1: Directory of people involved in this Working Group

First Name	Last Name	Affiliation	Address	e-mail
Sergio	Moya	CIC biomaGUNE	De Miramon Ibilbidea, 182, 20009 Donostia, Gipuzkoa, Spain	smoya@cicbiomagune.es
Rune	Karlsson	Nordmiljö AB	Strandvägen 2, 671 29 Arvika, Sweden	rune.karlsson@nordmiljo.se
Rudolf	Reuther	Nordmiljö AB	Strandvägen 2, 671 29 Arvika, Sweden	rudolf.reuther@nordmiljo.se
Roberto	Labrador	Nanologica AB	Drottning Kristinas Väg 45, 11428 Stockholm, Sweden	hanoi@nanologica.com
Wolfgang	Unger	Federal Institute for Materials Research and Testing	Unter den Eichen 87, 12205 Berlin Germany	wolfgang.unger@bam.de
Wolfgang	Parak	Philipps University of Marburg	AG Biophotonik, Renthof 7, 35037 Marburg, Germany	wolfgang.parak@physik.uni-marburg.de
Eugene	Mahon	Centre for Bionano Interactions	School of Chemistry and Chemical Biology, University College Dublin, Belfield, Dublin 4, Ireland	eugene.mahon@cbni.ucd.ie zeljka.krpetic @cbni.ucd.ie
Zeljka	Krpetic			
Lesley	Tobin	Institute of Nanotechnology	Lord Hope Building, 141 St James Rd, Glasgow, G4 OLT, UK	lesley.tobin@nano.org.uk

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Working Group 2

Hazard

Website: http://tinyurl.com/wg2hazard
Chair: Flemming Cassee Co-Chair: Teresa Fernandes

Table 1 Working Group Contributing projects List.

No.	Project Acronym	Role in Working Group	Representative
1	NanoMile	Hosting meetings of WG2 Chair	Flemming Cassee
2	MARINA	Co-Chair	Teresa Fernandes
8	Please see member list as too extensive to list here.		

Contents

1 Summary

This working group covers a large number of topics. This has lead to / will result in the creation of various teams, each with their own webpages, group leaders and members. Current groups include:

- Information exchange of methods available within the Cluster
- Harmonised methods/requirements for biokinetic investigation
- Create summaries of key results for further use by the dissemination group (cooperation Working Group 7)
- Immunosafety focus group
- Marine Ecotox focus group
- Biomembrane Nanosafety focus group
- Genotoxicity Focus group.

Hazard is by nature a very broad theme, and covers both human and environmental hazard. As the vast majority of EU Fp7 Nanosafety projects address some aspect of hazard, almost all are represented in this WG. Membership is thus quite diverse, and ranges from highly active members to those in a more observational role at present who will take on more active roles as new tasks and activities arise.

2 Scientific and technological challenges

Key challenges driving the remit of WG2 (Hazard) are the need for agreed and harmonised approaches to hazard assessment, to reduce the uncertainty regarding the safety of nanomaterials, and provide a scientific rational for differences in observed responses to nominally identical materials reported by different groups, such as different biologically available doses as a result of different dispersion media, or differential ageing behaviour again resulting from differences in media.

3 Objectives (short, medium & long term)

With about 100 subscribers Wg2 and 4 subgroups WG2 is trying to facilitate the communication across the various HSE projects on nanomaterials

- Information exchange of methods available within the Cluster
- Harmonised methods/requirements for kinetic investigation
- Harmonised methods/requirements for appropriate toxicological endpoints

- Harmonised methods/requirements for appropriate in vitro tests (e.g. cell lines)
- Harmonised protocols for testing if appropriate
- Comparison in-vitro versus in-vivo results
- Validation of appropriate testing methods including identification of limitations
- Requirements for screening tests
- Description of correlation between biological effects and material properties (cooperation Working Group 1)
- Description of correlation between bioavailability/bioaccumulation/ADME and NP properties (cooperation Working Group 1)
- Description of correlation between biological effects and exposure aspects and LCA (cooperation Working Group 3)
- Provision of results and outcome to the database (cooperation Working Group 4)

4 Workplan for 12 month horizon

We will start developing a WIKI to facilitate sharing knowledge. This could be outcomes from project that contribute to specific hazard questions and also do's and don't's during experiments, sharing protocols. See:

(http://hazard.nanosafetycluster.eu/mediawiki/index.php/NSC_WG2-Hazard_Wiki)

In addition to this, WG2 will try to align with European societies such as EUROTOX, SETAC and the Environmental Mutagen Society.

5 Progress and Outcomes to date

See website.

6 Expected Impact

Provide an overview of most important achievements on hazard assessment in nanosafety research sponsored by the EU.

7 Directory

Table 1 Directory of people involved in this Working Group.

