Compendium of Projects in the European NanoSafety Cluster

2013 Edition

February 2013

Editor:

Michael Riediker, PD Dr.sc.nat.
Institute for Work and Health, Lausanne, Switzerland
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PREFACE

This is the fourth edition of the Nanosafety Cluster compendium. It documents the status of important projects on nanomaterial toxicity and exposure monitoring, integrated risk management, research infrastructure and coordination and support activities.

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 papers and research results, as this task is covered by scientific conferences and the peer reviewed press.

The compendium aims to bring researchers 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 available laboratory infrastructure. 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, I 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. A limited number of printed paperback copies of the compendium are also available.

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

ACKNOWLEDGMENTS

I would like to thank the project 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. I 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).

Michael Riediker, Editor and Chair of the Dissemination WG of the NanoSafety Cluster
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QUOTE

Michael Riediker and (Ed.)
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CONTENTS

Overview matrix : Research themes of the NanoSafety Cluster projects ....................................................... IV
Foreword .......................................................................................................................................................... 1
ENNATOX ...................................................................................................................................................... 3
ENPRA ............................................................................................................................................................ 15
EuroNanoTox .................................................................................................................................................. 27
HINAMOX ..................................................................................................................................................... 35
INSTANT ...................................................................................................................................................... 45
ITS_NANO ..................................................................................................................................................... 51
LICARA ......................................................................................................................................................... 57
MARINA ........................................................................................................................................................ 61
MembraneNanoPart ....................................................................................................................................... 71
MODERN ....................................................................................................................................................... 75
ModNanoTox ............................................................................................................................................... 81
Nanodetector ............................................................................................................................................... 85
NANODEVICE .......................................................................................................................................... 91
NanoFATE .................................................................................................................................................. 99
NanoHouse ................................................................................................................................................ 115
Nanolyse ................................................................................................................................................... 123
NanoMICEX ............................................................................................................................................. 133
NanoMILE ............................................................................................................................................... 141
NanoPolyTox .......................................................................................................................................... 151
NanoPUZZLES ....................................................................................................................................... 159
NanoReTox ............................................................................................................................................... 165
NanosafePACK ....................................................................................................................................... 175
NanoSTAIR ............................................................................................................................................. 185
NanoSustain ............................................................................................................................................ 191
NanoTransKinetics ................................................................................................................................. 201
NanoValid ................................................................................................................................................ 211
QualityNano .......................................................................................................................................... 225
REACHnano ........................................................................................................................................... 239
SANOWORK ........................................................................................................................................... 245
Scaffold .................................................................................................................................................... 253
SIINN ........................................................................................................................................................ 261
SMART-NANO ....................................................................................................................................... 269
## Overview matrix: Research themes of the NanoSafety Cluster projects

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Foreword

Dear Readers of the Compendium, Dear Friends,

The NanoSafety Cluster Compendium is again ready to disseminate knowledge about European Commission funded research projects on various aspects of nanosafety. What was earlier, during the 5th and the 6th Framework Programmes, a relatively limited activity, research for the promotion of nanosafety, has grown to one of the globally leading research programmes within the 7th Framework Programme for Research and Innovation. Clearly, due to the active funding efforts of the Commission, and due to the research efforts of the European nanosafety research community, Europe has been put into a unique position to take a global leadership of nanosafety research. This development has major benefits for the EU, its research community, and the wealth of EU and its citizens. The Commission has identified uncertainties around the safe use of engineered nanomaterials and nanotechnologies a major obstacle of nanotechnology innovations, and investments into this technology. The Commission has considered nanotechnology to belong to the key-enabling technologies in the EU, one of the most important technology drivers of the future economic growth of the Innovation Union.

By reducing uncertainties about safety and health effects of engineered nanoparticles and nanotechnologies to workers, consumers and the environment, the European research efforts can have a major boost benefiting nanotechnology through assuring its safety and bringing safety into the core of nanotechnology business thinking. This impact of nanosafety research is thereby the added value that is provided by increased understanding of the true nature of engineered nanomaterials characteristics for their categorization, more thorough understanding of exposure and release of these materials, discovering their novel hazard mechanisms, and utilization of all these data in more reliable risk assessment and management of engineered nanomaterials.

To fulfil the expectations put forward by the Commission and different stakeholders, the European nanosafety research projects have to produce high quality research, and to come up with scientific breakthroughs. Only scientific quality, both conceptual discoveries and technical quality assuring the reliability of the scientific findings, will have the potential to make a change. This means increased understanding of those material characteristics that determine the possible harms of these materials on human health and the environment, and possibilities to create exposure determinants that have predictive value in the assessment of risks. The nanosafety research projects have jointly accepted the challenge about increasing quality of research put forward in the Editorial of Nature Nanotechnology (Editorial, Nature Nanotech, 7, 545, 2012).

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.

Best regards

Kai Savolainen, MD, Research Professor
Director, Nanosafety Research Centre at the Finnish Institute of Occupational Health
Coordinator of NANODEVICE and NANOSOLUTIONS Projets
Helsinki, Finland
ENNSATOX
ENgineered Nanoparticle Impact on Aquatic Environments: Structure, Activity and Toxicology

Contract Agreement: NMP4-SL-2009-229244
Website: http://www.ennatox.eu
Scientific Coordinator: Professor Andrew Nelson, School of Chemistry, University of Leeds, UK
Project Manager: Dr Karen Steensom, Faculty of Engineering, University of Leeds, UK

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Contents

1 Summary ....................................................................................... 3
2 Background – The ENNSATOX Project ........................................ 4
   2.1 Introduction, Scientific / Industry Needs & Problem Addressed.................................................................................. 4
   2.2 Scope & Objectives ............................................................... 5
   2.3 Technical Approach, Work Description & Achievements....... 6
   2.4 Conclusions from the Third Year of the Project............... 11
3 Directory ...................................................................................... 12
4 Copyright ..................................................................................... 13

1 Summary

Project Duration: 1 July 2009 – 30 June 2012
Project Funding: €3,655,316 Total Budget; €2,816,500 EC Contribution

The use of engineered nanoparticles (NP) in cosmetics, pharmaceuticals, sensors and many other commercial applications has been growing exponentially over the past decade. EU and Member States’ research into the environmental impact of these materials, particularly in aquatic systems, is at an early stage. There is a large uncertainty into the environmental risk posed by these new materials. ENNSATOX addresses this deficit through a comprehensive investigation relating the structure and functionality of well characterised engineered nanoparticles to their biological activity in environmental aquatic systems.

ENNSATOX takes account of the impact of nanoparticles on environmental systems from the initial discharge to the uptake by organisms. Accordingly an integrated approach will assess the activity of the particles in a series of biological models of increasing complexity. Parallel environmental studies will take place on the behaviour of the nanoparticles in natural waters and how they modify the particles’ chemical reactivity, physical form and biological activity. A comprehensive theoretical model will be developed describing the environmental system as a series of biological compartments where particles transport between a) compartments by advection-diffusion and b) between phases by a transfer function. Following optimisation of the transfer functions a generic predictive model will be derived for the environmental impact of each class of nanoparticle in aqueous systems. The project will include the use of unique biological membrane models not only to understand better the interaction of nanoparticles with cell membranes from an organism health point of view but also to develop suitable nanoparticle screening procedures which can substitute for the more lengthy in vivo tests. ENNSATOX will generate: 1) Exploitable IP of screening devices and simulation software; 2) Set of standard protocols; 3) Global dissemination of results; 4) Creation of an EU laboratory service; 5) Tools and data to inform EU Regulation; and, 6) Risk assessment procedures.
2 Background – The ENNSATOX Project

2.1 Introduction, Scientific / Industry Needs & Problem Addressed

Nanomaterials are becoming increasingly important in their applications and uses in many industries, consumer products and healthcare (The Nanotech Report, 6th Edition, Lux Inc, New York, 2008). Current worldwide sales of products incorporating nanomaterials are €1.1 trillion and are expected to rise to €4.1 trillion by 2015. Engineered nanoparticles represent a major part of this growth. However an understanding of their toxicological properties has not kept pace with the exponential rate of increase of research into their synthesis, characterisation and applications. Research into their behaviour, impact and fate in aquatic environments is at a very early stage. Out of 14 funded EPS/FP6 nanotoxicology projects only one is dedicated fully to this area (“EU Nanotechnology R&D in Health and Environmental Impact of Nanoparticles” http://cordis.europa.eu/nanotechnology/home.html report, Jan 2008). This report details the member states with the largest number of nanotoxicity research projects as follows: UK (46), Switzerland (24) and Denmark (12) of which the numbers dedicated to the fate of nanoparticles and their impact in the aquatic environment are UK (6), Switzerland (2) and Denmark (1) respectively. The majority of risk studies are concentrated on airborne particulates. A similar situation is also seen in the recently updated US National Nanotechnology Initiative (NNI) Strategy for Nanotech-related Research for the Environment, Health and Safety Research, Feb 2008 (http://www.nano.gov/NNI_EHS_Research_Strategy.pdf) where the focus for aquatic environmental research is into environmental transport mechanisms and standardisation of nanoparticles rather than their ecotoxicological effects.

The toxic effects of nanomaterials are poorly understood and their effects on aquatic wildlife are largely unknown. In the absence of such basic toxicological information, it is difficult to set environmental quality standards or perform risk assessments for these materials. As a result two EU Member States have recently recommended a voluntary moratorium on the release of engineered nanoparticles into the environment backed by a voluntary reporting system. These Member States are Germany (Nanotechnology: Health and Environmental Risk of Nanoparticles, Joint Working Party Report, Aug 2006, http://www.baua.de/nn 49456/en/Topics-from-A-to-Z/Hazardous-Substances/Nanotechnology/pdf/draft-research-strategy.pdf) and the UK (Council for Science & Technology Review of Government Progress of its Action Plan for Nanoscience and Nanotechnology, March 2007, http://www.baua.de/nn 49456/en/Topics-from-A-to-Z/Hazardous-Substances/Nanotechnology/pdf/draft-research-strategy.pdf), to be administered by the Department of Environment, Food and Rural Affairs (DEFRA). On 17th January 2008 the UK’s Soil Association, the national organic food certification body, issued a complete moratorium on the use of engineered nanoparticles for organic food production (http://www.soilassociation.org). More recently, Poland et al. (20th May 2008), described important findings relating the dimensional characteristics of carbon nanotube and inorganic fibres to the inability of macrophages to prevent mesothelioma risks in rat lungs. http://www.nature.com/nnano/journal/v3/n7/abs/nnano2008111.html

ENNOSATOX addresses this crucial uncertainty by seeking to relate the structure and functionality of a well known class of nanoparticles of varying morphology to its biological activity at successive levels of molecular, cellular and organism organisation. Its research will focus in particular on the impact of nanoparticles on these biological systems in aqueous environments with relevance to the interpretation of their effects on ecosystems. The work programmes will examine the importance of the biological membrane in the toxicology and bioaccumulation of nanoparticles in aquatic organisms. The study will thus operate at a series of levels and will take into account not only the responses of the individual organism to the specific agent but also relate this to the mechanism of activity of the agent. This goal will be achieved by engaging in a multidisciplinary approach and integrating the results in a multi component model. In so doing it will fill an important knowledge gap and inform the EU’s code of conduct for responsible nanosciences and nanotechnologies research, ftp://ftp.cordis.europa.eu/pub/nanotechnology/docs/nanocode-recommendation-peo0840c8424_en.pdf for the purpose of future regulation by the EU (REACH Directive) and Member states.

The underlying concept of the proposed research is to address the current uncertainty of nanoparticle toxicity and environmental impact using an integrated multidisciplinary approach.

The philosophy of ENNSATOX’s work plan is to initially produce and thoroughly characterise different morphologies and sizes of a model nanoparticle, such as zinc oxide (ZnO), using the most advanced state-of-the-art methods in physical chemistry and microscopy. This will be extended to additional classes of nanoparticles in particular silicon dioxide (SiO₂) and titanium dioxide (TiO₂). At the same time the programme will look at the nanoparticles’ activity towards a series of biological models of increasing complexity and organisation. Next, the behaviour of the nanoparticles in environmentally relevant aquatic systems will be examined see whether the environment alters the chemical and/or structural nature of these particles. Throughout the study an integrative model will be used to plan the activities and at the end of the programme, a predictive mathematical model will be developed incorporating all of the elucidated parameters.

The hypothesis is:

The biological activity and environmental impact of nanoparticles is directly dependent on their structure and functionality. By evaluating these relationships we can develop predictive models which can be deployed for statutory controls of nanoparticle use.

Toxicity assays will be performed using in vitro models of cell and tissue culture and in vivo models of several different aquatic species of key indicator organisms. As part of this proposal, all the procedures for toxicity testing will be selectively developed and optimised for nanoparticles. This means that streamlined protocols for nanoparticle toxicity testing will be formulated which can later be exploited as routine tests for nanomaterials.

The biological membrane and its dependent mechanisms play important roles in nanoparticle toxicity for two reasons. Firstly the biological membrane forms the boundary of the living cell which nanoparticles will need to cross and, secondly, the biological membrane hosts many of the physiological processes such as
respiration and nerve conduction and any disruption in its structure will lead to a disruption in the function of the incumbent processes. The effect of nanoparticles on biological membrane structure is entirely unknown as is the permeability of nanoparticles in cell membranes. This study therefore allocates considerable resources to look at the interaction of nanoparticles with biological membranes by using highly novel supported membrane models of successive complexity. These model membranes represent the most basic model for nanoparticle interaction and will deliver important preliminary structure-activity relationships which are used to guide the more complex in vitro and in vivo studies. Already one of the model membrane tests being deployed in this study is in the process of being patented1 as a generalised toxicity testing procedure which can be applied to investigate the activity of nanoparticles. We see a major outcome of this study as the delivery of calibrated and accredited toxicity testing protocols for nanoparticle biological activity. A very recent SETAC World Congress in Sydney (August 2008) had an extensive session on nanomaterials and it was apparent that there were many issues to be addressed concerning how the materials should be tested for biological activity and the mechanism of toxicity. ENNSATOX therefore has a great opportunity to make advances which could be a significant asset commercially.

2.2 Scope & Objectives

1) To source and comprehensively characterise a representative group of nanoparticles: initially ZnO and later SiO2 and TiO2 and other metal oxides of varying morphology and dimension. In-house synthesis is limited to special nanoparticles not obtainable commercially or from other projects. In these cases, the production methods are well defined. This objective will be continued as an iterative process throughout the programme of work. The success of this objective is directly measurable by the standardised particles which it delivers.

2) To characterise the interaction of the nanoparticles with the following biological models: supported phospholipid membranes of increasing complexity, in vitro models of cell and tissue culture, in vivo models of several different species of key indicator organisms. A feature of this objective is the direct comparison of the effects in the different groups which leads to the configuration of generalisations of nanoparticle biological activity.

3) To formulate direct and predictive structure-activity relationships between nanoparticle form and nanoparticle biological activity. Success in this objective will be achieved following results from objectives 1 and 2 and is a central feature of ENNSATOX.

4) To analyse the behaviour and fate of nanoparticles and their impact on models of biota in environmental aquatic systems. This advances on the initial structural-activity relationships by testing their application in the environmental aquatic situation.

5) To configure a mathematical model for the behaviour of nanoparticles in aquatic environments taking account of their interactions with biota of increasing complexity. This objective quantifies the interactions and will serve as a means of verifying and measuring previous objectives 1-4.

6) To draw up standard procedures for the exploitation and dissemination of the results for statutory planning and accredited use.

In order to accomplish the challenge ENNSATOX has assembled a group of RTD performers of unprecedented excellence from across Europe. The ENNSATOX Consortium has outstanding capabilities and achievements in:

- Nanoparticle manipulation, synthesis and characterisation (Leeds, Wageningen);
- Supported model membrane technology (Leeds, Naples, Wageningen);
- Environmental and molecular mathematical modelling (Lleida, Wageningen, Leeds, Antwerp);
- In vitro and in vivo biological models (Naples, Leeds, Antwerp, Wageningen, MBA);
- Surface and colloid chemistry (Leeds, Wageningen, Naples);
- Environmental impact assessment (Wageningen, Antwerp, MBA); and,
- Dissemination of best practice worldwide (MBA, SETAC).

The objectives directly address, in an integrated manner, the impact of the nanoparticles on the environment. Implicit in this is the approach towards understanding the environmental and biological fate, transport, and transformation of nanoparticles in various biological compartments in aquatic systems. It is clear that the above objectives incorporate investigations into the toxicokinetics, cellular and molecular mechanisms, behaviour and fate, bio-persistence and biokinetics of nanoparticles. This enables a fundamental understanding of the exposure, behaviour, mechanisms, consequences and potential effects to various endpoints of nanoparticle-biological entities interactions.

Contained within the objectives the following important questions will be addressed:

- What is the dispersion and solubility of nanoparticles in water?
- What are the most likely routes of exposure for environmentally relevant species?
- Can nanoparticles interfere with critical physiological mechanisms in aquatic organisms?
- Can nanoparticles bioaccumulate in aquatic organisms?
- Can nanoparticles be metabolised to less toxic forms?
- What biomarkers are relevant for determining nanoparticle exposure levels?
- What end-points are significant for determining risk of nanoparticles?
- What are the mechanisms of toxicity of nanoparticles in environmentally relevant systems?
- Does the presence of nanoparticles in the environment affect the toxicity of other compounds and vice versa?

2.3 Technical Approach, Work Description & Achievements

The scientific (RTD) activities are conducted within seven work packages (WP1-7), with two other work packages being specifically concerned with exploitation/IPR and pre-validation (WP7), and dissemination (WP8):

WP1: Synthesis and characterisation of a selected group of nanoparticles. To keep the study focused three groups of nanoparticles are being examined: silicon dioxide (SiO2), zinc oxide (ZnO) and titanium dioxide (TiO2), of different morphology and dimension. Although Leeds is responsible for the synthesis, sourcing and processing of the nanoparticles, their characterisation is being cross calibrated with Wageningen. Nanoparticle characterisation in the in vitro, in vivo and aquatic systems is being carried out throughout the programme as and when appropriate (WPs 2, 3, 4 and 5) in order to follow their behaviour and fate in the respective systems. Figure 1 shows a characteristic image from this study of TiO2 nanoparticles which have been sourced at Leeds and have very small constant particle size. These particles are representative of the many samples which have been distributed round the Consortium for testing.

WP2: Interactions of different classes of nanoparticles with model membrane systems. Leeds and Wageningen possess a whole suite of experimental model biological membrane systems of increasing levels of complexity (Figures 2 & 3). Wageningen have considerable expertise in surface and colloid chemistry and extensive expertise modelling membrane interactions, and are responsible therefore for correlating the model membrane-nanoparticle interactions with theoretical mathematical models using self consistent mean field theory. The form, structure and functionality of the particles are being related to their activity towards the model membrane systems. Anton Dohrn is examining the effects of nanoparticles at the level of single channels (HERG K+ channels). The principle is to understand how nanoparticles affect the organisation and fluidity of the biological membrane, how they influence the functioning of ion channels and enzymes located in the membrane environment and whether the nanoparticles are themselves permeable in the membrane structure. To test the biomembrane activity of all nanoparticles a custom built high throughput sensor has been developed. This device can assess the biomembrane activity of a nanoparticle dispersion in 10 minutes. Interesting results have been found using this sensor. For instance, SiO2 nanoparticles have been shown to adsorb on the surface of phospholipid membranes, as seen in SEM images of silica nanoparticles on phospholipid monolayers on electrode surfaces. The extent of contact of the SiO2 particles’ surface with the phospholipid membrane surface determines the effect on the membrane’s properties and is dependent on the particle size. The interaction of ZnO and TiO2 particles is also very significant. Unlike amorphous silica, ZnO and TiO2 particles have a strong tendency to aggregate. The nanosensor shows that in the first few minutes after aqueous dispersions are formed, ZnO particles have a high permeability in the membrane. The in-house synthesised ZnO and anatase TiO2 particles with smaller primary particle size (<8 nm) interact strongly with the membrane surface. The biomembrane activity of TiO2 particles strongly correlates not only with their primary particle size but also with their electrical charge carrying ability.

DOPC giant unilamellar vesicles (GUV) have been exposed to SiO$_2$ particles (see Figure 4). The interaction of SiO$_2$ nanoparticles with the GUV is a manifestation of the balance between the adsorption energy of the particles on the GUV and the elastic energy of the GUV. Small SiO$_2$ particles (<50 nm) adsorb on the GUV whereas the GUV breaks up and adsorbs on larger (>50 nm) particles.

Figure 4: Confocal laser scanning microscopy of DOPC GUV's after NPs interactions: (a-d) GUVs after 20 min interaction with 18 nm SiO$_2$; (a, b) reconstituted 3D images of GUVs with unusual curvature and stabilized holes; (c) a helmet shaped GUV; (d) confocal image of deformed GUVs and dextran fluorescence leakage after interaction with 18 nm 25 mg ml$^{-1}$ SiO$_2$ NPs; (e) 3D reconstruction of a GUV interacting with 182 nm SiO$_2$ NPs; (f) schematic view of our interpretation of the effect of size on the NP membrane interactions: small NPs adsorb on to the membrane (top) and the membrane wraps the large NPs (bottom). Depth profile is shown in Figure a, b, c, and e. Insets to c: 2D and 3D view of the same GUV; insets to d, additional deformed vesicles from the same experiment; inset to e: 3D view of a GUV undergoing a wrapping event.

**WP3: Interactions with in vitro models.** These studies are directed to nanoparticle interactions at both the cellular level and the tissue level. The test systems will be established on in vitro models. The cellular level will include test systems ranging from tissues and cultured cells to DNA. The tissue level includes nerve axons from the squid consisting of a single axon and glia, and ascidian embryos (rapidly developing chordate embryos to 12 hrs). The principle is to understand how the nanoparticles affect the structure and function of these systems using both real time assays and electron microscopy. The in vitro work is led by Anton Dohrn and is spread between Anton Dohrn and Leeds (WP 3). Anton Dohrn has extensive facilities in electron microscopy and biophysical and molecular biological techniques and considerable world expertise in electrophysiology.

Some of the most exciting recent work carried out by WP3 has been on the effect of ZnO particles on membrane proteins. NPs provided by WP2 were tested directly on HEK cells that heterologously express the hERG K$^+$ channel. This gave us the opportunity of assessing the impact of the NPs on defined membrane proteins directly. The range of concentrations used was 0.1-10 µg ml$^{-1}$ for both SiO$_2$ (dialyzed and non-dialyzed) and ZnO. Cells were held at -70 mV under voltage clamp and hERG K$^+$ channels were activated by patterns of voltage steps which produced outward ionic currents which were subject to biophysical analysis (Figure 5). The channel activity was stable for at least an hour without run-down although experiments were normally carried out in the first 20 minutes. Examination of the hERG current kinetics (activation / inactivation and peak currents revealed no effect of SiO$_2$ up to 10 µg mL$^{-1}$ but a notable selective effect of ZnO on channel kinetics (Figure 5). To establish if this effect was due to release of Zn$^{2+}$ ions from the NPs, we carried out experiments where increasing concentrations of ZnCl$_2$ were added and the peak currents measured. As can be seen in Figure 6, increasing the concentration of Zn$^{2+}$ begins to block the channel only in the mM range. The effect of the NPs in Figure 6 is the opposite of this, i.e. they increase the current. Therefore the NP effect cannot be due to residual Zn$^{2+}$.
Figure 7 depicts a model to explain the effect of the ZnO particles with the ion channel activity.

Figure 7. A simple model to explain the interaction between ZnO NPs and hERG.

**WP4: Interactions with in vivo models.** In vivo testing is being performed on at least eight different species to allow the construction of species sensitivity distributions for the selected nanoparticles. This also includes three standard toxicity species of which the acute and chronic toxicity is well documented and characterised for a variety of toxicants (e.g. Chlorella, Daphnia, and Danio). The in vivo experiments address three main issues: namely bioavailability, accumulation and toxicity. A series of chronic experiments are being performed in which effects on growth and reproduction are being determined. This work is led by Antwerp with an input from Anton Dohrn. Antwerp is one of the world leaders in molecular, cellular and whole-organism toxicology, and both experimental and predictive modelling. In the last year studies have concentrated on looking at the chronic toxicity and internalisation routes of nanoparticles. In a chronic exposure scenario over 21 days, the ZnO nanodispersion (Alpha Aesar NanoTek) is toxic to Daphnia magna at low concentrations (around 0.02 mg Zn/L), whereby especially the reproduction was affected. As it has already been observed in studies carried out in WP3, toxicity of the ZnO nanodispersion cannot be solely due to the Zn or Zn²⁺ toxicity (see Figure 8).

Results also showed the uptake and internalisation of the ZnO nanoparticles in Ciona intestinalis (see Figure 9). However, under the tested conditions, algae and daphnids did not take up the particles.

Figure 8: Chronic toxicity of ZnO nanodispersion on the reproduction of Daphnia magna (mean ± SD) after 21 days of exposure. Number of juveniles per adult Daphnia; one-way ANOVA with Dunnet’s post test, significant differences p < 0.001-0.01 (left) and sigmoidal dose-response curve on the inhibition of reproduction (right).

Figure 9: Feeding the animals with ZnO nanoparticles results in an accumulation of the particles in the stomach tissue. TEM analysis of this organ indicates that nanoparticles, found at the plasma membrane of the cells facing the lumen, pass into the cytoplasm and through the junctions (A). Clusters of ZnO nanoparticles can be observed at the edge of zymogen granules (B), in the mitochondria and scattered in the cytoplasm (C). Scale bars 0.5 μm.

An extensive series of chronic toxicity tests have been carried out on the effect of nanoparticle dispersions on indicator organisms. As shown in Figure 10 the toxicity of ZnO nanoparticles is marginally higher than that of ZnCl₂.

Figure 10: The chronic toxicity of ZnO nanosun (left) and ZnCl₂ (right) to the reproduction of Daphnia magna after 21 days. X-axis: exposure concentration (log mg Zn/L), Y-axis: Reproduction inhibition (%).
WP5: Nanoparticle environmental impact. The biophysicochemical behaviour of nanoparticles, and their ensuing bioavailability and toxicity characteristics, strongly depends on the nature and the extent of molecular interactions with organic and inorganic materials in the environment. Wageningen together with an input from Antwerp are responsible for analysing the influence of chemical conditions and binding of particular species on the biointeraction and bioaccumulation of nanoparticles. Wageningen have extensive experience in the relationship between the chemical speciation of dissolved and particulate material in ‘natural’ waters and its bioavailability. They are studying how the nanoparticles and the nanoparticle-water interface is modified when they enter a typical aqueous environmental system such as river, estuarine and sea water and how this affects their biological activity. Experiments are being carried out in laboratory controlled and relevant microcosms. The rate of the actual transfer of oxide nanoparticles across the cell membrane of a few selected aquatic organisms (microorganisms, invertebrates and fish); in relation to their local speciation and the physicochemical conditions at the outer side of the biointerface will also be investigated. The alteration of the nanoparticles during the in vivo experiments described in (WP4) is being investigated in this section and related to their effects. In the first half of the programme, studies have mainly concentrated on the surface chemistry of SiO\textsubscript{2} particles and their interaction with the soluble heavy metal ion Pb\textsuperscript{2+}. An important part of WP5's activity has been investigating the charge properties of the particle dispersions which reflects their overall reactivity in aqueous environments. Figure 11 below shows that the charge carrying properties of TiO\textsubscript{2} particles are highest for the anatase dispersion which has the smallest particle size and a charge carrying capacity five times the value of the other TiO\textsubscript{2} dispersions. Significantly as stated earlier in the work of WP2 the anatase dispersion is the only TiO\textsubscript{2} dispersion which is biomembrane active.

Figure 11: Titrations of sourced TiO\textsubscript{2} dispersions with at different concentrations of KNO\textsubscript{3} as indicated (in mol dm\textsuperscript{-3}).

WP6: Integrated Modelling. No environmental toxicological study is complete unless the various parts are integrated together in a theoretical model. This is essential not only for planning the study but also for assessing the final transfer parameters. Such a process is continuously iterative throughout the investigation until towards the end of the study, when the parameters are completely optimised, and predictions as to the impact of the nanoparticles on the aquatic environment can be made. The model is being developed along the lines of previous environmental ecological models which predicted the transport and fate of soluble contaminants. A compartmental model is being used where the compartments are represented by the cell membrane, cell organelles, total cell, tissue, and model aquatic organisms. The model based on ECoS\textsuperscript{3} (developed by Plymouth Marine Laboratory) allows the set-up and integration of sets of advection–diffusion equations representing multiple constituents interacting in a spatial context\textsuperscript{3}. WP6 has developed working models of the solubility of oxide NPs and the validity of these models has been tested experimentally. The first step in the development of reliable structure-activity toxicological predictive relationships has been carried out and the modelling of the amount of NP that effectively reaches an organism and is, subsequently, internalized by it, prior to the toxic effect itself is taken care of using this model (see Figure 10). The main advantage of this model is that it provides a prediction of the amount of NP attached (or absorbed) and internalized by a microorganism as a function of time, based on a simple physicochemical mechanism.

Figure 12: Schematic representation of the diffusion-adsorption-internalization model. Uptake is described through the following sequential steps: a) diffusion of NPs from bulk solution (blue); b) reversible adsorption/desorption of NPs on the biomembrane following a Langmuir-like kinetics (red); c) internalization of NPs through endocytosis or similar mechanisms (purple); and finally d) efflux of NPs out of the organism (green).

The diffusion-internalization models have been validated by experiments on nanoparticle uptake by cells displayed in Figure 13.

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developing accredited toxicity tests and assays for NPs in the environment. Significant regulatory developments are being carried out by the EU and of carrying out bioassays for toxicity. The MBA has been consolidating all tests around the Consortium and evaluating their results with their own. The majority of in vivo and in vitro tests within the ENNSATOX would tend to indicate that ZnO nanoparticles are more likely to cause deleterious effects than those composed of SiO2. However there are different requirements for Zn across taxonomic groups which consequently result in a range of sensitivities to excess metal. In vivo ZnO toxicity to the alga Isochrysis galbana was not markedly different across size ranges of NP tested here and were slightly less toxic than ionic Zn (Figure 11). Toxicity of the NP decreased in the sequence: nanotech suspension > Metals Basis undialysed > Metals Basis dialysed > powder (Sigma Aldrich). This implies that, over the studied size range, toxicity was highest for the larger Zn particles, though it is not clear if these differences were a function of some other characteristic (including actual Zn content of the stock suspensions rather than particle size per se).

WP7: Exploitation and pre-validation. The Marine Biological Association of the UK a leading environmental charitable organisation is leading this activity. MBA has a track record of coordinating EU contracts and of carrying out bioassays for developing environmental quality objectives, with expertise in transferring analytical technology and significant regulatory experience. This includes considering all the above issues as well as developing accredited toxicity tests and assays for NPs in the aquatic situation. An important output will be aiding environmental legislation on these materials. Another important outcome is guidance on as to an effective means of calibrating and accrediting the toxicity testing procedures being developed. The results of a point of contact high throughput test using marine algae which has been developed for cross-checking all other toxicity tests in the programme.

The MBA has been consolidating all tests around the Consortium and evaluating their results with their own. The majority of in vivo and in vitro tests within the ENNSATOX would tend to indicate that ZnO nanoparticles are more likely to cause deleterious effects than those composed of SiO2. However there are different requirements for Zn across taxonomic groups which consequently result in a range of sensitivities to excess metal. In vivo ZnO toxicity to the alga Isochrysis galbana was not markedly different across size ranges of NP tested here and were slightly less toxic than ionic Zn (Figure 11). Toxicity of the NP decreased in the sequence: nanotech suspension > Metals Basis undialysed > Metals Basis dialysed > powder (Sigma Aldrich). This implies that, over the studied size range, toxicity was highest for the larger Zn particles, though it is not clear if these differences were a function of some other characteristic (including actual Zn content of the stock suspensions rather than particle size per se).

WP8: Dissemination. Although this work package concerns dissemination it also feeds into WP7: Exploitation & Pre-validation. It identifies opportunities to publicise the achievements and capabilities developed under the auspices of ENNSATOX, engaging with potential end-users in industry, regulatory authorities, NGOs, academia, as well as the wider European and International public. The work package therefore also serves to aid Exploitation and will also have general marketing benefits for both the participating members of the ENNSATOX Consortium and the overall FP7 program.

WP9: Scientific Coordination & Project Management. This work package establishes effective coordination and decision structures that address the scientific and business needs of the project. It ensures that all project beneficiaries participate in decision-making and that the project is run efficiently on a day-to-day basis. It also maintains Quality Assurance (QA) on all procedures run by the Consortium. Finally it ensures the participation and representation of the ENNSATOX Consortium in the NanoSafety Cluster.

In Figure 15 the objectives and activities are set within an integrated strategic environmental framework. The figure shows the environmental discharge and behaviour of the nanoparticles in the left hand compartment (a) and, the impact of nanoparticles on the biological barriers in between the two compartments (b) and on the aquatic organisms in the right hand compartment (c). Activities WP1 and WP5 will focus on understanding processes in compartment (a). Activity WP2 focuses on interaction and transport mechanisms at the interface between the compartments (b). Activities WP3 and WP4 focus on interaction and bioaccumulation mechanisms in compartment (c). Activity WP6 will integrate and model all processes represented in the figure summarising the RTD activities in this project.
A list of deliverables arising out of these activities can be summarised as:

- Fundamental insight into nanoparticle interactions and transport in the aquatic environment and in living cells and organisms.
- Relation between structure and functionality and activity of nanoparticles and modified nanoparticles at all levels of biological organisation.
- Integrated model to assess and predict the fate and risks of nanoparticles in the environment.
- Protocols for screening nanoparticle activity to be accredited for statutory use.

The following has been achieved in the third year of the project:

- Synthesis of constant particle size <10 nm ZnO particles.
- Comprehensive characterisation of biomembrane activity of ZnO and TiO₂ particles of wide range of particle size.
- Charge characteristics of SiO₂ particles.
- Cross calibration of results throughout the Consortium.
- Chronic toxicity and bioavailability of indicator organisms to nanoparticles.
- Development of theoretical models simulating interaction of NP with phospholipid membranes, the transport and fate of nanoparticles in environmental aqueous systems and the uptake of nanoparticles by unicellular organisms.
- Interaction of ZnO with ion channel proteins.

2.4 Conclusions from the Third Year of the Project

Conclusions from the work programme associated with relevant work package are outlined below:

**WP1:**
- Water solubility of ZnO nanoparticles of varying size and functionality has been determined.
- Work has sourced and synthesised stable dispersions of ZnO nanoparticles, and provided a set of well characterised single class of ZnO nanoparticles to the Consortium.
- Characterisation of NPs after exposure to biological media and characterisation of NPs internalized by cells and organisms.
- Quality TiO₂ dispersions and powders have been sourced and characterised.

**WP1/WP2:**
- Full comprehensive relationship between the structure and functionality of SiO₂, ZnO and TiO₂ particles and their biomembrane activity and their in vitro and in vivo biological activity established.

**WP2:**
- Toxicity screens consolidated and inter-calibrated.
- Mechanisms of SiO₂, ZnO and TiO₂ particle interaction with biomembrane model determined using electrochemical methods of impedance.

**WP3:**
- Detailed assessment and comprehensive feedback of in vitro test results to WPs 2 and 4.
- EMs of NPs in cells and tissues.
- On-line in vitro assays for NP toxicity developed.

**WP4:**
- NP effects on Ciona intestinalis growth determined, correlation with specific/non specific response.
- Structure Activity (SA) relationships established with WPs 2 and 3.
- Chronic toxicity tests using Daphnia magna carried out.

**WP5:**
- Determination of the surface charge density and zeta-potential of the NP dispersions in the presence of other ions and organic substances fulvic and humic acid present in the aquatic environment.
- Analysis of the heterogeneity of metal ion binding by silica NPs, and comparison with heterogeneities observed in the metal ion binding by macroscopic silica surfaces.
- Verification of the lability of Pb²⁺/SiO₂ nanoparticulate complexes by Eigen type reconstruction of the kinetic steps involved in the surface complex formation Surface characterisation of ZnO NPs.
- Permeability of NPs in hydrogels/model cell walls. Physiochemical conditions on permeability rate established.
- Form, permeation of SiO₂ and TiO₂ determined. Rigorous kinetic analysis of permeation.
- Charge characteristics of TiO₂ dispersions.

**WP6:**
- Advection/diffusion of NPs in aquatic environment studied.
- Results from WPs 2 and 5 integrated into model.
- Aggregation and sedimentation of model nanoparticle dispersions simulated and validated.
- Uptake of model nanoparticles by cells simulated and validated.

**WP7:**
- Intercalibrative testing of ZnO and TiO₂ on MBA indicator organisms.
## Directory

Table 1 Directory of people involved in ENNSATOX.

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Coordinator: Lang Tran, Institute of Occupational Medicine, Edinburgh (UK)

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<td>US</td>
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<tr>
<td>17</td>
<td>Duke University</td>
<td>Duke</td>
<td>US</td>
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</table>

US PARTICIPANTS
The following partners are linked to the project by a Memorandum of Understanding signed by their legal representatives

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<th>No.</th>
<th>Benefitary name</th>
<th>Short name</th>
<th>Country</th>
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<tr>
<td>18</td>
<td>US Environment Protection Agency</td>
<td>EPA</td>
<td>US</td>
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<td>19</td>
<td>National Institute of Occupational Safety &amp; Health</td>
<td>NIOSH</td>
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<td>20</td>
<td>National Institutes of Health - National Institute of Environmental Health Sciences</td>
<td>NIH-NIEHS</td>
<td>US</td>
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<tr>
<td>21</td>
<td>The Woodrow Wilson Center</td>
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<td>US</td>
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Contents

1 Summary ...................................................................................... 16
2 Concept ........................................................................................ 16
2.1 Aim and Objectives .................................................................. 16
2.1 Overall view of the Workplan .................................................. 17
3 Directory...................................................................................... 22
4 Copyright..................................................................................... 25
1 Summary

Engineered Nanoparticles (ENP) are increasingly produced for use in a wide range of industrial and consumer products. Yet it is known that exposure to some types of particles can cause severe health effects. Therefore it is essential to ascertain whether exposure to ENP can lead to possible health risks for workers and consumers. We have formed a consortium of well-known scientists from European Universities and Research Institutes, with over 100 publications in the field of Nanotoxicology. Our aim is to develop an approach for the Risk Assessment of ENP (ENPRA). Our objectives are:

- to obtain a bank of commercial ENP with contrasting physico-chemical characteristics and measure them;
- to investigate the toxic effects of ENP on 5 (pulmonary, hepatic, renal, cardiovascular and developmental) target systems and 5 endpoints (oxidative stress, inflammation; immuno-toxicity; fibrogenicity; genotoxicity) using in vitro animal/human models;
- to validate the in vitro findings with a small set of carefully chosen in vivo animal experiments;
- to construct mathematical models to extrapolate the exposure-dose-response relationship from in vitro to in vivo and to humans;
- to use QSAR like models to identify the key ENP characteristics driving the adverse effects;
- to implement a risk assessment of ENP using the Weight-of-Evidence approach;
- to disseminate our findings to potential stakeholders.