Name	E-mail address
Albert Duchl	albert.duschl@sbg.ac.at
Andy Booth	andy.booth@sintef.no
Carla Falugi	carlafalugi@hotmail.it
Ilaria Corsi	corsii@unisi.it
Dave Spurgeon	dasp@ceh.ac.uk
Diana Boraschi	diana.boraschi@gmail.com
Flemming Cassee	flemming.cassee@rivm.nl
G. Janer	gjaner@leitat.org
Arno Gutleb	gutleb@lippmann.lu
High Byrne	Hugh.Byrne@dit.ie
Geoffrey Hunt	huntg@smuc.ac.uk
Inge Nelissen	inge.nelissen@vito.be
Irina Estrela-Lopis	Irina.Estrela-Lopis@medizin.uni-leipzig.de
Wolfgang Kreyling	kreyling@helmholtz-muenchen.de
Luigi Calzolai	luigi.calzolai@jrc.ec.europa.eu
Maria Dusinska	maria.dusinska@nilu.no
Dr Song	nwsong@kriss.re.kr
Peter Hoet	peter.hoet@med.kuleuven.be
Richard Handy	rhandy@plymouth.ac.uk
Rob Vandebriel	rob.vandebriel@rivm.nl
Vicki Stone	v.stone@hw.ac.uk
Valaria Matranga	valeria.matranga@ibim.cnr.it
Andrew Nelson	A.L.Nelson@leeds.ac.uk>
Diana Conduto	diana.conduto@jrc.ec.europa.eu
Joanma Bednarcyk	joanna.bednarczyk@inop.poznan.pl

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WG5 - RISK

Website: http://www.nanosafetycluster.eu/working-groups/5-risk-wg.html
Chair: Prof. Lang Tran Co-Chair: Janeck J. Scott-Fordsmand/Agnes Oomen

Table 1 Working Group Contributing projects List.

No.	Project Acronym	Role in Working Group	Representative
1	MARINA	Chair	Lang Tran
2	MARINA	Co-Chair	Janeck J. Scott Fordsmand
3	MARINA	Co-Chair	Agnes Oomen
4	**Please see member list!**		

Contents

1 Summary

There is an urgent need for sufficient knowledge to allow reliable assessment of the risks associated with nanomaterials (NMs).
Given the world-wide nature of the nano related risk work, the need to harmonization the WG5 and the wish for EU to take a leading role in this the work is closely coordinated with the EU_US community of Research initiative.
The Working Group on Risk (WG5) – launched in September 2013 has established communication pathways, send around questionnaires regarding risk tools to all NSC projects, and are working on developing conceptual and regulatory implementable frameworks and tools for risk assessment.

2 Scientific and technological challenges

There is an urgent need for sufficient knowledge to allow reliable assessment of the risks associated with nanomaterials (NMs). The formulation of a grouping/categorization concept that allows safety assessment across materials is required to overcome the current need of testing each NMs on a case-by-case basis (see ITS-Nano), and that is useful for all stakeholders, though developing this grouping/categorisation concept is highly challenging both from a scientific point of view as well as for the process. An Intelligent Testing Strategy (ITS) integrates data from in vivo and in vitro tests, non-testing methods and physico-chemical properties for a specific material as efficiently as possible with regards to costs, the number of experimental animals and time in order to reach a conclusion on potential risks. Such concepts should be used in a risk assessment strategy for NMs to come to a coherent approach.
Such pursuit for a risk assessment and intelligent test strategy protocols is ongoing with various EU projects and in other projects worldwide. Based on these EU initiatives first suggestions have been published e.g. Oomen et al 2013 .
However, there is still a long way to go and this work must continue to get coherent, validated and implementable tools, which compass the expected future development for NMs.

3 Objectives (short, medium & long term)

Based on the initial discussions between the members of WG5, the following objectives have been identified as important starting points:

Short-term:
Identify communication systems/strategies between projects and between WGs

Mid-term:
Draft of an overview of tools and methods (tools) available for Risk Assessment of Nanomaterials

Long-term:
Draft of the concepts useful for the risk assessment of Nanomaterials

4 Activities and deliverables:

Activities

1. Risk WG session various international events e.g. NSC cluster meeting Birmingham 2013, US-EU meeting Washington 2013, NSC cluster meeting Antalya 2014, plus regular phone conferences.
2. Organization of workshop (by MARINA) on NMs risk assessment tools and methods in 2012, 2013, and 2014 (to come).

Deliverables

1. Apart from draft documents, meeting minutes etc., there are no deliverables at present (which is according to plan).

5 Progress and Outcomes to date

Given the worldwide implication of the risk work, it was decided by the WG5 coordinators that the work of WG5 should be closely coordinated with the ongoing work in the risk group of the US-EU Community of Research (CoR).

Communication strategies:
It was decided to keep the communication systems/strategies between projects and between WGs, to e-mails, to NSC website and to face-to-face meeting in connection to NSC meetings or international other meetings. This approach was taken in order to avoid many parallel communication pathways, making progress difficult to follow.

Tools and methods (tools) available for Risk Assessment:
A questionnaire (by MARINA) has been send to all the project leaders regarding the tools used for risk assessment purposes within each project; this information is now being retrieved and analysed. The contribution includes e.g. risk management tools (MARINA), long-term tools (SUN), and web-based tool (GuideNano).
The WG5 participants[2] have also been asked regarding their progress in the risk related area, although low response rate, this has been reported.

Concepts for the risk assessment:
This work is ongoing with the MARINA project, and concepts from other projects has been included e.g. the NSC strategic research agenda and the ITS-nano (see NSC website http://www.nanosafetycluster.eu/working-groups/5-risk-wg.html). Later in the process it is expected to get input from other projects e.g. SUN, NanoValid and GuideNano. The work is continuously fed into the NAnoREG project, to ensure coherency.
The risk related conceptual progress is also made within the CoR initiative, here the WG5 coordinators ensure that there is a continuous information flow between NSC-WG5 and US-EU Cor.