To harmonize the research activities between our EU group and the US, we have established links with scientists from US Universities (Duke, Rochester) and Government Agencies (NIH/NIEHS, NIOSH and EPA) with on-going research in Nanotoxicology.

Our objectives here are:

- to share information and agree on experimental protocols;
- to avoid duplication of work;
- to further validate the findings of this proposed study.

2 Concept

Nanotechnology is one of the key industries in Europe. The estimated economic impact of nanoparticles in industrial, consumer, and medical products will be US$ 292 billion by 2010 and US $1 trillion by 2015. The prosperity of our continent depends on the safe and sustainable development of this emerging technology 2. Every new technology brings with it new risks and for nanotechnology, the potential health risks to workers and consumers are paramount. They can arise from exposure to nanomaterials either at work or through consumer products. These risks, if not assessed and managed properly, can prevent economic growth and deprive us of a much needed competitive edge, but more importantly could have grave potential consequences for human and environmental health 2,3. Being aware of the health issues concerning engineered nanomaterials, in 2006, some of the ENPRA partners have written an article, published in Nature4, outlining the grand challenges for the safe handling of nanotechnology. It is clear that the production of safe nanomaterials is essential to establish and sustain the confidence of end users. This confidence is the ultimate guarantor for nanotechnology growth. It is therefore essential to develop an effective approach for improving the assessment and management of potential health risks from exposure to engineered nanoparticles (ENP)5. This is the overall aim of ENPRA.

2.1 Aim and Objectives

The principal aim of ENPRA is to develop and implement a novel integrated approach for ENP Risk Assessment (ENPRA). This approach is based on the Exposure-Dose-Response Paradigm for ENP (Figure 1). This paradigm states that exposure to ENP of different physico-chemical characteristics via inhalation, ingestion or dermal exposure is likely to lead to their distribution, beyond the portal-of-entry organ to other body systems. The cumulative dose in a target organ will eventually lead to an adverse response in a dose-response manner. Our approach will adapt the traditional Risk Assessment approach to ENP and will cover: Hazard Identification; Dose-Response Assessment; Exposure Assessment and Risk Assessment, Management.

The specific objectives of ENPRA are: (i) for Hazard Identification: To characterize a panel of commercially available ENP carefully chosen to address the relevant hazards, properties and potential mechanisms; (ii) for Dose-Response Assessment: To assess the hazards of these ENP by means of in vitro toxicology tests based on five body systems: (1) pulmonary; (2) hepatic; (3) renal; (4) cardio-vascular and (5) developmental, and five endpoints: (a) oxidative stress; (b) inflammation and immune-responses; (c) genotoxicity; (d) fibrogenicity and (e) developmental toxicity; (iii) To verify the in vitro findings with in vivo models; (iv) for Exposure and Risk Assessment: To use data from this project and other sources (including US data) to: (1) model exposure and the exposure-dose-response relationships by means of mathematical

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1 The ENP selected represent a subset from a panel of ENP chosen as reference materials for testing in a UK government (DEFRA) funded project and is very likely to be fed into the OECD plan for reference materials testing. The samples were chosen with contrasting properties on size/surface area (TiO2), charge (silica), shape (MWNT), surface chemistry (silver, iron).
modelling such as PBPK and QSAR-like methods, and extend these deterministic models into probabilistic models (2) to conduct the risk assessment with uncertainty analysis; (v) for Risk Management: To develop and implement a strategy for dissemination to maximize the anticipated high impact of our findings.

The design of our in vitro and in vivo studies takes into account the need to promote the principles of 3R.

Exposure Assessment: We will review existing exposure models in the public domain; Collect exposure information from existing EU and National Project and from our US partner; Construct a model of ENP exposure in occupational settings; Extend the traditional risk assessment approach by quantifying the uncertainty in ENP exposure.

Risk Assessment: We will extend the current risk assessment approach to ENP by building mathematical models of exposure-dose-response, including uncertainty analysis, to be used in estimating the DNEL and make comparison to the values obtained in Exposure Assessment.

Risk Management: We will implement a communication strategy to bring the ENPRA results to stakeholders including government agencies and Nanotechnology industry.

The approach proposed by ENPRA is in line with the grand challenges described in our article in Nature4. The rationale of ENPRA is summarised graphically in Figure 2.

The ENPRA Consortium To implement the ENPRA plan, we have assembled a consortium of 21 partners (15 Europeans and 6 Americans) with an excellent academic record measured in hundreds of publications on Nanotoxicology (and three relevant articles in Nature and Nature Nanotechnology). Our partners also include prominent members of government bodies, participating in the regulatory process, on both sides of the Atlantics (e.g. JRC and US EPA, NIOSH). Most importantly, different groups within the ENPRA consortium have experience in working together in FP projects as well as other national projects and will be able to share their extensive experience on working with ENP in achieving the objectives laid out in ENPRA.

The main deliverables of ENPRA are:

A novel risk assessment approach – with uncertainty analysis - specific to ENP;

In vitro and in silico models of exposure-dose-response relationships for 5 target organs and 5 endpoints to be used for the hazard assessment of ENP and considered for high-throughput screening tests;

In vivo models for the hazard assessment of ENP to complement the REACH and OECD guidelines.

The rationale of the ENPRA project is to generate essential data on ENP characteristics and toxicity to be used with data from other sources for a risk assessment of ENP.

Using the traditional Risk Assessment approach as starting point, our approach consists of:

Hazard Identification: We will implement (i) a comprehensive set of measurements of the physico-chemical characteristics of ENP, both in bulk samples and in body tissues; (ii) common protocols for ENP characterization and preliminary validation of measurement techniques; (iii) relationship between particle characteristics and hazards.

Dose-Response Assessment: We will implement a development of in vitro testing systems using models representing the most important target systems affected by ENP.

These in vitro tests need to be verified with in vivo models, (carefully designed to minimize the numbers of animals used and/or their inconvenience).

The verified tests will be validated by a round robin process between the ENPRA partners.

The selected in vitro tests could then be integrated as part of a low-cost, high-throughput screening test system, as a cost effective way of testing a large number of ENP expected to enter the EU market in the near future.

The in vitro data will be used to develop a QSAR model linking ENP characteristics with the adverse effects.

The in vivo models will also be considered as additions to OECD guidelines for regulatory toxicology tests of ENP.

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The project has reached the mid-term (18 month) milestone. The work performed by each WP and the main results are summarised below:

### WP2 EU-US Collaboration

The progress of WP2 in the first reporting period is according to the original workplan and we have achieved the current milestones. The notable achievements of WP2 so far are:

1. The ENPRA website which is available for internal and external use.
2. The continuing collaboration between EU and US partners in the supply of ENP samples and protocols.

In the second reporting period, we have implemented the following activities:

1. Partner 17 performs complementary ICP-analysis of the catalast particles in MWCNT to cross compare with data from ENPRA.
2. The first in vitro set of results from Partner 19 with MWCNT has been sent to Partner 1 to incorporate in the ENPRA database for use in WP6.
3. Partner 18 and Partner 1 are designing the data templates for transfer the TOXCAST data over to ENPRA for WP6.

### WP3 Hazard Identification through characterization of the Physico-chemical properties of ENP

The progress of WP3 for the first reporting period is according to the ENPRA workplan. Specifically, we have achieved the following:

1. Test materials have been identified or produced and distributed.
2. The 2% serum-water-based batch dispersion protocol (± use of 0.5 vol% EtOH) has been developed and adopted within ENPRA and beyond.
3. Size distributions of batch dispersions have been collated and distributed informally to ENPRA.
4. Primary physicochemical characteristics have been obtained and a report is currently being prepared.

For the second reporting period,

1. All primary physicochemical characterization has been completed and analyses of organic coatings have been completed.
2. Extensive supporting analysis for the toxicity testing has been completed.
3. A complete database on physical and chemical characterization of ENP has been distributed for incorporation in the ENPRA database.

We continue to measure the hydrochemical reactivity and biodurability of ENP. We will also validate the developed protocols with real biological samples from WP and perform a final intra- and inter-homegeneity study will be done on the TiO2 samples. Finally, we prepare publications on the ENPRA dispersion protocol and co-authoring papers lead by researchers outside the WP.

### WP4 – Dose-response assessment I: Development of in vitro models for assessing the potential hazards of ENP

The progress of WP4 in the first reporting period is according to the ENPRA workplan. Specifically, we have achieved the following:

1. For the kick off meeting all tasks were detailed via a spreadsheet, dividing each task into target organ/cell types and the relevant endpoints for each target.
2. Using the spreadsheet, each partner identified which target and endpoints they were to be responsible for in order to identify areas for collaboration and potential gaps. This was discussed via teleconference in order to allow coordination of collaboration. No gap was identified.

3. Key users of protocols that spanned multiple targets were identified and these groups generated standard operating procedures for these protocols. These protocols have been shared amongst partners via the ENPRA website. In addition they have been provided to NanoImpactNet for conversion into NIN protocol format.

4. A panel of 10 ENP were distributed to all partners via Mercator.

5. Using the agreed protocols, each group tested all of the particles for cytotoxicity in the relevant target cell types in order to determine LC₅₀ values.

6. LC₅₀ values have been assembled into a summary spreadsheet to enable future strategic decision making for WP₄ and WP₅. This spreadsheet has allowed identification of relatively high toxicity materials (ZnO and Ag) and relatively low toxicity materials (MWCNT and TiO₂), but it has also allowed identification of cell type - particle type interactions which are relatively sensitive (e.g. macrophages and long MWCNT). This information, combined with the information from the characterisation WP, will allow prioritisation of particles for mechanistic studies and in vitro studies.

The most notable results so far for WP₅ are:

1. The clear consistency between all partners that the ZnO and Ag particles are relatively toxic;
2. all other ENP are generally of no significant toxicity at the doses tested.

For the second reporting period, we continue with the following:

1. Each group has tested all of the particles in the relevant target cell types in order to determine LC₅₀ values.
2. LC₅₀ values have been assembled into a summary spreadsheet to enable future strategy decision making for WP₄ and WP₅.
3. All Cytotoxicity data has been submitted for data analysis and database generation in other WPs.
4. Much of the cytokine data has been submitted for data analysis and database generation in other WPs.
5. Mechanistic studies now focus on a minimum of the 2 MWCNT, the Ag, uncoated ZnO and one TiO₂.
6. One publication is already in press for the genotoxicology studies and a number of other manuscripts are in advanced stages of preparation.

WP₅ – Dose-response assessment II: Using in vivo models for a kinetics study and verification of in vitro results

The progress of WP₅ in the first reporting period is according to the ENPRA workplan. Specifically, we have achieved the following:

1. The major part of the study has been conducted
2. The dose-response relationships of the full panel of ENP after acute exposure have been executed. The study has been performed using the finalised dispersion protocol to make the particle suspensions and the finalised instillation protocol. The data are being collected and currently analyzed.

The most notable results so far for WP₅ are:

1. Results of kinetic study using TiO₂ have been reported. ENP taken up into the lungs cross the lung membrane and reach the blood stream. This leads to an accumulation in organs in a size dependent manner: the smaller the ENP, the higher the accumulation in the organs.
2. The intratracheal instillation has been performed for the acute dose-response study. Preliminary results show a very low acute inflammatory potential, except for ZnO.
3. In contrast to the in vitro results, nano-silver does not evoke an acute inflammatory response.
4. The MWCNT seem to be less acutely toxic compared to other MWCNT studies described in literature, but a detailed comparison in particle characteristics needs to be done to give an idea why that is.
5. Data from the in vitro studies and the preliminary results from the acute in vivo study have lead to a critical re-evaluation of the study set-up for the in vivo studies in mice with a pre-existing risk factor.

For the second reporting period we have continue with the following:

1. Bio-kinetics inhalation studies with Gold and Silver ENP have been performed and the data analysed. The gold ENP undergo the same metabolic and biokinetics when comparing inhalation versus instillation. Rather unexpectedly the entire NP translocation across the air-blood-barrier did not differ significantly and the retained fractions in prominent secondary organs like liver and spleen were also not significantly different. This result is insofar important as it suggests that the rather inexpensive method of instillation provides a rather simple application for NP delivery to the lungs including comparable translocation towards secondary organs compared to the application by inhalation – the gold standard of lung toxicology research.
2. In vivo studies to determine toxic effects after acute (24hrs) exposure for the full panel of ENPs have been conducted. The data have been analysed and dose-response relationships have been established for a range of endpoints. The intratracheal instillation of ZnO NM-110 and NM-111 will be repeated for 2 reasons. 1. Due to technical difficulties during blood drawing after administration of NM-110, no blood analysis was possible while effects are seen in blood parameters with ZnO NM-111. 2. Due to advances with ZnO toxicity testing in other projects, there could be indication for early changes in lung tissue. In the current protocol
The most notable results so far for WP6 are:

1. The ENPRA database templates have been produced for data collection from WP3, 4 and 5.
2. The ground work for Exposure modelling, QSAR, PBPK and PD modelling is ongoing. When data will be available, in the first quarter of 2011, results will be generated.


The progress of WP6 in the first reporting period is according to the ENPRA workplan. Specifically, we have achieved the following:

1. The ENPRA database templates have been produced for data collection from WP3, 4 and 5.
2. The ground work for Exposure modelling, QSAR, PBPK and PD modelling is ongoing. When data will be available, in the first quarter of 2011, results will be generated.

The most notable results so far for WP6 are:

1. on preparatory work for modelling, including uncertainty analysis using Monte Carlo simulation. This is done in collaboration with US partner 19. Period 1 work has concentrated on data generation, with much of the WP6 work so far being preparatory. In Period 2, significant and important results from the Risk Assessment will be available.

In the second reporting period, the data available from other WP are now increasingly available. The notable progresses in WP6 are:

1. The collection of data from WP3, 4 and 5 and later also from the US partners into the ENPRA database for QSAR analysis.
2. Partner 1 and Partner 14 have met to discuss the transfer the data for analysis
3. Bio-kinetics data from WP5 have been used for PBPK modelling
4. In vitro and in vivo endpoints commensurate for comparison have been identified
5. Model of exposure of ENP are being constructed using data from Partner 19.

WP7 – Dissemination Strategy to maximise impact

The progress of WP7 in the first reporting period is according to the ENPRA workplan. Specifically, we have achieved the following:

1. The main contributors (P14, P5 and P1) of this work package met face-to-face during the kick-off meeting (where a general agreement on the initial 6 months work plan and working procedures was achieved) and several times virtually by teleconference to agree organise further steps. Also the annual management meeting and the expert panel meeting offered opportunities to meet.

2. The dissemination strategy was implemented as foreseen by:
   - holding the first annual workshop with participation of the main stakeholders (EU CAs, Industry, NGOs, COM Services, OECD, other FP6/7 projects) and disseminating its outcome
   - organising the three experts’ panel meeting and producing and disseminating the two EONS reports
   - participating in a number of workshops/conferences both in the EU and the US
   - participating in one OECD experts’ meeting

The most notable results for WP7 so far are the regular and high-impact events to disseminate the idea and results of ENPRA. Overall, the work package is well on track and the foreseen milestones and deliverables have been reached without major problems. This seems to be the case as well for the next milestones and deliverables.

3.1. Expected final results and their potential impact and use (including the socio-economic impact and the wider societal implications of the project so far),

ENPRA has reached the Mid-Term Milestones. As demonstrated above, the major achievement of ENPRA so far are the results from the in vitro (WP4) and in vivo tests (WP5) together with the measurement of the ENP physico-chemical characteristics for Hazard Identification. So far, there is remarkable concordance between in vitro and vivo results (with the exception of nano silver). The ENPRA team is currently analysing and formulating new hypotheses regarding the low toxicity of the ENP samples in contrast to the published results in the public domain.

In the second reporting period we continue with the following:

1. The 2nd Annual ENPRA Workshop and Stakeholders Panel Meeting took place in Somma Lombardo (Italy) from 10 to 12 May 2011 organised by the JRC-IHCF. This time the workshop theme was “Challenges of Regulation and Risk Assessment of Nanomaterials” and it was organised in the frame of the JRC Enlargement and Integration Action, that allowed to support the participation of scientists from EU and Associate Candidate Countries. Attendance of members of the Competent Authorities for REACH and Classification and Labelling Sub-Group on Nanomaterials (CASG Nano) and the European Chemicals Agency (ECHA) was supported. Also other strategic stakeholders as OECD, NGO’s (representing environment and workers’ protection organisations), the contractors for the RIP-oNs projects and chemicals and nanomaterials industry and EFSA were involved. Some related projects under FP7 Research Program participated too. It involved about 80 participants. Generalist and specialised press were also present. During the workshop, 34 experts from 26 different organisations informed the participants on the latest scientific progress in the field of nanoparticles risk assessment produced within the ENPRA and other related projects and presented and discussed recent developments concerning legislation in the EU and
A full report of the event is currently in the final steps for publication and distribution. Information can be found in the JRC site [http://ihcp.jrc.ec.europa.eu/events_workshops/joint-jrc-nano-enpra-2011](http://ihcp.jrc.ec.europa.eu/events_workshops/joint-jrc-nano-enpra-2011).

The results of the 2nd workshop will be published online on the JRC site (presentations already online, full report by mid November) and widely distributed among targeted stakeholders.

The 4th EONS report (state of the art report summarising the discussion of the 4th ENPRA expert panel meeting held in Brussels on March 31st) was published on June 2011 (D7.2 – M24). The report (10 experts’ article commentaries) was then disseminated.

The 5th ENPRA expert panel meeting will be held in Paris on October 13, 2011. The event will gather 13 participants including ENPRA partners (P1, P5, P6, P8, and P14) and French OMNT experts (5).

1. Analyse the body of experimental data generated by the WP4 and 5 as well as data contributed by US EPA and NIOSH
2. Relate the information to the physico-chemical characteristics of ENP in WP3 by means of modelling.
3. Assess the potential health risks with regards to the tested ENP
4. Fully develop and validate a model of Exposure to ENP; Use the PBPK as a tool for Risk Management.
5. Conduct a round robin tests for a chosen in vitro model from WP4 with selected ENP.
6. Finalised the in vivo experiments in WP5.

In summary, ENPRA has generated a comprehensive in vitro, in vivo dataset together with the characterisation of the ENP and a dispersion protocol. The database of results was used for modelling, the process of ENP exposure, the passage from Exposure to internal dose in the portal of entry organ and its distribution across secondary organs.

In particular, for the final period of ENPRA, the key achievements were:

1. The combination of the Exposure and Toxicity profile of the ENP in order for risk assessment.
2. The use of in vivo results to calculate the safe airborne exposure levels for the ENP for risk management.
3. The use of QSAR modelling to derive the relationship between ENP characteristics and their adverse responses using the characteristics of ENP and the in vitro data, including data from our US partners.

**Fig 3.** Flow chart describing the information flow between different WP of ENPRA (solid lines) and the process of coordination, management and collaboration (dotted lines)
### 3 Directory

**Table 1 Directory of people involved in this project.**

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<tr>
<th>First Name</th>
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### NanoSafetyCluster Compendium 2013

**Compendium of Projects in the European NanoSafety Cluster**

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<tr>
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EURO-NanoTox
European Center for Nanotoxicology

Funded by: Federal Ministry of Science and Research
Website: http://www.EURO-NanoTox.eu
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Contents
1 Introduction.................................................................................. 28
2 Background ................................................................................ 28
2.1 From international to Austrian needs in the field of nanotoxicology ....................................................... 29
2.2 Interdisciplinary Network ....................................................... 29
3 Core-Activities of EURO-NanoTox ............................................. 29
4 Organisation of EURO-NanoTox.................................................. 30
5 EURO-NanoTox Expertise-Portfolio ........................................... 31
6 EURO-NanoTox-Letters – ONLINE-Journal................................. 31
6.1 Aim of EURO-NanoTox-Letters................................................ 31
6.2 Background of EURO-NanoTox-Letters .................................. 31
7 SUMMARY and OUTLOOK ........................................................... 31
8 Directory ................................................................................... 33
9 Copyright .................................................................................. 33
10 References ................................................................................. 34
1 Introduction

Nanotechnology is, along with biotechnology and information technology, a key technology of the 21st Century that has far-reaching implications for science, industrial development and the creation of new products. Therefore, it is considered highly important for successful economic development over the coming decades. The subject of nanotechnology is a collective term that relates to different techniques in the nanometer range, the production, study and in the application of structures. Molecular materials, internal interfaces and surfaces with critical dimensions or production tolerances ranging from a few to about a hundred nanometers are studied structural factors. In the most important industries, it is increasingly recognized that the control of structural and functional properties of novel materials - so-called "Advanced Materials" - on the nanometer scale is the key to technological advances and new products that will conquer emerging markets [1]. Besides the use of nanotechnology in material science, there are great impacts of nanotechnology that are expected to alter medicine. Nano-based techniques in the fields of diagnostics (e.g. imaging, biosensors) and therapy (e.g. drug delivery, drug targeting or regenerative medicine) are creating new possibilities in medicine [2, 3]. Cancer therapy along with the treatment of viral and a number of degenerative diseases has shown significant progress with nano-based techniques [4-8].

However, despite the obvious benefits of such advanced materials, there are potential adverse effects on the environment and people due to the fact that humans are exposed to nanoparticles through various routes: inhalation via the respiratory tract, dermal absorption/penetration through hair follicles, ingestion by the gastrointestinal tract, and injection. Nevertheless, the toxicology of these materials has been investigated insufficiently.

Regarding the degradation processes of advanced materials (e.g. waste deposit, air, and groundwater), nanostructured materials are being distributed in the environment. Until now, it has not been possible to show whether nanoparticles that are ingested or inhaled from the environment are systemically absorbed on a larger magnitude and if it is possible to calculate their long-term effects [9, 10].

In addition to the desired physio-chemical changes, a modification of toxicological behaviour is observed due to the structuring of the materials on the nanometer scale. Systematic studies exist regarding the effects of environmental nanoparticles (ultratine particles) in relation to a reported increase in the incidence of cardiovascular disease and propensity for asthmatic disease [11-15]. The change of the toxicological potential, which is due to the reduction of the material in the nanometer range, was negated a few years ago. In many cases, the importance has only been recognized in recent years. People and the environment are permanently exposed to nanostructured materials as a result of an ever-widening use and also from their release from within their life cycle; these effects are not negligible. Therefore, a profound knowledge of the toxicological potential of nanostructured materials, breakdown products, penetration of and metabolism in the human body, and their emission is of enormous importance.

The knowledge of toxicology, the possibility of critical assessment of the potential danger of using standardized testing procedures and the systematic studies carried out on nanomaterials are hereby determined by public acceptance. Public acceptance is a prerequisite for the sustainable and successful development of nanotechnology. A poor acceptance (e.g. caused by a lack of awareness in the field of toxicology) could probably lead to a negative trend in perception similar to that of genetic engineering.

Due to the fact that nanomaterials are increasingly present in our environment, international experts have a growing interest regarding this issue. It is increasingly clear, however, that there is a tremendous need for standardization. A portion of data published to date, which has been used, contains insufficiently characterized nanostructured materials. Moreover, many of the in vivo studies carried out with mice or rats have used overly high doses of the investigated nanostructured materials. This demonstrates that the results are not conclusive and that a classification of the key parameters for assessing the toxicity of nanostructured materials is urgently needed.

2 Background

In the field of toxicology in recent years, a paradigm shift towards a proactive risk assessment has been identified. The public reporting has increased significantly, making the need for the objective communication of risks paramount. Public opinion and acceptance of nanotechnology contribute to four main areas and therefore must be given special consideration: (a) public attitudes, (b) public perception, (c) the role of the media, and (d) trust from those who communicate the risk in public behaviour and attitudes [16]. This development is also taken into account on an international scientific level and is recognized by The European Commission. Janez Potocnik, a member of the Commission for Science and Research until November 2009, cited this: "Nanotechnology is a key area where Europe leads the way and we must ensure that this remains so. The potential of nanotechnology for European industry and society is enormous so we need to research a clear strategy and effective measures in this area. At the same time we must consider eventual health, safety and environmental risks and address them as early as possible." [17]. The bidding of the 7th Framework Program of the European Union reflects this trend by making a specific call for clarifying research in the field of toxicology.

Another initiative in this field sets the Organization for Economic Co-Operation and Development (OECD) with the "Sponsorship Program for the Testing of Manufactured Nanomaterials". This program pools expertise and funds the safety testing methods of specific manufactured nanomaterials (NMs). The priority list includes 14 NMs for testing based on materials which are in, or close to, commerce: Fullerences (C60), single-walled carbon nanotubes (SWCNTs), multi-walled carbon nanotubes (MWCNTs),
silver nanoparticles, iron nanoparticles, carbon black, titanium dioxide, aluminium oxide, cerium oxide, zinc oxide, silicon dioxide, polystyrene, dendrimers and nanoclays [18].

In 2004, Donaldson and colleagues claimed, ‘We suggest that a discipline of nanotoxicology be built up to address the new potential threats that widespread use of new nanoparticles could bring in support of the growth of a safe and sustainable nanotechnology industry’[20]. The term ‘nanotoxicology’ was introduced into the literature and Austria became involved with various activities right from the beginning.

2.1 From international to Austrian needs in the field of nanotoxicology

The network NanoNet Styria was the first in Austria to touch upon the topic of nanotechnology and has dealt with nanotoxicology from the beginning. Initiated through these regional activities in nanoresearch, the Austrian Nanoinitiative was founded at the federal level. The group, which has been working within NanoNet Styria with bianonotechnology, has evolved into BioNanoNet Forschungsgesellschaft mbH. In 2004, the Austrian Nanoinitiative announced the program “National cooperative research and technological development in collaborative projects.” BioNanoNet worked as an administrative coordinator for the proposal of the joint project "Nano-HEALTH - Nano-structured materials for drug targeting, release and imaging" (www.nano-HEALTH.at). This project deals with nanostructured materials and integrated the toxicological concerns as a sub-project. International experts have critically evaluated the submitted research project. They have positively reviewed the project twice. The budget for “Nano-HEALTH” was allotted at 8 million Euros for the time period of 2005 to 2012. The toxicological work under this project was the basis for the establishment of the European Center for Nanotoxicology.

In 2007, giving active support to decision makers to implement an Austrian strategy on nanotoxicology, Helge Torgersen and Frank Sinner laid down the basis for the joint recommendation to the Austrian Federal Ministry for Transport, Innovation and Technology (BMVIT) regarding the questions of risk and societal issues in nanotechnology. These recommendations summarized the state-of-the-art in nanotechnologies and risk assessment as well as the possible effects on human health. Additionally, they outlined the knowledge gaps that need closing and the methods that will ensure safe and sustainable development of the entire field of nanotechnologies. The authors also proposed a set of strategies to implement these recommendations on a short, medium and long-term basis. Some aspects of these recommendations were implemented by the funding of the project Nano-Trust which is managed by the Austrian Academy of Science.

The core of Nano-Trust is to provide a point of contact for issues dealing with the potential health and environmental risks of nanotechnology for citizens, government and politicians. Furthermore, a multidisciplinary team has established an annotated literature database that covers different aspects of nanotechnologies including the effects on human health, ecotoxicity, and governance. The team consists of the Austrian Academy of Science, Environmental Agency, BioNanoNet Forschungsgesellschaft mbH and the Austrian Agency for Health and Food Ltd (AGES).

2.2 Interdisciplinary Network

The key to the development of safer nanomaterials (including the factors mentioned above) is the establishment of interdisciplinary networks of nanomedicine and the transference of existing knowledge in Austria. In order to focus on this necessary expertise in Austria, the European Center for Nanotoxicology (EURO-NanoTox) from the BioNanoNet Forschungsgesellschaft mbH was founded in 2007, funded by the Austrian Federal Ministry of Science and Research (BMWf).

The European Center for Nanotoxicology (EURO-NanoTox) is the Austrian hub for scientific knowledge in the field of human nanotoxicology. The Center’s science and networking industry contributes significantly to improving safety in the workplace when dealing with nanostructured materials. EURO-NanoTox is designed to address all aspects of nanotoxicology and is a national contact point with international visibility for researchers and industries. The EURO-NanoTox is managed by the BioNanoNet Forschungsgesellschaft mbH, a non-profit network company active in the field of pharmaceutical development. The partners of the EURO-NanoTox are Joanneum Research, the Medical University of Graz, the Karl-Franzens-University of Graz, Seibersdorf Laboratories GmbH, BioMed-zet Life Sciences GmbH, the University of Salzburg, Mondi Uncoated Fine & Kraft Papers GmbH – Department Research & Development, and the University of Vienna. The variety of the scientific backgrounds and the techniques offered by the partners allows the Center to describe biological actions of nanoparticles from different perspectives (see standard-method-catalogue on www.EURO-NanoTox.at). The EURO-NanoTox is in collaboration with national and international working parties. EURO-NanoTox is an open network that is accessible to all Austrian groups active in or interested in the field of nanotoxicology. Furthermore, EURO-NanoTox establishes a strong cooperation to key institutions on European level. EURO-NanoTox is involved in all major activities in the field and is a data contributor in the OECD Working Party on manufactured nanomaterials (WPMN) on the international level.

3 Core-Activities of EURO-NanoTox

The Center is active in the following areas:

1. Development and structuring of the field of nanotoxicology in Austria.
2. The Development, establishment and implementation of standardized in vitro and in vivo toxicological methods for nanostructured material
3. The development of national and international research projects on nanotoxicology
4. It provides industry with a tool kit of methods for the in-vitro and in-vivo measurement of the toxicological potential of nanostructured materials as well as carrying out and interpreting these tests
5. The active establishment of international contacts with key players in the area of nanotoxicology
6. The active monitoring of relevant literature and the providing of an information point for interested scientists and industry partners
7. Participation in and organization of comparative studies including ring studies.

The core function of the Center, however, is to develop and implement standardized in vitro and in vivo tests for the determination of the toxicity of nanostructured materials. This is an absolute necessary basis for the systematic investigation of toxicological effects as well as for toxicological mechanisms. Hence, the EURO-NanoTox was conceived as a vehicle that will bring all these aspects together. Through the application of standardized methods in a quality assured environment, expensive failures in product development and/or potential hazards occurring upon product release can be avoided.

The toxicological profile of a given nanostructured material is determined by multiple parameters, including, but are not limited to: size, payload, composition and geometrical structure. Thus, it is essential to develop, in each case, an individual toxicological strategy tailored to each unique nanostructured material. The strategy should reflect current literature-based knowledge and enable an approach that is both cost-effective and well structured (see figure 1).

The systematic in-vitro toxicology is based on cytotoxicology and hemotoxicology concerning the effect of the port of entry into the human body (pulmonary, dermal, nasal, buccal, oral, and endothelial) and the effect onto specific organs (liver, kidneys, spleen). Additionally, a 3D liver model can be used for testing metabolic activity, cell viability, cell toxicity, biochemical assessment of ROS generation (oxidative stress), CYP450 activity (xenobiotic metabolism), stress and genotoxic as well as inflammatory responses. Genotoxic effects are identified by the assessment of changes in the structure of chromosomes and DNA. Evaluations of the in-vivo effect of nanoparticles include blood count and clinical chemistry (serum parameters for liver damage, kidney function, inflammation, and immune response), histopathology and immunohistochemistry, all of which address specific questions (proliferation, inflammation, oxidative stress etc.).

An improved understanding of tissue specific toxicology of nanoparticles is critically dependent on the development of procedures that are able to sample the tissue microenvironment in a manner that enables continuous sampling, i.e. without taking biopsies. Open Flow Microperfusion [OFM] enables such an approach to be realized in a highly effective and elegant manner given that it: (i) is a minimal invasive procedure, (ii) allows continuous sampling and (iii) enables the full spectrum of analytes to be harvested from the surrounding milieu, i.e. ranging from small molecules to nanoparticles (micro dialysis in contrast employs a catheter containing a semi-permeable membrane).

The latter features allow a broad spectrum for analysis of all potential nanoparticles and substances (electrolytes, small molecules, peptides or proteins) to be performed. All these expertise are collected in the “Assessment of Toxicological Effects by in-vitro and in-vivo Assays and open flow microperfusion”-folder available on the EURO-NanoTox Homepage (www.EURO-NanoTox.at).

4 Organisation of EURO-NanoTox

The pooling of the scientific expertise of all partners involved and the formation of a link with the structured network of BioNanoNet Forschungsgesellschaft mbH has facilitated the creation of a broad base for a toxicology Center. The embedding of this know-how in international research and development landscape in collaboration with regulatory bodies and authorities will lead to the extension and further development of EURO-NanoTox as an international hub. EURO-NanoTox is also eager to pursue strategic collaborations with other European nanotoxicology centers which will lead to the establishment of a European nanotoxicology network.
The core functions of the Center, however, are: (i) to serve as the Austrian junction point at which industry and science can submit their nanostructured materials for investigation regarding human toxicity and (ii) to develop and implement standardized in-vitro and in-vivo methods for the determination of the toxicity of nanostructured materials (including workplace safety). This is absolutely necessary because without this basis of determining toxicity, no systematically investigation of the toxicological effects will be possible.

Therefore, EURO-NanoTox was conceived as a vehicle by which the coordination of these aspects is possible. Through the application of standardized methods in a quality assured environment, costly failures product development or potential hazards due to product release can be avoided. Besides the applied aspects of nanotechnologies for scientific expertise, EURO-NanoTox builds in the area of workplace safety. Furthermore, the Center elaborates upon the requirements for a European information platform in order to ensure that the workers and decision makers, who are responsible for the safety of the employees, have access to important emerging knowledge in the field of nanotechnology.

Aspects of converging technologies have the capacity to be viewed in a negative way by the public. The development of scientific expertises, provisions for the availability of information and management for public expectation will be important parts for the acceptance of this innovative technology.

5 EURO-NanoTox Expertise-Portfolio

During the first two years of the project, EURO-NanoTox created a methods-catalogue, and in 2010 already revised and expanded this document. The assessment of toxicological effects induced by conventional drugs, nanoparticles or medical devices includes a series of in vitro and in vivo tests. The EURO-NanoTox partners offer a first assessment of toxicological effects for producers of chemical substances and especially of nanoparticles.

The document is available online: http://www.euronanotox.at/images/stories/folder_euronanotox_webversion.pdf

6 EURO-NanoTox-Letters – ONLINE-Journal

EURO-NanoTox-Letters is a new journal in the biomedical field that fills the gap between material science orientated and medical journals. The main aim of EURO-NanoTox-Letters is to increase the knowledge in the field of nanotoxicology and to help to pave the way from the present case-to-case to a holistic approach. This journal should help to ensure a sustainable development of the entire field of nanotechnology. The journal will publish in vitro, ex vivo and in vivo studies elucidating NMs behavior in physiological environment. It will describe absorption, distribution, metabolism and elimination of NMs in order to find out to which extent toxicity testing guidelines for drug products can be used for the toxicological assessment of these materials.

The following top-level category structure is proposed for EURO-NanoTox-Letters:
- Interaction of nanoparticles with cells
- Changes of nanoparticles by interaction with physiological fluids
- Absorption, distribution, metabolism and elimination of nanoparticles
- Physico-chemical characterization of nanoparticles
- Bio-persistence of nanoparticles
- Interference with test systems

The journal publishes original articles on all aspects of nanotoxicology as well as on toxicological issues in nanomedicine; reviews- these inform readers of the latest advances in nanotoxicology and short papers- these feature exciting research breakthroughs in the field are available resources.

Neither the authors nor their institutions will be charged for publication processing fees.

The editors are looking forward to receiving high quality papers from experts in the field of nanotoxicology and nanomedicine in order to make this journal a leading publication in the field.

6.1 Aim of EURO-NanoTox-Letters

The aim of EURO-NanoTox-Letters is to increase knowledge on interactions of nanoparticles in the physiological context by investigating adsorption, distribution, metabolism and elimination of nanoparticles in order to find out to which extent toxicity testing guidelines of drug products for nanoparticles can be used for the assessment of nanoparticles' toxicity.

6.2 Background of EURO-NanoTox-Letters

Research on the toxic effects of nanoparticles was started by reports that nano-sized combustion products may cause health problems. The potential toxic effects on workers exposed to nanoparticles, which are generated either as by-products during the production or that consist of the final product itself, are another important topic for nanotoxicological research. Increasingly, nanoparticles are also designed for drug-delivery, medical devices and imaging in medicine. Although products, which are used in medical treatment, are subjected to strict guidelines, these guidelines may not apply for nanoparticle-based therapeutics.

7 SUMMARY and OUTLOOK

EURO-NanoTox pursues the main goal to condense and structure all available scientific expertise in Austria and to develop standardised methods for toxicology assessment of nanostructured materials. The catalogue of Austrian nanotoxicology expertise, which is available online (http://www.euronanotox.eu/images/stories/folder_euronanotox_webversion.pdf)
has been updated, new methods have been, and continuously will be included. EURO-NanoTox is the AUSTRIAN hub for nanotoxicology and serves as the port for all scientific driven aspects of nanotoxicology and human health.