6 Expected Impact

The ongoing work covers the fundamental development of risk assessment and intelligent testing strategies including grouping/categorization, in addition finding ways of implementing this in a regulatory context. The expected impact is hence a directly scientifically based risk assessment/management tool directly applicable for regulators across Europe, but also across Europe and US given the US-EU CoR collaboration. It is expected that the work has wider consequences since, partners from other parts of the world and included in the project e.g. from China, Japan and Russia.

7 Directory

Table 1 Directory of people involved in this Working

Name	eMail
Adriele Prina-Mello	prinamea@tcd.ie
Agnes Oomen	agnes.oomen@rivm.nl
Albert Duschl	albert.duschl@sbg.ac.at
Aleksandar Jovanovic	jovanovic@risk-technologies.com
Aleksandar Jovanovic	lcanano@risk-technologies.com
Alok Dhawan	alok.dhawan@ahduni.edu.in
Amina Tijani	amina.tijani68@free.fr
Anatoly Zherdev	zherdev@inbi.ras.ru
Andrea Haase	andrea.haase@bfr.bund.de
Andrea Richarz	a.richarz@ljmu.ac.uk
Andy Booth	andy.booth@sintef.no
Anja Glinschert	aglinschert@akademie-fresenius.de
Anna Costa	anna.costa@istec.cnr.it
Antoine Barbier	abarbier@cea.fr
Antonietta Gatti	gatti@nanodiagnostics.it
Antonio Pietroiusti	pietroiusti@med.uniroma2.it
Araceli Sanchez	araceli.sanchez@iom-world.org
Arno Gutleb	gutleb@lippmann.lu
Asa Gustafsson	asa.gustafsson@foi.se
Axel P. Mustad	axel@nqcg.com
Barry Park	barry.park@gbpconsulting.co.uk
Benoit Hazebrouck	bh@eu-vri.eu
Bjorne Olsson	bjorne.olsson@ivl.se
Camila Viana	camilaoviana@gmail.com
Carlos Fito Lopez	cfito@itene.com
Christian Sanchez-Espinel	cristianespinel@nanoimmunotech.es
Christoph Meili	christoph.meili@unisg.ch
Danail Hristov	danail.hristozov@unive.it
David Carlander	david.carlander@nanotechia.org
David Mairal	davidmairal@me.com
Derk Brouwer	dick.brouwer@tno.nl
Diana Boraschi	diana.boraschi@gmail.com
Dimiter Prodanov	dimiter.prodanov@imec.be
Doreen Fedrigo	dfedrigo@etui.org
Dr. Miriam Baron	baron.miriam@baua.bund.de
Dr. Stefan Seeger	stefan.seeger@bam.de
Edmunds Tamanis	edmunds.tamanis@du.lv
Elena Semenzin	semenzin@unive.it
Emeric Frejafon	emeric.frejafon@ineris.fr
Enrico Bergamashi	enrico.bergamaschi@unipr.it
Fadri Gottschalk	contact@etss.ch
Falk Andreas	andreas.falk@bionanonet.at
Francesca Larese	larese@units.it
Frederic Amblard	frederic.amblard@cea.fr
G. Janer	gjaner@leitat.org
Gautam Kaul	gkndri@gmail.com
Geoffrey Hunt	huntg@smuc.ac.uk
Gerald Chung	gerald_chung@hsa.gov.sg
Hugh Byrne	hugh.byrne@dit.ie
Il Je Yu	u1670916@chollian.net
Ilaria Corsi	corsii@unisi.it
Isabella Buttino	isabella.buttino@isprambiente.it
James Njuguna	j.njuguna@rgu.ac.uk
Janeck Scott-Fordsmand	jsf@dmu.dk
Jos Bessems	jos.bessems@rivm.nl
Karel Lach	karel.lach@zu.cz

Karl Hoehener	karl.hoehener@temas.ch
Katarzyna Dembowska	k.dembowska@innobaltica.eu
Kerstin Hund-Rinke	kerstin.hund-rinke@ime.fraunhofer.de
Lang Tran	lang.tran@iom-world.org
Lena Wallenhorst	wallenhorst@hawk-hhg.de
Lesley Tobin	lesley.tobin@nano.org.uk
Liliana Tudoreanu	liliana_tudoreanu223@yahoo.com
M.L. Fernandez-Cruz	fcruz@inia.es
Małgorzata Okrasa	maokr@ciop.lodz.pl
Margrethe Winther-Nielsen	mwn@dhigroup.com
Mark-Oliver Diesner	m.diesner@oeko.de
Markus Berges	markus.berges@dguv.de
Martie van Tongeren	martie.van.tongeren@iom-world.org
Martine Reynier	martine.reynier@inrs.fr
Maurice Brennan	M.E.Brennan.1@bham.ac.uk
Michael Faust	faust@fb-envico.com
Michael Riediker	michael.riediker@iom-world.sg
Michael Steinfeldt	mstein@uni-bremen.de
Murphy Finbarr	finbarr.murphy@ul.ie
Mustafa Culha	mculha2@gmail.com
Mustafe Culha	mculha@yeditepe.edu.tr
Natasa Janev	natasa.janev@gmail.com
Nathalie Thieriet	nathalie.thieriet@anses.fr
Nicolas Segebarth	nicolas.segebarth@ec.europa.eu
Nina Jeliazkova	jeliazkova.nina@gmail.com
Olga Hendrickson	odhendrick@gmail.com
Pavel Danihelka	pavel.danihelka@vsb.cz
Peter Bos	peter.bos@rivm.nl
Phil Reeves	phil.reeves@apvma.gov.au
Richard Handy	rhandy@plymouth.ac.uk
Richard Marchese Robinson	R.L.MarcheseRobinson@ljmu.ac.uk
Sarah Robinson	sarah.robinson@nicnas.gov.au
Seyed Ali Johari	sajohari@gmail.com
Shareen	s.h.doak@swansea.ac.uk
Simon Wilkins	s.a.wilkins@leeds.ac.uk
Sinead O'Doherty	sinead.odoherty@ul.ie
Stefano Bellucci	bellucci@lnf.infn.it
Teresa Fernandes	t.fernandes@hw.ac.uk
Tobias Walser	tobias.walser@bag.admin.ch
Tomasz Puzyn	t.puzyn@qsar.eu.org
Vicki Stone	v.stone@hw.ac.uk
Volkan Ozguz	vozguz@sabanciuniv.edu
Willie Peijnenburg	willie.peijnenburg@rivm.nl
Yves Sicard	yves.sicard@cea.fr