In future, EURO-NanoTox will additionally serve as a scientific foundation for regulatory aspects as for example worker safety/workplace safety. To enhance safety for workers EURO-NanoTox actively tested workplaces and assessed the risk and safety aspects at these workplaces. The EURO-NanoTox partners additionally provide valid scientific data and perform validated scientific experiments to address the potential toxic profile of nano-structured materials. Extract of the publications from EURO-NanoTox and its partners:


Teubl, BJ; Meindl, C; Eitzelmayr, A; Zimmer, A; Fröhlich, E; Roblegg, E (2013) In-vitro Permeability Studies of Neutrally Charged Polystyrene Particles Through the Buccal Mucosa. Small DOI: 10.1002/smll.201201789


Fröhlich, E; Meindl, C; Roblegg, E; Ebner, B; Absenger, M; Pieber, TR; (2012) Action of polystyrene nanoparticles of different sizes on lysosomal function and integrity Part Fibre Toxicol 9:26


Furthermore, EURO-NanoTox is setting up a European-wide network of national hubs for nanotoxicology in collaboration with already existing platforms. This network will help to interchange recent developments and developed methods between different European countries and promote the development of European standards to help ensuring the successful development of nanotechnologies as a key for European growth. EURO-NanoTox actively contributes to European projects dealing with regulatory aspects of nanotechnology (e.g. NANOFORCE) and plans to enlarge its initiative in this field in future projects.
# Directory

Table 1 Directory of people involved in this project.

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10 References

HINAMOX

Health Impact of Engineered Metal and Metal Oxide Nanoparticles: Response, Bioimaging and Distribution at Cellular and Body Level

Contract Agreement: NMP4-SL-2009-228825 - HINAMOX  Website: http://www.hinamox.eu
Coordinator: Sergio E. Moya, Centro de Investigación Cooperativa en Biomateriales – CIC biomaGUNE

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Contents

1 Summary ................................................................................................................. 36
2 Project Background ............................................................................................. 36
3 What is HINAMOX ................................................................................................. 36
   3.1 Project description ......................................................................................... 36
   3.2 Partners ............................................................................................................ 39
   3.3 Dissemination activities .................................................................................. 40
   3.4 Citations ........................................................................................................... 40
4 Directory ................................................................................................................ 41
5 Copyright .................................................................................................................. 44
1 Summary

Project number: 228825

Project Duration: 1 October 2009 – 30 September 2012

Project Funding: EUR 2,297,337.26

HINAMOX was a 7th Framework project dedicated to the study of metal and metal oxide nanoparticles (NPs) such as TiO2, ZnO, Al2O3, CeO2, as potentially hazardous to biological organisms.

HINAMOX has accomplished an advanced work in the field of in vivo and in vitro studies of NPs. The importance of this work lays on the fact that will set the basis for proper dose relation quantifications and distribution studies both at cellular and body level. This represents indeed a milestone for the future definition of nanosafety regulations, standards definitions and for the assessment of the health effects of NPs on humans.

Research in HINAMOX focused on the correlation of materials properties to their toxicological endpoints.

HINAMOX generated knowledge on the biodistribution and biological fate of metal oxide NPs. To achieve this goal procedures for NP radiolabelling were developed to allow for the application of Positron Emission Tomography to trace the NPs in animals.

In parallel to the in vivo studies the HINAMOX consortium worked in establishing quantitative data and practical procedures to determine the concentration and distribution of NPs at cellular level applying Ion Beam Microscopy, Confocal Raman Microscopy and Transmission Electron Microscopy techniques.

The consortium studied as well the cytological and pathological response to NPs, targeting innovative work of the inflammatory response of the alveoli as a possible vehicle for the introduction of NPs in the body. Detailed analysis of NP leaching and dissolution for the assessment of NP biodurability and residence times in tissue and lung-lining fluids will be developed.

All together, these studies will make an important contribution to a deeper understanding of NP toxicology and will be fundamental in defining regulations where dose effect relation are required both at the cellular and body level.

2 Project Background

Among the immense variety of industrial and medically important NPs, the HINAMOX proposal will focus on metal and metal oxide NPs as potentially dangerous to biological organisms. Metal oxide and metal NPs are widely used in various industrial processes and common products.

Metal oxide and metal NPs may be dangerous for humans because of two reasons: their special catalytic activity coming from the properties of their nanointerface may interfere with numerous intracellular biochemical processes and the decomposition of NPs and subsequent ion leakage could heavily interfere with the intracellular free metal ion homeostasis, which is essential for cell metabolism.

A very specific problem dealing with metal oxide NPs is the difficult of localizing and quantifying them in cells and organs. Obtaining dose effect relationships for these NPs is not simple, because of the unknown amount of material present in affected cells.

Previous research and hypothesis suggest that the particle size, shape, chemical composition and the chemistry of the capping agent determine the catalytic properties and surface activity of NPs as well as the materials where the NPs are incorporated. These properties are important for the applications of the NPs and must be studied in the context of their effects on human health. The substantiated evaluation of the health risk, associated with the exposure to metal oxide and metal NPs, requires a concerted action. Our approach has been a close collaboration in a highly interdisciplinary consortium with expertise in synthetic chemistry, production technology, particle physics and characterization, biochemistry, toxicology, immunology and occupational hygiene.

3 What is HINAMOX

3.1 Project description

The integrated study of NP health effects in our project involved the following steps:

1) Characterization of commercially available NPs, and the fabrication and characterization of NPs with specific properties and with either fluorescence or radioactive labelling.

The company PlasmaChem, which was a member of our consortium, provided us with metal oxide NPs. CeO2 and ZnO NPs have also been purchased from Evonik, Germany. At least one example of each of the metal oxide NPs with the most relevant applications or potential, according to the Nanomaterial Roadmap 2015 of the 6th Framework programme, was considered for study.

The consortium worked in the design of NPs endowed with either fluorescence or radio labels. An important aspect of this project was indeed the fabrication of NPs with proper labelling to trace the fate of the NPs both in vitro and in vivo. This task implied the development of proper routes of labelling both NP core and capping agent. Two strategies were followed for the radiolabelling of NPs. In one the NPs were synthesized with 18 O, and activated with a proton beam to produce 18F-labelled NPs via the 18O(p, n)19F nuclear reaction. The other procedure involved the direct activation of commercial NPs.

NPs were fluorescently labelled applying surface chemistry. Core labelled ZnO NPs were prepared as well. Detailed physic chemical characterization was performed on the NPs to assess the influence of the labelling on the NP properties such as aggregation, size, charge, morphology and crystallinity, which can have a direct impact on the interaction of the NPs with cells and organs and in their toxicity.

Characterization of the structural properties of the commercial NPs and those fabricated by the consortium was a key aspect in this project. Surface and structural properties of NPs will be related to their toxicological effect and a strong effort will be made in understanding and relating to the characteristics of the materials the differences in toxicity, uptake or distribution among NPs of the same materials but from different sources.
Transmission Electron Microscopy (TEM) images (a, d), X-Ray diffractograms (b, e) and size distribution determined by Dynamic Light Scattering (DLS) (c, f) for Al₂O₃ nanoparticles (NPs) synthesized with [¹⁸O]H₂O before (a, b, c) and after (d, e, f) irradiation.

2) Single Photon Emission Computed Tomography (SPECT) and Positron Emission Tomography (PET) for the analysis of the uptake, distribution and release of NPs in vivo.

At the organism level, we did Positron Emission Tomography (PET) to study NP biodistribution and fate and organ inflammation. PET allows for the three dimensional mapping or imaging of organs and functional processes in the body through the detection of radioactive species. PET was used to directly follow the uptake, distribution and release of the NPs in animal models by different ingestion ways: topical, oral, intravenous, and inhalation. Whole-body analysis using direct imaging techniques of potentially toxic NP distribution and kinetics has been accomplished. The relative amount of NPs per organ following the different ingestion routes could be quantified by activity measurements. This data can bring fundamental information for risk assessment.

There is a profound lack of knowledge concerning the amount of NPs present in a cell for an applied NP dose. In other words, a quantitative relation between dose and uptake of NPs at both organ and cellular level is missing. The particle uptake depends on the activity of the cells, as well as the size, shape and physico-chemical properties of the nanomaterial. Therefore, the absence of dose-effect relationships represents a serious drawback for proper risk evaluation of special intracellular developed effects. At cellular level, the localization and quantification of metal and metal oxide NPs were performed by Ion Beam Microscopy (IBM), Electron Microscopy (EM), Confocal Raman Microscopy (CRM) and Confocal Laser Scanning Microscopy (CLSM or LSCM).

IBM is a unique and very powerful technique capable of localizing and quantifying these particles as well as performing elemental map distributions inside cells. It does not require particle labelling and relatively thick specimens can be investigated. The IBM technique is based on the targeting of a sample with high energetic ions (with approximately 2-3 MeV energy), which penetrate the targeted sample interacting with the electrons and nuclei present. This leads to an excitation of electron shells, which rearrange themselves under emission of electromagnetic radiation (X-rays and light).
PIXE elemental mapping of A549 cells treated during 72 h with various NPs: A – CeO2; Sulfur mapping is used for cell visualization.

Since the interaction processes depend on the encountered atoms, on the structure of the sample and on the sort and energy of the ions, the detection of secondary products of the interactions allows the determination of the elemental content and distribution in a sample. IBM has been successfully applied for the quantification of CeO2 and TiO2 NPs (Menzel et al., 2004). Ion mapping was performed in cells exposed to these NPs. From IBM data the amount of NPs per cell could be quantified obtaining relations between exposed dose and “real” intracellular dose. Since the IBM technique is time consuming, TEM and CLSM were used as supporting techniques. CLSM required the labelling of the NPs with fluorescent molecules. In most of the cases this is done at the surface of the NPs and can alter NPs properties. We applied CRM to visualize CeO2 and Al2O3 NPs in cells without labelling them. The NPs were detected in the cells measuring their own raman bands.

TEM images of H460 cells incubated with TiO2 NPs taken after 4, 12, 24, and 48 h incubation

IBM was also applied to image NPs in lung tissues of mice exposed to NPs in an inhalation studies.

4) Understanding the interaction of NPs with cellular and extra-cellular components.

For assessment of the fate and interaction of NPs in the organism, investigations of NP-protein interaction and stability of NPs in different biological compartments was carried out by biochemical methods focusing on measuring complementary agents and by means of binding studies with Fluorescence Correlation Spectroscopy (FCS). In FCS fluctuations in the fluorescence intensity from a confocal volume in a sample, which are caused by diffusional and rotational processes are measured and correlated temporally (Haustein et al., 2003). These results can be related to aggregation, association, polymer dynamics and, most importantly, in the proposed research, binding reactions. The technique has been successfully applied to measure binding constants and association of biomolecules. It has the advantage of only requiring very few fluorescent molecules in the confocal volume, and this can be applied in combination with CLSM to measure binding and association within cell compartments. The stability and corrosion of NPs is being investigated by biodissolution tests in an environmentally controlled, stirred batch reactor.

5) Determination of physiological effects of NPs in vitro.

It is a well-accepted hypothesis that reactive oxygen species may play an important role in particle-induced toxicity (Limbach et al., 2007; Xia et al. 2006; Sayes et al., 2006). Great differences in toxicity have been found between different oxide NPs, and in vitro studies suggest that the levels of toxicity may correlate to the reactive oxygen species (ROS) formation capacity of the NPs (Limbach et al., 2007; Jeng & Swanson, 2006; W. Lin et al. 2008), and also the release of ions. Comparatively more work has been completed on quantum dots, fullerenes and carbon nanotubes (CNTs) (Cui et al, 2004; Monteiro-Riviere et al, 2005; Lam et al, 2004; Maynard et al, 2004; Derfus et al, 2004; Kirchner et al, 2005; Hoshino et al, 2004; Schrand et al., 2007). Immune competent cells are specialized in the recognition of external factors in the skin, mucosa, blood, digestive and lung tissue, etc. They are also responsible for the subsequent production of signal molecules (cytokines), which activate other mechanisms of the immune defence system such as antibody production, macrophage activation, lymphocyte activation and proliferation. In addition, humoral factors such as complement, or acute phase proteins, participate actively in the inflammatory process and in the destruction of foreign elements. The subsequent changes in cell physiology induced by the presence of the NPs will have a tremendous impact on the induction and course of the immune reactions as a whole. For example, NPs can modify endogenous protein, inducing allergy processes, or induce macrophage activation in lungs, leading to a chronic inflammation with fibrosis (Lam et al., Crit Rev Toxicol. 2006). ATII cells are by far the most frequent cell in the alveolar lining. They are, among other functions, responsible for secretion and recycling of lung surfactant and a number of defence proteins.
Apoptosis and necrosis of A549 in presence of ZnO NPs. Cells were exposed to different NP concentrations (3, 10, 30 µg/ml) and incubation times: 6 h (A), 12 h (B), 24 h (C), 48 h (D) and 72 h (E).

6) Risk of exposure and toxicological effects of metal and metal oxide NPs.

The knowledge generated by the different workpackages of HINAMOX was used to make an assessment of the risks associated with these kinds of NPs following European standards suggested by the Scientific Committee on Emerging and Newly Identified Health Risks (SCENIHR, 2007). The data gathered in this project utilizing in vitro and in vivo models will be used to support integrated risk assessment. Integrated Risk Assessment Framework (IRA), as identified in the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH EC/1907/2006), was utilized.

3.2 Partners

HINAMOX project fully adheres to the European Recommendation of 07/02/2008 on a code of Conduct for Responsible Nanosciences and Nanotechnology Research. Furthermore, HINAMOX strongly identifies with the objectives of sustainability expressed by the Code of conduct that states that research activities in Nanotechnology and Nanosciences (N&N) must be safe, ethical and contribute to sustainable development. N&N research activities should not harm or create a biological, physical or moral threat to people, animals, plants or the environment at present or in the future. Therefore, HINAMOX will strive for the generation of a culture of responsibility and precaution to protect not only the researchers taking part in the project but also professionals, consumers, citizens and the environment that may get involved in the activities to be developed in the course of the research activities of HINAMOX.

The HINAMOX consortium is formed by eight different academic institutions and companies located in Europe, Asia and Latin America. The consortium blends a wide range of expertise ranging from the synthetic skills and the physical characterization to the bioimaging, including molecular biology, immunology and microscopy.

CIC biomaGUNE is a non-profit research organization created to promote scientific research and technological innovation at the highest levels in Spain, in order to help strengthen and further develop the new business sector based on biosciences in the country. CIC biomaGUNE blends a unique mixture of expertise. The institute combines synthetic chemistry, material science, physical and biophysical characterization, with in vivo and in vitro imaging. CIC biomaGUNE is endowed with a cyclotron, radiochemistry labs for hot chemistry and animal PET, SPECT and MRI cameras. State of the art techniques for material characterization are presented in the institute such as TEM, SEM, NMR, Raman, FITR, light scattering, etc. Among the different research lines in the institute there is a compromise to develop nanomedicine tools and to become a leading institution in in vivo studies concerning nanotechnology. CIC biomaGUNE was the coordinating partner in the project, its role is the characterization of commercial NPs, the synthesis and characterization of radio and fluorescently labelled NPs and “in vivo” studies with animal models.

The University of Vigo (UVIGO) is a recent University (1990) located in Galicia (Northwest Spain), covering many educational disciplines including Biology, Chemistry, Engineering, Law, Economics, etc. The Immunology Area was set up on 1996 in the Department of Biochemistry, Genetic and Immunology, and brought in researchers with experience in Medical Immunology and Biology. Since then, UVIGO has developed and trained scientists with experience in basic and applied Immunology, development of monoclonal antibodies and immune responses to vaccines. The role of the University of Vigo in HINAMOX was to study the cytotoxicity of NPs in different human cell lines (lymphoid, lung origin), interaction of nanomaterials with complement and serum proteins, effect of sterility and immunogenicity of nanomaterials in vitro and in vivo.

The University of Leipzig is one of the largest and oldest universities in Germany, covering all educational disciplines. The proposed research work will be conducted in close collaboration with two different faculties (Physics and Medicine), and in three different departments or Institutes.

1) Institute of Medical Physics and Biophysics: The institute has its focus on membrane and cell biophysics for medical applications.

2) Institute for Experimental Physics II, Division of Nuclear Solid State Physics: The focus of the research of the accelerator laboratory, using the high energy ion nanoprobe Lipson, is on spatially resolved quantitative trace element analysis in neuroscience, cell biology and in elemental analysis of natural and artificial micro- and nano-structures and ion beam modification of materials with sub-micrometer resolution.

3) Medical Hospital, Department of Pneumology: The department is responsible for the in-patient treatment of severe pulmonary disorders such as asthma, pneumonia or lung carcinoma. In parallel, clinical research is carried out to improve the treatment of pneumological disorders.

The role of the University of Leipzig in the project was the quantification of NPs in cells by means of IBM techniques and FCS, and studies of the lung function in presence of NPs, uptake and immunological response of lung cell lines under different breathing regimes.

PlasmaChem GmbH is an SME with research facilities dedicated to the development, production and sales of medical devices, analytical equipment and nanomaterials and their formulations. PlasmaChem GmbH was founded at 1993 in Mainz. In 2005 the company moved to Berlin. The main area of the company concerns nano-materials, detonation-, vacuum-, plasma- and ultra-thin film technologies and their biomedical and technical applications. The main technology focus of PlasmaChem concerns the development of processes, induced by low temperature plasma on different surfaces, in atomically flat, inorganic solids and in liquid interfaces. The important business line of PlasmaChem GmbH is production and sale of new industrial products - Nanopowders (NanoDiamonds, NanoCeramics, NanoMetals and composite Nano-particles - Nano-capsules). In 2005 PlasmaChem launched the world's first General Catalogue on Nanomaterials and related products. PlasmaChem performs chemical and low temperature plasma modification of nanopowders, with the purpose of functionalizing the nano-particle's surface with assistance from a new plasma chemical method developed by PlasmaChem for ultra-dispersed materials. Along with new nano-particles, PlasmaChem develops new, ready-to-use industrial nano-products like nano-
abrasives, additives to engine oils and composite nano-suspensions for electroplating and electroless-plating of metals. The role of PlasmaChem in HINAMOX was the design, fabrication and scale up of NPs.

**National Research Centre for the Working Environment (NRCWE)** is a Danish governmental research institute in the field of occupational health and safety under the Ministry of Employment. NRCWE’s goal is to generate and disseminate knowledge contributing to a safe, healthy and developing work environment in accordance with the technical and social development of the Danish society. NRCWE contributes to securing the coordination of Danish work environment research and monitors national and international work environment development and research. The knowledge is disseminated via NRCWE’s Working Environment Information Centre. Health risks from occupational exposure to NPs is one of NRCWE’s seven strategic research areas. The role of NRCWE in HINAMOX was the study of biodurability of NPs, genotoxicity, exposure of NPs and risk assessment.

**Centro de Investigaciones Químicas Aplicadas** (CIQA) is one of 27 Mexican public research institutions covering the major fields of scientific and technological knowledge funded by the Consejo Nacional de Ciencia y Tecnología (CONACYT). CIQA’s focus is on research and development in polymers and advanced materials and has a full time faculty of about 45, 70 technicians and 120 PhD and MSc students. CIQA has broad expertise in new materials synthesis and characterization including nanoscale structures and metamaterials, and in polymer synthesis, processing and engineering. Sate-of-the-art techniques for material characterization at CIQA include TEM, SEM, SQUID magnetometry, magnetoelectric capability and ISO-9000 facilities.

The role of CIQA in the project was to support in the design routes for NPs to be suitable of being labelled and the application of High Resolution TEM for characterization.