References

1. Oomen A et al 2014. Concern-driven integrated approaches to nanomaterial testing and assessment – report of the NanoSafety Cluster Working Group 10. Nanotoxicology, 8(3):334–348
2. Wg5 cover people outside EU projects and some (but not all) EU projects.

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WG7

Dissemination

Website: http://www.nanosafetycluster.eu/working-groups/7-dissemination-wg
Co-Chair: Lesley Tobin Co-Chair: Iseult Lynch

Table 1 Working Group Contributing projects List.

No.	Project Acronym	Role in Working Group	Representative
1	QualityNano	NSC Compendium	Iseult Lynch
2	NanoValid	NSC Newsletter and Events calendar	Lesley Tobin
3	nanoSTAIR	Standardisation Sub-group	Benoit Hazebrouck
4	NanoTransKinetics	NSC Website	Andrzej Fima
5	QualityNano	Administrative support	Karen Griffin

Contents

1 Summary

The Working Group for Dissemination develops distinct platforms of communication to facilitate the multi-directional information flow between all the Working Groups, Project Partners of all NanoSafety Cluster Projects, and the wider community of nanosafety interest groups and stakeholders.

2 Objectives (short, medium & long term)

The main objectives are:

1. Serve as a communication platform for the NSC as an EU hub of information on nanomaterial safety, characterisation, and measurement knowledge

2. Using appropriate channels and media, make the wider community aware of the resources and exploitable outcomes being developed within the projects

3. Maintain, develop and identify new tools and channels for pan-directional targeted dissemination and communication between members of the NSC and the wider community.

3 Workplan for 12-month horizon

In the coming year, Working Group 7 aims to continue to develop and maintain communication tools and platforms for the NSC as an EU hub of information on nanomaterial safety, characterisation, and measurement knowledge, and to this end it will engage in the following activities:

- Aggregate all relevant updates, output, results and information from NSC project partners, advisory bodies and complementary initiatives
- Identify, develop and maintain tools and channels to receive information
- Identify, develop and maintain tools and channels to deliver information

In addition, Working Group 7 aims to make the NSC and wider communities aware of the resources and exploitable outcomes being developed within the projects (see figure 1), and to this end it will:

- Develop existing subscriber and dissemination databases of stakeholders to extend the reach of the NSC
- Ensure active involvement of stakeholders from different industries as well as continuous interaction and exchange with all relevant end-user groups concerning their needs and experiences.
- Ensure targeted delivery to different stakeholder groups via a combination of electronic platforms and channels, and physical engagement for cross fertilisation of information, updates and news.

Stakeholders	Push-pull platforms & channels	Tools
Standardization bodies Regulatory authorities Academia Industry RTD providers Instrument suppliers Metrology Policy-makers NGOs Wider public	NSC website Partners'/EAB websites Industry websites Industry newsletters Community websites Professional networks News aggregators Committees - eg CEN YouTube / Vimeo Industry / Trade fairs Conferences, workshops Meetings/ dissemination events Polls & surveys Peer reviewed journals Network memberships Popular press	News items Bulletins Press releases Fact sheets Case studies Online reports Word-of-mouth Flyers Posters Presentations Webinars Podcasts Manuals Articles Blogs Webcasts

Figure 1. Target stakeholders, platforms and tools for dissemination

Working Group 7 aims to achieve these objectives using the following tools and platforms:

a. The NSC Website: www.nanosafetycluster.eu

The Nanosafety cluster website has been developed by the project administration team at University College Dublin, with Dr. Andrzej Fima as webmaster, under the remit of the FP7 NeuroNano project, and continues to be maintained by UCD via the QualityNano project with support from NanoValid for WG7.

The Nanosafety cluster website is intended to serve several functions for the community, including:

- as a first point of contact with EU-funded nanosafety research, e.g. via the Nanosafety cluster compendium and the links to the websites of all past and current FP-funded nanosafety projects;
- as a platform to facilitate the exchange of information and data between FP7-funded projects thereby reducing duplication, mainly driven by the Working Groups (WGs) and discussion fora; and, in the longer term;
- as a means of centralising and hosting the many scientific and technical outputs from FP6, FP7 and future projects, thereby acting as an archive. Collection of all public deliverables from projects and links to publications will commence shortly.