**Zhejiang University** is located in Hangzhou, Zhejiang Province, China. It was initially founded in 1897, and is the third oldest University in China. It has 5 campuses and occupies a total area of 518 hectares. It is a key comprehensive university whose fields of study cover eleven branches of learning, namely philosophy, literature, history, education, science, economics, law, management, engineering, agriculture and medicine. The university now has 112 specialties for undergraduate studies, and it is entitled to confer Masters’ degrees in 317 programs and Doctoral degrees in 283 programs. Under its administration, there are 14 Key National Laboratories, 2 National Engineering Research Centres and 3 National Engineering Technology Centres.

The role of Zhejiang University in the project was the study of the cellular uptake and distribution of NPs by means of TEM and CLSM.

**The role of FIOH** in HINAMOX was the integration of the knowledge generated in HINAMOX in a final risk assessment.

### 3.3 Dissemination activities

HINAMOX has participated in several international events related to its research topic, mainly workshops and conferences. Concerning publications, a list of journals, magazines and newspapers pertaining to the nanotoxicology subject has been identified and over a dozen publications have already been published. Both events and publications are an effective way to widely disseminate the project results among the appropriate communities.

In particular, the following conferences are of special interest:

- At **NanoBioMed 2011**, several of the HINAMOX partners had the opportunity of presenting their work: University of Vigo, University of Leipzig and CICbiomaGUNE. HINAMOX organized a seminar targeted to industrial stakeholders who are now disseminating on scientific and legal issues related to the toxicity of nanomaterials.

- The **Safety Issues of Nanomaterials along their Life Cycle** conference in Barcelona was co-organised with other two consortia, NANOPOLYTOX and NEPHH.

- The second edition of the conference **Safety Issues of Nanomaterials along their Life Cycle** (Nanolca II) in San Sebastian, co-organized with NANOPOLYTOX. NEPHH, EMPPRA and the Joint Research Center.

HINAMOX participates actively in the **EU NanoSafety Cluster** and the HINAMOX project contributes to the joint newsletter by sharing its findings. All events organised by HINAMOX are communicated to EU NanoSafety Cluster who kindly help with their dissemination. HINAMOX contributes this way to create an environment to foster discussion, exchange ideas and common activities together with other groups and European projects in the field of nanotoxicology.

### 3.4 Citations


Hoshino, A., Fujioka, K., Oku, T., Suga, M., Sasaki, Y.F., Ohta, T., Yasuhara, M., Yamamoto, K. “Physicochemical properties and


4 Directory

Table 1 Directory of people involved in this project:

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## 5 Copyright

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HINAMOX is a research project under the European Commission's 7th Framework Programme.

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

Contract Agreement: NMP4-SE-2012-280550 Website: www.instant-nps.eu
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Contents
1 Summary ..................................................................................... 45
2 Background ................................................................................ 46
3 What is INSTANT ........................................................................ 46
3.1 Summary of INSTANT’s key strengths ................................... 46
4 Organization of INSTANT ........................................................... 46
5 INSTANT Events and Reports ..................................................... 46

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, SiO2, TiO2, 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. 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, 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
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

<table>
<thead>
<tr>
<th>WP</th>
<th>Title</th>
<th>Topic</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Sensor development</td>
<td>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.</td>
</tr>
<tr>
<td>2</td>
<td>Sample preparation and standard materials</td>
<td>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.</td>
</tr>
<tr>
<td>3</td>
<td>Recognition elements</td>
<td>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.</td>
</tr>
<tr>
<td>4</td>
<td>Chemometrics and experimental design</td>
<td>WP 4 deals with data mining for extracting minute signals from the complex and probably noisy measurement data obtained by the sensor system(s).</td>
</tr>
<tr>
<td>5</td>
<td>System integration</td>
<td>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).</td>
</tr>
<tr>
<td>6</td>
<td>Management</td>
<td>This work package coordinates the overall financial, administrative, legal and contractual management of INSTANT.</td>
</tr>
<tr>
<td>7</td>
<td>Dissemination and exploitation of results</td>
<td>Dissemination activities beyond the consortium to the scientific community and towards a wider international audience through specialized events like conferences, fairs and exhibitions.</td>
</tr>
</tbody>
</table>

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 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. SMART-NANO, QNano). This will draw the attention from customers to the INSTANT technology and their possibilities.

### 5.1.2 Public midterm seminar

The midterm seminar will disseminate the results of the INSTANT project to an enlarged number of scientists in the field of sensor technology, nanotechnology and separation sciences, including representatives from other EU projects.

## 6 Directory

Table 2 Directory of people involved in this project.

<table>
<thead>
<tr>
<th>First Name</th>
<th>Last Name</th>
<th>Affiliation</th>
<th>Address</th>
<th>e-mail</th>
</tr>
</thead>
<tbody>
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</tr>
</tbody>
</table>
7 Copyright

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ITS-NANO

Intelligent testing strategies for engineered nanomaterials

Project Number: 290589  Website: http://www.its-nano.eu
Coordinator: Vicki Stone, Heriot-Watt University, Edinburgh, United Kingdom

<table>
<thead>
<tr>
<th>No.</th>
<th>Beneficiary name</th>
<th>Short name</th>
<th>Country</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Heriot-Watt University</td>
<td>HWU</td>
<td>United Kingdom</td>
</tr>
<tr>
<td>2</td>
<td>Veneto Nanotech SCPA</td>
<td>VN</td>
<td>Italy</td>
</tr>
<tr>
<td>3</td>
<td>Aarhus University</td>
<td>AU</td>
<td>Denmark</td>
</tr>
<tr>
<td>4</td>
<td>Fondazione Istituto Italiano di Tecnologia</td>
<td>IIT</td>
<td>Italy</td>
</tr>
<tr>
<td>5</td>
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<td>CRF</td>
<td>Italy</td>
</tr>
<tr>
<td>6</td>
<td>Fraunhofer-Gesellschaft zur Foerderung der Angewandten Forschung E.V</td>
<td>IME</td>
<td>Germany</td>
</tr>
<tr>
<td>7</td>
<td>Institute of Occupational Medicine</td>
<td>IOM</td>
<td>United Kingdom</td>
</tr>
<tr>
<td>8</td>
<td>Det Nationale Forskningscenter Forarbejdsmiljo</td>
<td>NRCWE</td>
<td>Denmark</td>
</tr>
<tr>
<td>9</td>
<td>JRC – Joint Research Centre – European Commission</td>
<td>JRC</td>
<td>Transnational (EU)</td>
</tr>
<tr>
<td>10</td>
<td>European Research Services gmbh</td>
<td>ERS</td>
<td>Germany</td>
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</table>

Contents

1 Summary ........................................................................................................ 51
2 Background................................................................................................... 52
3 What is ITS-NANO........................................................................................ 52
3.1 Summary of ITS-NANO key expected impacts........................................ 53
4 Organisation of ITS-NANO ........................................................................... 53
5 Activities of ITS-NANO ............................................................................... 54
5.1 Intelligent approaches to grouping of nanomaterials based on their properties and their subsequent biological impacts in order to intelligently design next-generation nanosafety evaluation and risk assessment strategies .................................................. 54
5.2 Quick screening and identification of high risk materials, and implementation of strategies to counter these risks ..................... 54
5.3 Increased integration and advancement of EU policy on nanosafety evaluation and communication............................................. 54
5.4 Meetings, consultations and workshop ................................................. 55
6 Directory ...................................................................................................... 55
7 Copyright ..................................................................................................... 56

1 Summary

The background, concept and objectives of ITS-NANO are straightforward. The volume of information on hazard characterisation of ENM is increasing fast. In parallel with the scientific development, regulation orientated initiatives are also taking place to identify needs.

The ITS-NANO concept is 1: Gather scientific evidence by communication with leading scientists. 2: Develop an initial assessment (document) of the available knowledge, focussed on an intelligent approach to grouping ENMs based on their properties and on their subsequent biological impacts in order to intelligently design next-generation nanosafety evaluation and risk assessment strategies (www.its-nano.eu/the-project/project-output). 3: Assemble stakeholders for a dialog on how this relates to their aims/needs and how to make a consent-driven strategy. 4: Revise the initial assessment document with the input from the stakeholder sent around for commenting, presenting the next draft for a smaller group for final commenting. 5: Publish it

The work focuses on the following areas: Human toxicology, ecotoxicology and risk assessment. The project will last fifteen months, and organize two events to discuss the development of the testing strategy with the researchers and the stakeholders dealing with the issues. After these workshops, and after online consultation with the widest community, a roll-out event will be organized to present project results to all key stakeholders, including industry, legislators, and funding agencies, and to raise awareness in the media.
2 Background

The volume of information on hazard characterisation of ENM is increasing fast. In parallel with the scientific development in toxicology and eco-toxicology, regulation orientated initiatives are also taking place at the international as well as European and nation state levels. The main initiatives are the OECD Sponsorship Programme for the testing of Nanomaterials, the ISO classification of nanomaterials, and the REACH guidance on regulatory safety testing for chemicals, which is to be updated for ENM. Together, all these sources of information form the state-of-the-art landscape of the safety testing of ENM. The ITS-NANO concept is to gather scientific evidence and assemble representative stakeholders for the consent on a research strategy for rational grouping of ENM and a risk assessment approach for ENM.

Moreover, the framework for future research aiming at grouping ENM according to (i) their physico-characteristics and (ii) specific endpoints will be based on the nanosafety landscape above and specifically on the knowledge gaps that need to be bridged in order for such rational grouping to be made. However, as expected, there will be a considerable knowledge gap. Therefore it is essential to narrow this gap. Of course, with all the time and funding available, this could be eventually be achieved. However, time is critical and funding is restricted, so a rationale is needed for future research. This rationale will reflect the need for different types of evidence by industry, regulators (e.g. OECD WPNM, REACH) and scientists to prioritise the process for information generation (i.e. (eco)-toxicology tests) accordingly. This is why it is an ‘Intelligent Testing Strategy’. Our approach is also ‘intelligent’ at the testing level because we will identify:

- a battery of in vitro (eco)-toxicology tests with predictive power
- in vivo tests only where it is essential (in respect of the 3R principle).
- A risk assessment strategy is only effective if the key stakeholders (e.g. food industry, nano-material manufacturers, pharma- and health-related industry) approve and adopt it. This will be achieved by integrating, engaging them in the very shaping and development of the strategy.

3 What is ITS-NANO

The ITS-NANO consortium consists of 11 partners from 4 European Countries (Italy, United Kingdom, Denmark and Germany) and the Joint Research Centre of the European Commission; it includes industries, renowned universities, research centres of excellence, and a consultancy firm to provide a management structure in line with the needs of the European Commission.

The ITS-NANO consortium consists of experts in nanotechnology, nanotoxicology, nanocotoxicology, and risk assessment, from academia and industries with considerable experiences in collaborative research in FP6 and 7 NMP programme. Some of the collaborators are also members of the OECD Working Party on Manufactured Nanomaterials (WPNM), ISO-TC229, and are contributors to the review and extension of REACH for nanomaterials as well as other National initiatives. Project partners has a direct access to state-of-the-art knowledge, being involved in several initiatives already ongoing and about to start, including the two large NMP research projects MARINA and NanoValid. Finally, sustainable industrial implementation themes are also represented, due to the presence of industry, technology transfer experts but also occupational health research institutes.

ITS-NANO will receive further inputs from the integration of the most relevant stakeholders, regulatory bodies, policy makers, and industries, being the latter both the most significant actors of nanotechnology R&D at the moment, but also the end-users. Integrating such competences in the project, and also networking and interacting with the wide scientific community represented by the NanoSafetyCluster and by selected experts in the United States, will provide the consortium with a clear understanding of which are the priorities to be addressed in the future, according both to stakeholders’ needs, but also identifying the direction in which nanotechnology development and transfer is moving towards.

ITS-NANO first objective is to create a scientific basis for ensuring the safe and responsible development of manufactured nanoparticles and nanotechnology-based materials and products, and to support the determination of regulatory measures and implementation of legislation in Europe. Current (eco-) toxicological approaches to assessing nano-material hazard are based either on classical toxicology approaches or on novel multiplexed assays. These approaches do not provide a comprehensive assessment due to the many unique aspects of nanomaterials, such as the transport mechanisms (in the body and within cells) and, in particular, the relationship between the physico-chemical properties of the nanoparticles with:

1. the biological identity in situ (i.e. in various culture media);
2. the fate and behaviour (uptake, translocation, localisation);
3. the functional impacts at system and cellular level

Thus, new (eco)-toxicology approaches, which consider and exploit these unique aspects of engineered nanoparticles, are urgently needed. Specifically, an Intelligent Testing Strategy for Engineered Nano-Materials is required. This strategy is ‘intelligent’ both at the strategic level by identifying and setting a priority research agenda to reduce the research gaps according to the need of the stakeholders (industry, regulators) and at the tactical level, to be economical and ethical. This is the aim of the ITS-NANO proposal.

Specifically, our objectives are to develop:

- A framework for future research aiming at rational grouping, through well standardized methods, of engineered nanomaterials (ENM) according to their i) physical, ii) chemical, iii) biological characteristics.
- A framework for future research aiming at specific grouping of ENM according to the specific health risk they present towards the immunological, respiratory, reproductive, circulatory, etc... systems.
- A strategy to increase the integration among stakeholders (food industry, nano-material manufacturers, pharma- and health-related industry) for a shared, agreed-upon risk assessment strategy and
approach to conveying the appropriate, evidence-based information to the public.

3.1 Summary of ITS-NANO key expected impacts

- An integrated and agreed upon research strategy to address priority needs of key stakeholders.
- The integration of key stakeholders into the decision making process towards the definition of the Intelligent Testing Strategy.
- The definition of a reliable methodology for exposure assessment, hazard identification and risk assessment applicable in future research, speeding up the reliable generation of new knowledge.
- The promotion of innovative methodologies and concepts, to help in the clarification of molecular mechanisms involved in interactions between nanomaterials and biological systems.

4 Organisation of ITS-NANO

The overall goal of ITS-NANO is to bring together top specialists in nanoscience and technology, nanotoxicology, ecotoxicology, and environmental impact and risk assessment, from various countries of Europe (Italy, United Kingdom, Denmark, and Germany), and to take advantage of the existing broad European cultural wealth and variety to find best solutions for an integrated testing strategy to promote sustainable development of this increasingly growing economic sector.

ITS-NANO has a strong commitment to communication and openness, and will actively operate to invite all researchers and stakeholders to participate in the project activities. For this purpose, different layers of participation are foreseen: a small group of experts (the project partners) will identify key areas and knowledge gaps to be addressed by the Intelligent Testing Strategy, and will organise workshops with a core group of invited stakeholders (the Core Stakeholder Panel) to develop the initial strategy, which will be eventually evaluated and integrated by the widest scientific community of the NanoSafetyCluster, and other interest groups who wish to collaborate with the project.

The ITS-NANO work plan is broken down into four inter-related and interconnected work packages (WPs, see table below). Interaction and communication between the WPs is guaranteed by a network of interest groups who wish to collaborate with the project. In all work packages, existing data is taken into consideration and every attempt is made to include results and contributions from other ongoing projects.

Table 1 Workpackages (WP) of ITS-NANO

<table>
<thead>
<tr>
<th>WP</th>
<th>Title</th>
<th>Topic</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Project Management</td>
<td>This workpackage deals with the technical, financial, and administrative management. It supports the timely and efficient implementation of the work plan.</td>
</tr>
<tr>
<td>2</td>
<td>Goal-setting for Toxicology, Eco-Toxicology and Risk Assessment of ENM</td>
<td>This workpackage will identify and set the strategic goals for (eco)-toxicology testing and Risk Assessment of ENM. The experts represented in the consortium will use their knowledge and other information coming from other projects and reports, including the European NanoSafetyVision for 2015-2020, to identify which are the goals that need to be achieved, also integrating them with the foreseen of the potential development both in nanotechnology (to identify future changes/trends that will impact upon the types of human and environmental exposures that are likely to occur), and in the developments in biological and environmental sciences that can impact on how nano(eco)tox research would be performed. The results of WP2 are available at the project website, <a href="http://www.its-nano.eu/the-project/project-output">www.its-nano.eu/the-project/project-output</a>.</td>
</tr>
<tr>
<td>3</td>
<td>Intelligent testing strategy design</td>
<td>This workpackage aims to develop a structured research framework. The integrated test strategy will be developed starting from the work performed in WP2. Emphasis will be put on ensuring the inputs coming from the stakeholders contacted in the development of the testing strategy. The strategy will: i) be tailored towards the rational grouping of nanomaterials according to physico-chemical characteristics and common biological descriptors; ii) prioritize future research according to the necessity of the most important stakeholders, namely regulatory bodies and industries, and to foster the acceleration of the sustainable transfer of nanotechnology innovation from research to market; iii) propose the adoption of novel concepts and methods into nanotoxicology research, in particular fostering the adoption of innovative technologies and approaches into (eco)toxicological testing and risk assessment.</td>
</tr>
<tr>
<td>4</td>
<td>Integration of Stakeholders and Dissemination</td>
<td>This workpackage aims to maximise the communication and the integration of all the stakeholders, by: i) establishing a Core Stakeholder Panel, which will work in close collaboration with the project partners to define research priorities; ii) organising a series of workshops, international conference, and roll-out event, to promote the dissemination of project results, and to stimulate the wider scientific communities to provide a constructive feedback to project activities; iii) setting up an online consultation to promote the transfer of opinions, suggestions, and feedback from the widest range of stakeholders; iv) effectively disseminating project results and achievements, publishing newsletters, reports, articles on scientific journals.</td>
</tr>
</tbody>
</table>
5 Activities of ITS-NANO

5.1 Intelligent approaches to grouping of nanomaterials based on their properties and their subsequent biological impacts in order to intelligently design next-generation nanosafety evaluation and risk assessment strategies

While much of the function of nanomaterials is due to their core structure, the surface properties define much of their bioactivity, as it influences their interaction with the environment or the host organism. Several parameters may be used to describe the physicochemical characteristics of nanomaterials, some of them being conventional descriptors already used for chemicals (chemistry, size, size distribution, surface charge, water/octanol partition coefficient, etc...), whilst others becoming more important when considering engineered nanomaterials, like surface area, presence of nano-pores, absorption on different molecules (including biomolecules) on the surface, etc.

These factors influence strongly the interactions of the particles with the environment and exposed organisms, as they are responsible for events such as chemical reactions that might occur on the surface, for the compartmentalisation in different environmental matrices, for the altered binding of different biological molecules, and for the possibility to cross biological barriers. Such events that may, indeed, cause unwanted toxic effects. Only a few studies have been performed to quantitatively correlate such characteristics with possible biological activities (Gurello and Worth, 2010), the reasons being the lack of standard in vitro methods that univocally assign a precise toxicity value to the tested nanomaterials, the deficient knowledge regarding the mechanisms of action of these materials and the difficulty to mathematically describe a system which is too large as well as too complex for accurate calculations. The Delphi approach that ITS-NANO is going to develop will provide a first mean to elucidate a selection of physicochemical characteristics for which there is relatively clear evidence of impacts upon a series of exposure, toxicology and ecotoxicology endpoints. The first outcome of this Delphi consultation will be the creation of functional groups of particles according to their physicochemical characteristics that can eventually lead to given (eco)toxicological endpoints, but also to move in the opposite direction, clearly associating groups of endpoints to physicochemical characteristics.