Responsibility for developing content for the NSC website, including news updates, RTD developments, project newsletters and output, event promotions, surveys, etc lies with the community.

b. The NSC Newsletter

As the numbers of NSC projects, partners and stakeholders, and even working groups are increasing, the NanoSafety Cluster Newsletter was launched last Autumn to provide an outreach channel for news dissemination and communication for and by the NSC and wider community. Issued on a quarterly basis, the newsletter and/or a download link is sent out to 70,000 people, targeting the entire NanoSafety Cluster network, including all partners of every NanoSafety Cluster project, researchers, industrialists, policy-makers, regulatory and standardisation bodies, SMEs and NGOs. Moreover, links are sent to news aggregators such as NanoWerk, networks such as TINC, initiatives like NanoFutures, and authorities - including CORDIS. Back copies of the newsletter will be available on the website.

The Newsletter predominantly consists of items produced by the cluster and designed to inform readers about project activities, latest RTD initiatives, events and conferences, summer schools and workshops, new publications, job and partnering opportunities, and research breakthroughs. There are profiles of featured NSC projects along with the partners involved and updates from the 8 NSC Working Groups. There are also sections for publications, events (workshops, webinars and project meetings), job opportunities, calls for tenders, and special guest features, including opinion articles, and features on issues of interest to the community. New subscribers can register via the website.

c. The EU Compendium of NanoSafety Cluster Projects

This Compendium is the central resource for information about NSC projects, including descriptions of workpackages, outcomes, results, comprehensive contacts lists and much more. The compendium is compiled on an annual basis to ensure that information remains current. This 2014 edition has been compiled and produced by co-WG7 Chair Iseult Lynch at the University of Birmingham.

d. The NSC Events Calendar

The NSC Events Calendar is designed to facilitate collaborative events, prevent clashing schedules and aid planning among and between NSC project partners. It is hosted on the home page of the NSC website and users can search by month and date as well as event type: NSC Meetings, Project meetings, conferences, summer schools, workshops webinars and forums. An events submission form in the public domain allows people to submit information for inclusion in the calendar (see figure 2).

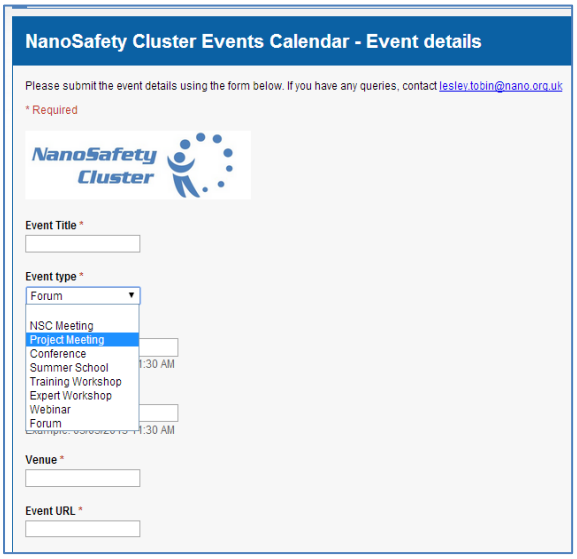

Figure 2. Event submission form

e. Social and Professional Networks

The NSC has its own LinkedIn group at http://www.linkedin.com/groups/EU-NanoSafety-Cluster-7471509. This group provides a discussion and networking platform for NSC project partners and stakeholders as well as a forum for problem solving and planning R&D activities among the wider community. The platform can be used to start discussions, contribute to current ones, expand networks, share output and expertise, or announce events, training and job opportunities and publications. Members are welcome to invite other colleagues and contacts to join too. The NSC also has a twitter account with 711 followers at @EUNanosafety.

f. Other

WG7 Chairs and members may discuss the possibility of producing flyers that provide a brief description of the NSC, its objectives and partners. It would also list the tools, platforms and channels that users can exploit in order to engage with the wider community. This would be distributed at events in order to increase visibility of the NSC and expand the network of contacts.

4 Progress and Outcomes to date

a. The NSC Website

Recent additions to the website's features include a Wiki for WG2 Hazard, a Newsletter subscription page, a news submission form complete with image upload, regular updates of news and developments within the NSC community from the Working Groups and the NSC projects, a button for direct registration and access to the LinkedIn group and a direct link to the twitter account.

Since its establishment in early 2010, the website has had over 28,900 visits and 118,075 Pageviews, as shown in the Google Analytics report from October 23rd 2013.

b. The NSC Newsletter

Since the 2013 Compendium, the NSC newsletter has been launched and two issues have been published as well as three bulletins. A news submission form has been implemented for access by the whole community. In total, over 60-70,000 stakeholders are directly contacted. The geographic distribution of the targets, based on the IoN database, comprises approximately 50% Europe, 25% US and 25% the rest of the world. Their listed organisation types include: research entities; industrial bodies; policy-making, regulatory and standardisation bodies; small and medium-sized enterprises (SMEs) and non-government organisations (NGOs).

In September 2013, an NSC Newsletter subscription form was implemented on the NSC website. All targeted stakeholders are regularly encouraged via the NSC website and Newsletter bulletins to subscribe to the Newsletter and their details are stored in a dissemination database. Since it was implemented, the number of subscribers has risen from 0 to 280 and continues to increase. Data for 220 subscribers at the end of January 2014 provides accurate demographic information for this sample of the readership, showing that readers are currently based in 41 countries.

Links: *subscribe to and receive the newsletter:*
http://www.nanosafetycluster.eu/home/subscribe-to-newsletter.html
submit news items:
http://www.nanosafetycluster.eu/news/submit-news.html

c. The EU Compendium of NanoSafety Cluster Projects

Since the 2013 issue of the Compendium, its usefulness and importance to the community has increased. Acknowledgement of this has led to the augmentation of contents to include roadmaps and updates from the 8 Working Groups, and dedicated sections for the EU-US Communities of Research, thereby adding to the body of information available to the EC and international entities.

d. The NSC Events Calendar

The Calendar, which was already on the website, has been moved to the home page to increase its visibility. A number of key events have been added, and the community has been informed of the possibility to submit events for inclusion via a user-friendly form at: http://www.nanosafetycluster.eu/news/calendar-add-event.html

e. Social and Professional Networks

A linkedIn group was established in March 2013. There are currently 68 members. The Nanosafety cluster Twitter account has 595 followers and continues to grow. Tweeters can either follow @EUnanosafety and the NSC will re-tweet the tweet content to the NSC for tweeting on their behalf.

5 Expected Impact

WG7 will continue to devise and adopt dissemination and communication strategies as well as maintain and develop a range of tools and platforms to maximise the synergies between existing and forthcoming projects addressing all aspects of nanosafety including toxicology, ecotoxicology, exposure assessment, mechanisms of interaction, risk assessment and standardisation.

Emphasis will be placed on establishing a counterbalance to the essentially Eurocentric nature of the project partnerships so that news, output and results can be further extended to the global nanosafety community and beyond. In this regard, it is anticipated that in the year March/April 2014-2015 the number and origins of website visits and page views will increase, newsletter readership will experience a growth in number and geographic spread in each of the stakeholder groups featured in figure 3.

Measuring impact:
The impact of WG7's dissemination efforts can be measured in a number of ways, as followed:

a. NSC Website
Numbers of website hits, page views, document downloads, comments received, requests for information received.
b. NSC Compendium
Numbers of page views, document downloads, comments received, requests for information received

c. Newsletter and bulletins
Number of items submitted, numbers of subscribers, expansion of network of contacts, number of requests to join subscription list after receiving issues from third parties.

d. Calendar
Page views, event submissions

e. Social and professional media
Linked In: members, frequency of discussion threads, origins of discussion threads, geographic spread, comments and likes.
Twitter: numbers of followers, accounts followed, tweets retweeted, favourited tweets

f. Flyers
Number of flyers & posters printed and distributed, number of events where they are displayed and/or distributed.

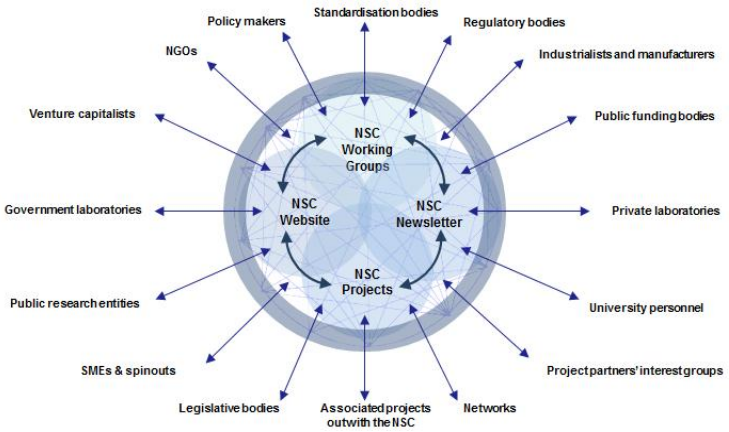

Figure 3. The NSC platforms, the targeted stakeholder groups. and multidirectional flow of information and communication between them.

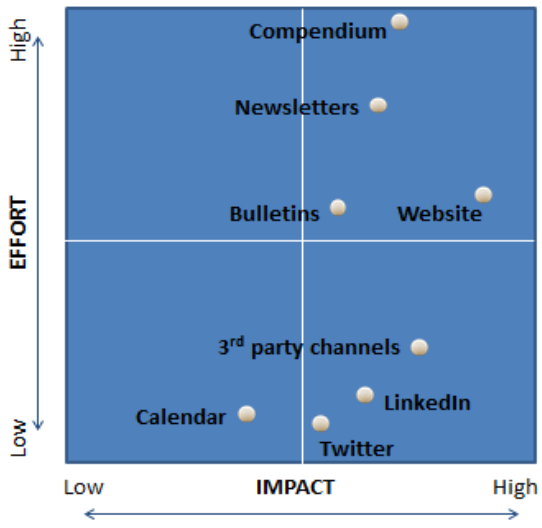

Figure 4. Gauging activity efficacy by measuring impact against effort.

The effectiveness of different aspects of the dissemination strategy can be assessed by assessing the richness of medium, the extent of its reach and the time and effort involved in implementing or auctioning it (see figure 4).

Ultimately, WG7 expects to raise the profile and prestige of the NanoSafety Cluster Working Groups and individual projects within the wider community of international stakeholders by sharing knowledge, encouraging shared innovative practice, fostering collaborations and promoting research excellence.

6 Directory

Table 1 Directory of people involved in this Working Group.

First Name	Last Name	Affiliation	Address	e-mail
Lesley	Tobin	Institute of Nanotechnology	University of Strathclyde Incubator, Graham Hills Building, 50 Richmond St, Glasgow G1 1XP	Lesley.Tobin@nano.org.uk
Iseult	Lynch	University of Birmingham	Edgbaston Birmingham B15 2TT	i.Lynch@bham.ac.uk
Benoit	Hazebrouck	EU-VRi - European Virtual Institute for Integrated Risk Management	Willi-Bleicher-Strasse 19 70174 Stuttgart, Germany	hazebrouck@eu-vri.eu
Andrzej	Fima	National University of Ireland, University College Dublin	Belfield, Dublin 4, Ireland	andrzej.fima@cbni.ucd.ie
Karen	Griffin	National University of Ireland, University College Dublin	Belfield, Dublin 4, Ireland	Karen.Griffin@cbni.ucd.ie

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WG8

Systems Biology

Website: http://www.nanosafetycluster.eu/working-groups/8-systems-biology-wg.html
Chair: Prof. Bengt Fadeel Co-Chair: Prof. Mark Viant

Table 1 Working Group Contributing projects List.