Furthermore, this rational grouping will have a direct impact to the definition of the techniques used in laboratory research. It is known (See OECD ENV/JM/MONO(2010)46, 01.12.2010) that several nanomaterials can interact with reagents used to assess their effect during laboratory assays; defining group of particles according their physicochemical characteristics, and associating such characteristics also with the experimental bias scientist may incur in when using tests that have been shown to be inappropriate for given groups, will avoid the production of biased results.

5.2 Quick screening and identification of high risk materials, and implementation of strategies to counter these risks

Rational grouping of nanoparticles and nanomaterials can be itself a way of screening high risk materials. In fact, functional groups of physicochemical characteristics may be associated to strong toxic behaviours, or to extreme likelihood of exposure in given circumstances. When these associations will become evident through our state-of-the-art analysis, the intelligent testing strategy representing the core output of ITS-NANO will be developed in order to contain a optimised set of tests to be used in rapid screening of toxic potential.

Through this strategy, it will also be possible to reduce risks associated to physico-chemical characteristics of nanomaterials. This strategy, in fact, can be transferred from the identification of risks to the design phase, suggesting to avoid to develop of nanomaterials with characteristics that can give them potential risks in term of release in the environment, human and ecotoxicity, or promoting the incorporation of chemical features that have been shown to be safer.

5.3 Increased integration and advancement of EU policy on nanosafety evaluation and communication

Research performed so far, based on a basic research approach, performed on pure commercial nanoparticles, often poorly characterised, has generated a significant amount of data, but this results obtained are now difficult to compare due to the absence on information of physicochemical characteristics. Furthermore, data achieved to date, do not represent a significant mean of predicting the actual behaviour of nanomaterials in the environment and when they will come in contact with humans, as in several cases they lack of the consideration and the assessment of which modifications occur during the whole life cycle of the product. These uncertainties are reflected in the general absence of a specific regulation for the use of nanomaterials in general applications. In fact, on November 20th, 2009, the European Union (EU) Council approved an updated cosmetics regulation that would require cosmetic products that contain nanoscale ingredients to be labeled as such. Concerning food applications of nanomaterials, the European Commission has explicitly stated that specific methods to assess the risk are developed, no food with nanomaterials should be put on the EU market, and is committed to only approve the marketing of food containing nanomaterials for which the food safety has been established. ITS-NANO will address this critical issue in nanotechnology risk assessment proposing an intelligent testing strategy that will try to respond to such relevant points. The first point that will be developed of this project is in fact the integration of the most relevant stakeholders, regulatory bodies, policy makers, and industries, being the latter both the most significant actors of nanotechnology R&D and at the moment, but also the end-users. Integrating such competences in the project will provide scientists with a clear understanding of which are the priorities to be addressed in the future, according both to stakeholders’ needs, but also identifying the direction in which nanotechnology development and transfer is moving towards. To understand this point, it is crucial to involve industries
in the discussion. The integration of scientific excellence, industrial and regulators’ needs, within the ITS-NANO consortium will set the backbone to design an prioritised research strategy, to be implemented in future research projects, but also in the EU projects already started and in which project partners are already actively participating. This research strategy will necessarily highlight which are the key issues to be addressed as a first priority, to help industries in designing safer particles for their application, to provide the regulatory bodies the mean to protect workers and consumers, but also to address the fears, justified or not, of the society, since we have already witnessed how a strong contrary reaction of the public opinion can block the possible development of promising technologies. Indeed, facilitating the understanding of the relevant toxicity mechanisms of nanomaterials will also benefit the possibility to respond to the safety concerns of the general public, as providing scientific dissemination agencies significant scientific data, achieved according to strategies and protocols agreed upon the independent scientific community.

5.4 Meetings, consultations and workshop

Firstly, the consortium will draft a concise set of goals for the strategy, based on the current state-of-the-art. This will be a conceptual draft, and the consortium will rely on its inherent expertise.

After 6 months this first draft will be discussed with a small panel of top-level experts (the Core Stakeholders Panel, to be defined as the project starts) in a focused workshop. This workshop aims to significant improvement of the first draft, of which an evolved version will be published for an open online consultation, starting month 9. This draft will include approaches to testing strategies, already.

Using an online consultation ITS-NANO will reach out to a much wider audience. Further, this way it will be possible collect opinions of certain stakeholders, including experts on the public perception to innovative technologies. While these positions may not add much in way of scientific facts, they are very important to make the researchers aware of present anxieties and fears, that need to be dealt with. Where possible the feedback from the online consultation will be classified using the scheme developed by Klimisch et al., which is used widely in OECD initiatives.

In month 12 ITS-NANO will organise a major international conference, to discuss a second draft, which includes conclusions of the online consultation. This event is the key element of our consultation procedure. The expert-panel members of our 6-month Workshop will chair sessions of the conference. In addition there will be high-level co-chairs representing the various stakeholder groups. This will in particular serve to integrate industry, legislators, and funding bodies strongly. The proceedings of this event will lay the foundation for an ongoing dialogue, and we will seek opportunities to secure an ongoing funding for such conference, after the project. Details of the meeting at the ITS-NANO website home page, www.its-nano.eu.

3 months after this conference, results will be presented to all key stakeholders: scientific community, industry, legislators, and funding agencies, in a roll-out event.

6 Directory

Table 2 Directory of people involved in this project.

<table>
<thead>
<tr>
<th>First Name</th>
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<th>Affiliation</th>
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</table>

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LICARA

Life cycle approach and human risk impact assessment, product stewardship and stakeholder risk/benefit communication of nanomaterials

Contract Agreement: 315494  Website: www.licara.eu
Coordinator: Esther Zondervan, TNO, Zeist, The Netherlands

<table>
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<td>Verein Mikro- und Nanotechnologie EUREgio Bodensee</td>
<td>NCB</td>
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<td>5</td>
<td>Freso technical-solutions</td>
<td>FRESO</td>
<td>Switzerland</td>
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<td>6</td>
<td>Research and Development of Carbon nanotubes S.A.</td>
<td>Nanothinx</td>
<td>Greece</td>
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<td>Eidgenoessische Materialprüfungs- und Forschungsanstalt</td>
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<td>Rent-A-Scientist</td>
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<td>9</td>
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Contents

1 Summary ..................................................................................... 57
2 Background ................................................................................ 57
3 What is LICARA? ......................................................................... 58
  3.1 Summary of LICARA’s key strengths ...................................... 58
4 Organization of LICARA ............................................................. 58
5 LICARA Events and Reports ...................................................... 59
  5.1.1 Workshops ........................................................................ 59
6 Directory ..................................................................................... 60
7 Copyright .................................................................................... 60

1 Summary

Project Duration: 1 October 2012 – 30 September 2014
Project Funding: 2,595,744.20 Mio. EUR

Nanomaterials have a great market potential for SMEs due to the high added values and the reduced batch sizes compared to their corresponding conventional bulk materials. Unfortunately the introduction of nanomaterials is hampered due to the unknown human and ecological risks. It may take many years to fill all knowledge gaps. However, the SMEs have to address the various different aspects and perceptions of risks, in communication with the various stakeholders. For this reason, SMEs need guidance to assess the risks and the benefits of their nanoproducts in comparison with the conventional (non-nano) products. This structured approach will be the base for a completely new service available to SMEs working with nanomaterials.

As proof of principle and concrete benefit for the SMEs, LICARA will deliver guidelines to the members of the SME Associations to support them in their communication with regulators, clients and investors and to improve the production processes and/or applications of their specific nanoproducts.

2 Background

The production of nanomaterials and nanoproducts is extremely attractive for SMEs. Firstly, the innovative functions of the product do have a high added value to the products, so SMEs can compete with large industry. Secondly, SMEs do not need much logistic infrastructure for handling large volumes of material, as the batch-
size of nanomaterials is normally only up to 10% of that of the corresponding conventional bulk materials.

A key issue hampering the implementation of nanomaterials is unknown human and ecological risks of the applied nanomaterials during their life cycle. Many initiatives have been launched to obtain data about the safety of nanomaterials. However, it will take many years to gather and analyse all the data required to perform a comprehensive risk assessment for nanomaterials. From the point of view of the life cycle assessment framework, the lack of reliable inventory data prevents so far even the calculation of their production impact on “traditional issues” like e.g. depletion of abiotic resources, ozone depletion potential, use of fresh water and land, or the impact on global warming. When considering on toxicological issues (like ecotoxicity or human toxicity), the lack is even more important – as also the respective characterisation factors for such an assessment are missing. In several cases the application of new substances without a proper prior risk assessment has led to unexpected costs and considerable suffering for industry and society (e.g. polychlorinated biphenyls (PCB), asbestos (EEA 2002, EU 2001)). On the other hand, the introduction of new material, e.g. nanomaterials, and applications, might have less impact on for example energy consumption or use of raw materials compared to other materials or product systems with comparable functionality. In communication with the various stakeholders, e.g. regulators, non-governmental organisations (NGOs), trade organisations etc., the SMEs have to address the various different aspects and perceptions of risks. For this reason, SMEs do need guidance to assess the risk and benefits of their nanoproducts in comparison with the risks and benefits of conventional (non-nano) products.

3 What is LICARA?

In LICARA, we will develop a life cycle approach and perform case studies to balance the risks and the benefits in the context of scarce data that contributes to the generation of solid guidelines for SMEs for specific products, despite scarcity of data (in the coming 10 years this can be assumed to be the case for nanomaterials!). This life cycle approach will be transferable to all kinds of nanomaterials and product systems. To do so, we will develop a new approach how to deal in a Life Cycle Assessment (LCA) with scarce life cycle inventory (LCI) data.

In specific case studies related to the nanoproducts of the involved SMEs, LCI data sets will be elaborated with the support of these SMEs as well as the involved SME-AGs. These LCA case studies serve to illustrate realistic environmental performance and the potential environmental benefits of nanoproducts and will indicate the priorities for information gathering / data gathering.

A challenge with respect to human risk assessment is to integrate or aggregate (1) the risks associated with the release of nanomaterials in (indoor) work environments or indoor use of nanoproducts (for specific subgroups like workers and consumer) and (2) the risks associated with emission (or release) to various environmental compartments for the general population. Pragmatic approaches for such aggregation of risks will be developed and applied for the case studies, indicated above.

In a further project step, the outcomes of the LCA and the Risk Assessment (RA) activities will be combined, resulting in this new life cycle approach mentioned above in which the risks and benefits can be balanced. Based on this balance the discussions between various stakeholders, e.g. governments, industry, NGOs, and research institutes about nanomaterials could be supported.

Finally, in a last step, guidelines and if possible “best practices” will be deduced from this life cycle approach in order to enable SMEs, in the project and beyond the project, to develop and produce safe and sustainable products by gathering relevant information. This information supports SMEs in business decisions on the production or application of nanomaterials. Our results will show under which conditions the use of nanomaterials have a favourable risk/benefit balance and what information is relevant to evaluate the risks and benefits (i.e. priorities for information gathering/data generation). A risk/benefit balance is a must for SMEs in communication with investors, clients and regulators. In addition, SMEs are committed to take a proactive role in informing the society about the risks and benefits of nanomaterials and to use the nanomaterials in a sustainable way.

With SMEs making up for 18% of the innovation potential of Europe, the support of SMEs at the early stages of a product development cycle is of utmost importance. The SME participating in this project foresee an increase in turnover in medium or long term. The member companies of the SME-Associations expect to establish commercial advantages from the conduct and results of the LICARA project through the achievements:

1. Provision of case-studies in an iterative format, allowing for the detailed review of product- and SME-specific data by the world-leading experts in LCA involved in this consortium

3.1 Summary of LICARA’s key strengths

- Development of a framework for life cycle assessment which properly addresses risks in data scarce situations.
- Application of the life cycle approach in case studies for 2 different types of nanomaterials in various applications.
- Compile guidelines for SMEs for nano-product risk-benefit balancing.
- Dissemination of the developed conceptual framework, the case studies and guidelines to SMEs.

4 Organization of LICARA

LICARA merges high ranked European partners with knowledge in the life cycle assessment, human risk assessment, environmental risk assessment and economic impact assessment. The three types of partners within LICARA are:

1. SMEs: FRESO (CH) and Nanothinx (GR). Both are companies that manufacture nanomaterial-based product systems. Their role in the project will be focused on providing the needed information and data for the risk-benefit analysis as aimed by the project.
2. SME Associations (SME-AG). One international SME-AG (NIA), and three national SME AGs, i.e. NIA–UK (UK), NCB (CH),
and SNT (SE). The main focus of the SME AGs in LICARA will be to link SME interests to research needs and the dissemination of the project results, i.e. guidelines for SMEs, to their members and their networks.

3. RTD performers, TNO (NL), EMPA (CH), and RAS (DE). These partners are needed to do the research on behalf of the SMEs and the SME AGs. TNO and EMPA have substantial expertise and capabilities with respect to life cycle analysis and risk assessment (TNO focusses on human health risks and EMPA on environmental health risks), which form the basic research parts of the project. RAS will focus on the efficacy testing to demonstrate benefits of nanomaterial-based product systems.

### Table 1 Work packages (WP) of LICARA

<table>
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<tr>
<th>WP</th>
<th>Title</th>
<th>Topic</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Management</td>
<td>This work package coordinates the overall financial, administrative, legal and contractual management of LICARA.</td>
</tr>
<tr>
<td>2</td>
<td>Conceptual Framework</td>
<td>To develop a life cycle based conceptual framework that supports decision-making based on the risks and benefits of the product systems of nanomaterials compared to the conventional product systems delivering the same functionality. To framework has to deal with data scarcity on the impact of nanomaterials on human health and the environment.</td>
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<tr>
<td>3</td>
<td>Risk Assessment</td>
<td>Development of a human (focus on the subpopulation of workers) and environmental risk assessment approach to enable an aggregated risk assessment of materials over the various stages of their life cycles.</td>
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<tr>
<td>4</td>
<td>Life Cycle Analysis</td>
<td>Comparison of products based on nanomaterials with conventional products in order to illustrate their risks and benefits for the environment. Determination of the environmental and socio-economic impact of these products.</td>
</tr>
<tr>
<td>5</td>
<td>Guidelines</td>
<td>The results of the work packages 2, 3 and 4 will be compiled into a guideline for SMEs. The guideline will be applicable in all of the different phases of the innovation life cycle i.e. starting innovation to close to the market introduction.</td>
</tr>
<tr>
<td>6</td>
<td>Dissemination</td>
<td>Dissemination activities beyond the consortium to the scientific community, international audience and to SMEs through specialized events like conferences, fairs and exhibitions.</td>
</tr>
</tbody>
</table>

### 5 LICARA Events and Reports

The project LICARA will provide the following:

- Conceptual Framework: review of decision techniques for scarce data situations; How to deal with data paucity and data uncertainty regarding the risk and benefits of nanomaterials?
- Approach for human health risk assessment
- Life cycle assessment for 4 case studies
- Guideline report
  - to indicate the key information that determines the outcome (and uncertainty) of the environmental and human risk assessment of their products communication and the value of (additional) information, i.e. what information is relevant to reduce uncertainties for SMEs, i.e. priorities for information gathering; The existing evaluation techniques and decision support tools will be used to structure the guidance;
  - to facilitate the communication of SMEs/SME AG on the risk-benefits balance with clients, consumers and regulators;
  - to support the systematic documentation of precautionary and control measures undertaken by SMEs and facilitate the risk-benefit communication among the partners of the value chain.

#### 5.1 Workshops

- The project will hold two major dissemination events in the form of workshops: M12, M24. The workshops will be designed to specifically meet the needs of SME, but will be open to all nanotechnology stakeholders, allowing for feedback to be received and implemented.
- Besides the final workshop described above, the project will hold final dissemination events in the form of a workshop in M24 on a national level in the United Kingdom, Netherlands, Sweden and Switzerland for those SMEs that are not able to be present at the major dissemination event workshop due to travel distance (or any other reason). The workshops will be designed to specifically meet the needs of SME, but will be open to all nanotechnology stakeholders, allowing for feedback to be received and implemented.
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## MARINA

**MANAGING RISKS of NANOMATERIALS**

Coordinator: Dr Lang Tran, Institute of Occupational Medicine, Edinburgh (UK)

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<th>No.</th>
<th>Participant Legal Name</th>
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<td>SME</td>
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<tr>
<td>2</td>
<td>European Research Services GmbH (ERS)</td>
<td>Germany</td>
<td>Industry</td>
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<tr>
<td>3</td>
<td>Aarhus University (AU)</td>
<td>Denmark</td>
<td>University</td>
</tr>
<tr>
<td>4</td>
<td>BASF (BASF)</td>
<td>Germany</td>
<td>Industry</td>
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<td>5</td>
<td>Commissariat à l’énergie atomique (CEA)</td>
<td>France</td>
<td>Research Organisation</td>
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<td>Das Institut für Energie- und Umwelttechnik (IUTA e.V.)</td>
<td>Germany</td>
<td>Research Organisation</td>
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<td>Finish Institute for Occupational Health (FIOH)</td>
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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.

The market size for nanotechnology is expected to grow to over $3 trillion by 2015 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, it 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.

Objectives

The specific objectives of MARINA are:

1. For Materials, to obtain reference nanomaterials for testing;
3. 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 monitoring 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. However, to date, the valuable results generated from these projects have in the main been unable 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 for risk assessment and management. To address this need, in MARINA, we have created a consortium consisting of first class scientists and organisations with a track records for research and development (NMP.2010.1.3-1: NanoREFORM); this involvement allows the establishment of added-value components to both projects 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 methodology for supporting decision making.

In summary, MARINA stands for integrated testing, integrated assessment and modular interconnection of knowledge and tools for Risk Assessment and Management.

- we recognise the relevance of our results to industry and regulators. To respond to this information and methodology for guidance on health and safety issues of ENM. Most importantly, in Health and Safety Issues of ENM, we have created a consortium consisting of first class scientists and organisations with a track records for research and development.
- 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 global effort in addressing this important issue and to promote our strategy for risk management of ENM globally.
- we will interconnect all MARINA activities 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.

NANOIMPACTNET, NANEX, ENNSATOX, NANOFACTE, 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.
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 to toxicology studies. The detection and characterisation 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 there is difficulty in extrapolating the results between studies. Also, almost no study has characterized the ENM that are actually released into the environment. 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\(^5\), 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\(^1\) like the heart, liver, kidney 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, TiO\(_2\) 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 Hund-Rinke et al\(^21\) who showed oxidative stress in algae and daphnids following TiO\(_2\) exposure. Since then data are emerging at increasing numbers every year. Almost all published ecotoxicological studies with ENM\(^22\) 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\(^23\). 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\(^27\)), 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\textsuperscript{39}. 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\textsuperscript{50–52}. Of particular importance, is FP7 NANEX\textsuperscript{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\textsuperscript{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\textsuperscript{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\textsuperscript{33}. Formation of aggregates or agglomerates can take place between ENM or with natural organic and/or inorganic colloids\textsuperscript{35–38}, and is influenced by environmental factors like pH and ionic strength\textsuperscript{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\textsuperscript{40,41}. The physico-chemical distribution of ENM between dissolved, colloidal and particulate phases is largely unknown\textsuperscript{42,45} 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) 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. TiO\textsubscript{2} – in different size, shape and surface charge, SiO\textsubscript{2}, Ceria Oxide, ZnO, nanoAg, Multi-Wall Carbon Nanotubes (MWCNT) – in different lengths) as Reference Nanomaterials (RN) 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 RN will be characterised, assessed for homogeneity, stability and described shelf-life according to the OECD WPMM SG\textsuperscript{57} endpoints and criteria. We will use these RN 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/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 characterisation 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. We will work with other successful projects in FP7 NMP-2010.1.3.