No.	Project Acronym	Role in Working Group	Representative
1	NANOSOLUTIONS	Chair	Bengt Fadeel
2	NANOMILE	Co-Chair	Mark Viant
3	SUN	Member	Mónica J.B. Amorim
4	MARINA	Member	Janeck J. Scott-Fordsmand
5	MODERN	Member	Carlos P. Roca
6	NANOFATE	Member	Francesco Dondero

Contents

1 Summary

Systems biology approaches have enormous potential in the nanosafety field, enabling high-throughput integrative assessment of the safety of nanomaterials and dissection of the underlying mechanisms of toxicity. However, implementation of systems biology-based strategies in nanosafety research requires joint efforts and collaborations between scientists representing different disciplines such as toxicology, material sciences, biostatistics, and OMICS. The Working Group on Systems Biology (WG8) – launched in September 2013 – aims at creating bridges between nanosafety researchers and systems biology experts. One of the main objectives of WG8 is thus to bring together nanosafety researchers in the EU working with systems biology approaches and to facilitate the standardisation of protocols and to establish associated good working practices in systems biology/nanosafety research. One important tool in the development of best practices for systems biology approaches in nanosafety research is the organization of educational training courses, lectures and workshops. There is an emerging critical mass of systems biology research in nanosafety, and it is important to capitalize on this and also to avoid unneccessary duplication of efforts between the various ongoing and new nanosafety projects in the EU Nanosafety Cluster.

2 Scientific and technological challenges

Before systems biology approaches are applied for the assessment of nanomaterial effects on biological systems – in the context of either human toxicology or eco-toxicology research –the nanomaterials in question must be properly characterized. On the other hand, toxicologists also need to gain an understanding of systems biology techniques. Therefore, it is important to develop guidelines and other best practice documents for systems biology approaches in nanosafety research, and to do so in the context of interdisciplinary collaborations between material scientists, toxicologists, biologists, OMICS experts, bioinformaticians, and modellers. Notably, nanomaterial characterization remains an important prerequisite for such research, if we are to generate meaningful data. Data sharing is also an important element; furthermore, applying OMICS techniques may yield plentiful data, but the data needs to be appropriately analysed using bioinformatics tools and should be corroborated in further biological experiments to assess the predictive potential of the systems biology approach. Ultimately, systems biology can afford a global view of mechanisms and pathways that are activated or perturbed by engineered nanomaterials and systems biology may provide an important approach to the development of safe nanomaterials.

3 Objectives (short, medium & long term)

Based on the initial discussions between all the members of WG8, the following objectives have been identified as important starting points:

1. Best practice recommendations for systems biology approaches in nanosafety research as a result of better communication between OMICS scientists, toxicologists and material scientists, achieved through the organization of educational training courses, lectures and workshops.
2. Development of protocols for the analysis of microarray data and other OMICS data in nanotoxicity studies, which ensure correctness and reproducibility of results. These protocols may consist in a recommendation of choice among currently available methodologies, their modification or the proposal of new methods. Close attention to the applicability of the methods for assessment of nanomaterials, in light of potential interference.
3. Development of computational tools for the identification of activated pathways in high-throughput data, from transcriptomics, proteomics or metabolomics assays. The challenge here is two-fold: (a) to offer tools able to process data of species important in toxicology studies, and (b) to provide analysis methodologies which improve the situation concerning, correctness and reproducibility of results.

4 Workplan for 12 month horizon

Activities and deliverables:

1. Systems Biology session in 7th International Nanotoxicology Congress (April 23rd-26th, 2014, Antalya, Turkey) with one NANOSOLUTIONS speaker (D. Greco) and one NANOMILE speaker (F. Falciani) plus one US speaker. Chair of session : B. Fadeel [also co-chair of the congress].

2. Mechanistic toxicology session in 24th SETAC meeting (15 May 2014, Basel, Switzerland). This session will present recent achievements on systems biology and other mechanistic studies (uptake, distribution, toxicokinetics and toxicodynamics) in eco-toxicologically relevant species. Results from FP7 projects, such as NANOFATE) will be presented.

3. Organization of short course on systems biology «Toxicogenomics, high-throughput data & network analysis in NANO» (September 1st-5th, 2014, Aveiro, Portugal), including a combination of hands on and theoretical, with lecturers from SUN (M. Amorim), MARINA (J. Scott-Fordsmand), NANOSOLUTIONS (B. Fadeel), and MODERN (C. Roca). Course organizer : M. Amorim.

4. WG8 session in the Nanosafety Cluster meeting-symposium (October 7th--9th, 2014, Syracusa, Italy) ; program remains to be determined, focus on young scientists, showcase results from FP7 projects, eg. NANOSOLUTIONS, NANOMILE, MARINA, SUN, MODERN, NANOFATE, etc.

5. Organization of conference-workshop "Systems Biology in Nanosafety Research" (3rd Mini-Conference on Nanotoxicology), in Fall 2015, Karolinska Institutet, Stockholm, Sweden. The conference will be hosted by the NANOSOLUTIONS project (B. Fadeel, J. Kere) and the suggestion is to co-organize with NANOMILE (M. Viant) and other members of the Systems Biology WG (planning phase).

6. Standardization activities aimed at good working practices in systems biology in nanosafety research ; for instance, take steps towards making our datasets Open Access through dataset journals such as *Gigascience* and *Scientific Data*, and make efforts to promote emerging and established international data standards, sharing and promoting the use of consistent protocols for omics studies, through the collection of SOPs and associated papers on the WG8 portion of the NanoSafety Cluster website.

5 Progress and Outcomes to date

The Systems Biology WG was launched in September 2013. B. Fadeel (NANOSOLUTIONS) was elected as Chair of the WG and M. Viant (NANOMILE) as co-chair. Refer to the WG8 section of the NanoSafety Cluster website for meeting minutes from the inaugural meeting.

Within the scope of the WG, a two-day workshop on mechanistic toxicology and high-throughput molecular approaches has been organized during the joint QualityNano, NanoFATE & NanoMILE Joint Meeting held in Birmingham, UK on March 5th -6th 2014. NanoFATE scientists Dr. Francesco Dondero and Prof. Peter Kille chaired the two sessions. On the first day experts in the fields of RNA transcriptomics and proteomics met eco-toxicologists and nano-scientists from different disciplines to present cutting-edge methodologies and the most recent systems biology and systems toxicology data obtained so far in FP7 projects and beyond. A rich panel discussion followed highlighting the added value of high-throughput molecular approaches in nanotoxicology, including biomarker discovery and identification of mode of action of toxic nanomaterials. The second day started with an in-depth focus on current proteomic techniques pointing out benefits and limitations in nanotoxicology. An instructive hands-on training session aimed at reducing complexity in the output of RNA Seq analysis was then carried out using real data obtained in NanoFATE and the Bioconductor open source package. The two-day workshop highlighted that there were a number of technical resources (reference genome/transcriptomes, bioinformatic pipelines, metabolic system model) in a range of species of value for addressing fundamental and applied questions in nanotoxicology.

The major themes addressed will be expanded on in talks in session organised and chaired by the same researchers at the SETAC Europe XXIV Annual Meeting in Basel 11-15 May 2014 (see above).

Additionally, a session on Systems Biology has been incorporated into the program of the 7th International Nanotoxicology Congress, chaired by Prof. Bengt Fadeel, with lectures by Dr. Francesco Falciani (NANOMILE) and Dr. Dario Greco (NANOSOLUTIONS), and others.

MARINA work is progressing in regard to system biology measures comparing human and environmental samples, following exposure to nanomaterials.

SUN ongoing work covers human and test organisms from different media and various levels of organization (molecular to organism) NANOSOLUTIONS work is aiming to develop nanomaterial safety classifier, integrating various systems biology data.

6 Expected Impact

If applied in a proper way, systems biology approaches will allow for integration of a huge amount of heterogeneous nanotoxicity data into predictive models thus enabling the identification of molecular "signatures" of different nanomaterials. To note, close interactions with other WG in the NanoSafety Cluster will be important for the successful implementation of these approaches, including WG on exposure, hazard, databases, and modelling. Moreover, because most effects are preceded by molecular events, a linked approach between the various levels (eg. gene expression and organism effect) is expected to advance the field of nanosafety research in terms of test sensitivity and time cost-effectiveness.

7 Directory

Table 1 Directory of people involved in this Working Group.

First Name	Last Name	Affiliation	Address	e-mail
Bengt	Fadeel	Karolinska Institutet, Institute of Environmental Medicine	Nobels väg 13, Stockholm, Sweden	bengt.fadeel@ki.se
Olesja	Bondarenko	Karolinska Institutet, Institute of Environmental Medicine	Nobels väg 13, Stockholm, Sweden	olesja.bondarenko@ki.se
Mark	Viant	University of Birmingham, School of Biosciences	Edgbaston B15 2TT, Birmingham, UK	m.viant@bham.ac.uk
Mónica	Amorim	University of Aveiro, Department of Biology & CESAM	Campus Universitario de Santiago, 3810-193 Aveiro, Portugal	mjamorim@ua.pt
Robert	Rallo	Rovira i Virgili University, Department of Computer Science and Mathematics	Av. Paisos Catalans, 26 43007 Tarragona Catalonia, Spain	robert.rallo@urv.cat
Carlos P.	Roca	Rovira i Virgili University, Department of Chemical Engineering	Av. Paisos Catalans, 26 43007 Tarragona Catalonia, Spain	carlosp.roca@urv.cat
Janeck	Scott-Fordsmand	University of Aarhus, Dpt. Biosciences	Vejlsovej 25, PO BOX 314, DK-8600, Silkeborg, Denmark	jsf@dmu.dk
Rob	Vandebriel	Centre for Health Protection, National Institute for Public Health & the Environment	PO BOX 1 3720 BA Bilthoven	rob.vandebriel@rivm.nl
Francesco	Dondero	Università del Piemonte Orientale	Via Michel 11, Alessandria	fdondero@unipmn.it
Dave	Spurgeon	Centre for Ecology & Hydrology	Benson Lane, Crowmarsh Gifford, Wallingford, UK	dasp@ceh.ac.uk
Peter	Kille	Cardiff University	Cardiff University, Main Building, Cardiff CF10 3AT	Kille@cardiff.ac.uk

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