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 toxicity 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 3 R 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 NAPRAHub 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 labs, industrial manufacturing, processing 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. Other 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 Committees, 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 - TiO2, ZnO, SiO2, CeO2, 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 can 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.

The relation between the WP is illustrated in Figure 1.

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 methods 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 NANOVALID. 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 MWNT. 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 partners. 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 will continue to support the NANOSAFETY school to be held in March 2013 and a workshop to implement exposure and hazard in risk assessment strategy for nanomaterials is already scheduled for April 2013.

Currently, the MARINA partners are intensive working towards the end of the first reporting period at month 18.

6 References


3. NANOCARE
   http://www.nanopartikel.info/cms/Projekte/NanoCare

4. Harn, Celpen, Refnano and Emergnano
   http://www.nanopartikel.info/cms/Projekte/NanoCare


Figure 1 Flow chart of MARINA

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MembraneNanoPart

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

Contract Agreement: NMP4-SL-2012-310465 Website: TBA
Coordinator: Vladimir Lobaskin, School of Physics, University College Dublin, Belfield, Dublin 4, Ireland

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Contents
1 Summary................................................................................................................. 71
2 Background............................................................................................. 71
3 What is MembraneNanoPart................................................................. 72
4 Organisation of MembraneNanoPart.............................................. 73
5 MembraneNanoPart: progress to date............................................... 73
6 Directory........................................................................................................ 73
7 Copyright...................................................................................................... 74

Project Duration: 1 January 2013 – 31 December 2015
Project Funding: 1 Mio. EUR

1 Summary
The goal of MembraneNanoPart is to develop physically justified models and computational tools to quantitatively describe and understand the molecular mechanisms of nanoparticle-cell membrane interactions, which we consider to be a crucial point in any predictive model of nanoparticle toxicity. We will 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 plan 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 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. A development of a joined approach is necessary, where nanomedicine and nanosafety will rely on the same body of knowledge, based on the understanding of the interactions between nanoscale objects and living systems. 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 (NP). 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.

We intend to address the issues of NP-cell membrane interaction by computer simulation. Molecular level computer simulation is now a well-recognized tool for studying intermolecular interactions, self-assembly, and structure of biomolecules or their complexes. Compared to other theoretical methods, computer simulation has the advantage that, within various levels of approximation, allows one to separately probe different physical mechanisms and chemical pathways, and assess, both qualitatively and quantitatively, their importance for studied phenomena. The reliability and predictive character of molecular modelling has improved significantly during the last few years, with development of new, carefully parameterized force fields, simulation algorithms, and greatly increased computer power. 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 What is MembraneNanoPart

In this project, we will attempt to develop a predictive approach to the problem of NP toxicity based on quantitatively consistent 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.](image-url)
4 Organisation of MembraneNanoPart

MembraneNanoPart includes six RTD and one management work package:

Work package 1 deals with data exchange and acquisition from external sources and provides the material for model parameterisation and validation, such as NP characteristics and cell viability data.

In Work package 2, we will do atomistic modelling of the NP surface, blood plasma components, glycans, and lipids. We will calculate the NP-protein binding energies, parameterise CG models of the NP-lipid interactions, and CG models for selected components of blood plasma and of the membrane to feed into large scale simulation in WP3-5. We will also independently evaluate the NP descriptors to be used in WP5.

In Work package 3, we will use the CG models of NP, blood plasma, and membrane in DPD or LD simulations to predict the NP equilibrium states in plasma and describe the effective nanoparticle, the entity that will be seen by the cell (and used in WP4). We will also calculate the NP-membrane interaction potentials to be used in WP4-5.

In Work package 4, the atomistic and CG models of (effective) NP and (effective) membrane will be used to study the possible scenarios of NP - lipid membrane interaction: attachment, translocation, inclusion, envelopment. We will calculate the energy profiles for the NP across the membrane to be used for predictive models in WP5.

In Work package 5, we will study the aftermath of the NP-membrane interaction and quantify the specific cytotoxicity effects: membrane reorganisation, leakage of the cytosol material, and NP genotoxicity potential. We will calculate the NP partition with respect to the membrane and attempt to correlate the toxicity with the NP descriptors.

Work package 6 contains dissemination and exploitation tasks.

Work package 7 accumulates the management tasks.

5 MembraneNanoPart: progress to date

The project started officially on January 1st 2013 and had a successful kick-off meeting in Lanzarote, Spain on February 16th and 17th, 2013. A harmonisation meeting with representatives of other projects in the NMP.2012.1.3-2 Programme “Modelling toxicity behaviour of engineered nanoparticles”: ModEnpTox, Modern, NanoPuzzles, PreNanoTox, is planned on 4th and 5th June 2013 in Brussels. All the teams agreed to establish a Cluster Steering Committee to facilitate the coordinated actions of the projects and hold joint meetings throughout their duration, and signed a Memorandum of Understanding, which regulates the data exchange between the participating consortia.

6 Directory

Table 1 Directory of people involved in this project.

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MODERN
MODelling the EnviRonmental and human health effects of Nanomaterials

Contract Agreement: 309314
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MODERN is cooperating with all ongoing modelling projects within the FP7-NMP Programme.

Contents
1 Summary ................................................................. 75
2 Introduction .......................................................... 75
3 Background ............................................................. 76
4 Project Description and Organisation ......................... 78
5 MODERN activities .................................................. 79
6 Directory ................................................................... 79
7 Copyright ................................................................... 80

1 Summary
Nano-sized materials are a common element in many industrial processes mainly due to their unique properties that lead to the production of high technology products. The widespread use of nanotechnology requires the consideration of the environmental and human health risks that may result from the introduction of engineered nanoparticles (eNPs) into the environment. Although toxic effects for certain types of eNP have been recently reported, there is still a lack of knowledge about their possible long-term effects in biological systems.

The project focuses on the understanding of the processes governing the interactions of nanoparticles with biological systems and their associated mechanisms of toxicity, which are essential for eNP safety assessment. Information on the effects of well-characterized eNPs will be obtained from literature and other data repositories. Targeted in vivo and in vitro experiments will be also carried out to overcome the limitations of data availability and for model validation. Computational methods will be applied to model both nanostructure-property relationships and the complex and highly non-linear nano-bio interactions and to diminishing the need for animal testing.

The main goal of MODERN is to establish new modelling approaches suitable for relating nanotoxicity with the intrinsic molecular and physicochemical properties of eNPs at environmental exposure levels and to implement safe-by-design nanoparticle design strategies. This implies three specific objectives: (i) To apply computational models for the characterization of the structural and physicochemical properties leading to QNPRs and safe-by-design strategies for eNPs; (ii) to develop in silico models (QNAR) of biological activity of eNPs in the body and in the environment; and (iii) to establish a categorization and hazard ranking protocol for eNPs based on structural similarity principles and in the analysis of their toxicological profiles.

2 Introduction
As a growing applied science, nanotechnology has considerable global socioeconomic value, and the benefits afforded by nanoscale materials and processes are expected to have...
significant impacts on almost all industries and areas of society. By 2015, the nanotechnology economy is estimated to be valued at 2.2 trillion €. Currently, in the conditions of the worldwide global economic recession, exponential growth of population, shortage of food, feed, fuel and raw materials and increasing environmental and societal problems, nanotechnologies have big expectations in almost every domain, from energy production to medicine. Moreover, nanotechnology has been referred to as the next industrial revolution. However, the safe application of nanotechnology at an industrial scale requires the careful consideration of the potential environmental and human health risks that may result from the introduction of engineered nanoparticles (eNPs) and nanomaterials in the environment. Nanomaterial means a natural, incidental or manufactured material containing particles, in an unbound state or as an aggregate or as agglomerate and where, for 50 % or more of the particles in the number size distribution, one or more external dimensions are in the size range 1 nm - 100 nm. ENPs may have a protective effect or pose new potential hazards towards living organisms, in part because their structure and properties are new and have not been previously introduced into the natural evolution processes. As hazard evaluation involves toxicity testing that is very laborious, limited (e.g., not all relevant issues can be tested), costly and still heavily relies on animal experiments, the introduction of in silico/QSAR (quantitative structure-activity relationships) approaches to nano(eco)toxicology is crucial, important and a challenging task. The need for cost-effective high throughput methods is even more obvious for eNPs than for 'regular' chemicals, since increasingly produced eNPs vary in composition, size and coating, leading to a huge number of combinations of chemical entities with different physicochemical properties (and thus different toxicity potential).

Understanding the implications of nanotechnology in our society and assuring its safe use requires a multidisciplinary research effort aimed to establish the basis for updating current regulatory framework regarding the safe use of these technologies. Indeed, there is an increasing risk of exposure to eNPs since the number of applications and consumer products on the market involving nanomaterials is increasing rapidly. For example, nanoscale iron for the remediation of contaminated groundwater, TiO₂ and ZnO nanoparticles for personal-care products (toothpaste, beauty products, sunscreens, and textiles) manufacturing, and nano-silver (n-Ag) as an antimicrobial additive in detergents, food packaging and clothing, such as socks and underwear. In addition, exposure at the workplace may adversely affect human health, especially in occupational exposure scenarios related to eNPs production, manipulation, and research. The study of eNPs effects in diverse biological systems is currently of great interest. Although there is a growing availability of eNP data (primarily for n-Ag and TiO₂ and carbon-based eNPs), the potential harmful effects and mechanisms of action are still not fully understood. As of 27/01/2016, the ISI Web of Science had more than 100,000 publications retrievable by keyword “nanoparticles,” and within this set of records only 87 were retrieved by refining the search by keyword “occupational”, 73 by “hazard” and just 16 by “ecosystem”. Although conventional toxicity data are rapidly emerging and High Throughput Screening (HTS) and High Content Screening (HCS) are contributing to the understanding of eNP - organism interactions, there is still a lack of fundamental understanding regarding these mechanisms. The acquisition of this knowledge is critical to characterize the hazard of eNPs, which is in turn indispensable for proper corresponding risk assessment and the development of appropriate environmental and health regulatory policies.

3 Background

Recent advances in nanotechnology and the corresponding increase in nanomaterials use in everyday products have resulted in uncertainties regarding their environmental and human health impact. The environmental risk assessment of eNPs requires information on their potential emission sources, properties in the nanoscale, intermedia distribution, transformations and persistence, and on their adverse effects. Every step of this process is uncertain, starting from the emission estimates and rates of entry and mobility in the environment to the eNPs effects on specific endpoints. This is a huge task and there are extensive knowledge gaps. Thus, this task has to be simplified, to yield meaningful information in relatively short time and using reasonable amount of economic resources. Analogous challenges are currently met by chemical industries due to the implementation of REACH regulations where Integrated (or intelligent) Testing Strategies (ITS) play a key role.

Despite the impressive knowledge gaps, existing risk assessment/classification tools may still provide helpful insight. In addition, there is ample strategic information accumulated during several decades of human health and eco-toxicological studies on bulk chemicals (e.g., choice of test organisms and validation of test protocols) as well as new toxicogenomic methods suitable to obtain new mechanistic knowledge on eNP-induced stress response on relevant model organisms. This information should be considered when categorizing and ranking eNPs. Ranking of eNPs requires understanding the mechanisms that govern their interactions with biological entities. It has been recently demonstrated that it is possible to establish quantitative nanostructure-activity relationships (QNAR) to describe the effects induced by eNPs in living cells/organisms, as done for conventional chemicals.

The importance of developing QNARs for category formation, hazard ranking and ultimately risk assessment and safe nanoparticle design has been recognized in specific EU workshops (e.g., COST Exploratory Workshop on QNTR, Maastricht, 2011). The effects of eNPs in environmental conditions strongly depend on their bioavailability and toxicity mechanisms, which in turn are modulated by their physicochemical and structural properties. Therefore it is fundamental to gather and integrate the experimental data available in the literature and in public data repositories to develop a well-characterized nanoparticle knowledge base necessary to drive the computational effort required to develop predictive nanotoxicity models. In addition, it is essential to develop a new generation of molecular descriptors suitable to describe eNP property profiles from their structure. The above eNP property profiles together with the in vitro/in vivo screening of toxicity will facilitate the generation of eNP...
signatures suitable for establishing categories based on similarity criteria.

Computational characterization of structural and physicochemical properties for safe-by-design nanoparticles

The identification of the hazard potential of nanoparticles to prevent harm to humans and the environment is of utmost importance for the widespread implementation and acceptance of nanotechnology. The production of safe nanomaterials requires understanding of the interactions between nanoparticle structure, properties and biological activity. Safe-by-design strategies for engineering nanoparticles can be implemented by introducing changes in the structure of eNPs, which in turn modify their intrinsic properties and effects. These strategies can be implemented by doping (introducing foreign element in the crystal lattice of the parent oxide - substitution or interstitial - to change the property of the material significantly with no detectable changes in the crystal structure) or by surface functionalization (i.e., surface adsorption of chemical species with a specific functional group responsible for the change in NP properties). The adoption of safe-by-design strategies either by introducing a dopant in the lattice or by functionalizing NP to generate a specific binding site for cellular proteins (protein corona) will help in the rational design of eNPs with reduced environmental and human health risks.

Therefore, specific nanoparticle descriptors have to be developed to describe intrinsic eNP properties such as surface chemistry, area, charge and reactivity, and structure-dependent electronic configuration using computational models. From the view point of molecular modelling, (1) nanoparticles are rather large and complex systems relative to single molecules and (2) the respective properties do not only depend on the molecular structure but also on physical properties of the nanoparticles. Currently, the number of existing models to address the physicochemical properties and biological activity of eNPs is very limited. However, until present no theoretical (molecular) descriptors have been developed that address the true properties of eNPs that depend on particle size. Moreover, available descriptors are only applicable to a limited range of eNPs (e.g., metals, metal oxides and carbon structures) without the capability to describe the properties of all possible eNPs. Therefore, a promising approach for developing QNARs is to centre on the surface-area and degree of agglomeration. However, it is generally assumed that for easily soluble eNPs, such as zinc, toxicity may be related to both released ions and to particle properties. Thus, mechanistic data are controversial, mostly due to the limited amount of available information but also due to intrinsic methodical difficulties such as limited solubility and aggregation/agglomeration in the test media. Several in silico methods for bulk chemicals have been developed as an alternative for in vivo and in vitro toxicity data generation within the framework of REACH. For eNPs, there are still few self-consistent toxicity databases and in silico models despite the amount of data published in the literature. The lack of well-structured and complete data repositories regarding structure, properties and activity of eNPs hinders the development of reliable in-silico nanotoxicity models and the subsequent eNP hazard ranking.

Even though concentration and size effects for eNPs have been reported, there is still the need for more universal QNAR models capable of predicting in vitro/in vivo activity profiles of current and novel eNPs and to provide a sound basis for the design and manufacturing of better, cheaper and safer products. In addition, preliminary studies on the environmental distribution of nanomaterials delineate exposure scenarios involving much lower nanomaterial concentrations than the ones typically used for in vitro HTS nanotoxicity assays and modelling. Genomic and proteomic studies demonstrated that exposure to low concentrations of eNPs may disrupt basic biological functions at the cellular and sub-cellular levels, which not always translate to observable cytotoxicity effects. These perturbations, however, may act as early indicators (similar to biomarkers) of much severe nanoparticle impacts and long-term effects (Fig. 1).

![Image](https://example.com/image.png)

**Figure 1.** Knowledge extraction from HTS datasets

Nanoparticle categories and hazard identification and ranking

Despite the incipient efforts in nanotoxicity modelling, the large number of possible nanoparticle types (e.g., diverse combinations of core, surface modifications and functionalizations) hinders the development of universal QNPR and QNAR models. It is thus fundamental to develop similarity...
metrics (based on nanostructure descriptors, physicochemical property profiles and biological activity) suitable to group nanoparticles into homogeneous categories where highly accurate and reliable models can be developed and validated. The establishment of eNP categories will also facilitate the ranking of their environmental and human health impact and will pave the way to the development of a risk assessment framework for nanomaterials.

4 Project Description and Organisation

The Project is structured in three research work packages (WP) designed to fulfil the specific objectives outlined above. Two additional non-research packages will account for the dissemination and exploitation of results and for the management and coordination of the project. The description of the S&T WPs and their knowledge domains (Fig. 2) is as follows:

WP1. Physicochemical, molecular and structural properties of eNPs (Lead: UFZ)

Main Research activities:
1. Development of new molecular descriptors and their associated calculation methodologies suitable to describe eNP molecular structure and properties.
2. Synthesis and characterization of eNPs targeted for QNPR model validation and to test safe-by-design hypothesis.

WP2. In silico profiling of environmental and human health impact of eNPs (Lead: URV)

Main Research activities:
1. Gathering literature data on in vitro/in vivo profiling of eNP effects on relevant environmental endpoints in aquatic and terrestrial ecosystems.
2. Development and analysis of integrated signatures describing structural, physicochemical and toxicity profiles of eNPs suitable for model development.
3. Development, validation and mechanistic interpretation of QNPRs.

WP3. Identification of eNP categories and basic hazard ranking (Lead: AU)

Main Research activities:
1. Development of a data mining framework suitable to identify similarity patterns on eNP signatures.
2. Development of a hazard ranking scheme suitable to rank eNPs and their categories according to their potential environmental and human health impact.

Project data sources and data quality control

The eNPs used in the project and their corresponding in vivo/in vitro toxicity data will be selected according to the last OECD, EU and US-EPA guidance documents. An initial subset of metal and metal oxide eNPs already available within the MODERN consortium will be considered in WP1 and shared across WPs. It will be used to develop QNPRs and safe-by-design approaches based on either solubility/redox potential mediated toxicity for metal oxide eNPs or on surface reactivity controlled by functionalization for metals. The MeOs in this subset with the necessary biological activity information will be used to develop nanotoxicity models and the hazard ranking scheme assuring that MODERN main goals can be attained without an excessively risky dependence on external data sources. Information on other nanomaterials, including those that could be outside the current EC definition, will also be collected from the literature and other sources (e.g., data available at the Institut Catala de Nanotecnologia (ICN) and the Centre for Nanobiosafety and Sustainability) (CNBSS); to complete the above first subset of eNPs. Additional nanomaterials may be added based on data availability and modelling requirements. The environmental effects of nano-scale chemicals (essentially metal and metal oxides) will be contrasted with the effects of the respective bulk chemicals whenever possible.

Consortium in vitro data will include diverse toxicity-related endpoints for protozoa, bacteria, yeast and algae. Data corresponding to in vivo assays will be collected for soil invertebrates, fish and mammalians. Regarding human health effects, human bronchial epithelial cells and murine macrophages will be used to evaluate toxic and inflammatory effects via a combination of single and multi-parameter assays. In addition to all the above data collected from diverse data sources available within the consortium, targeted in vitro experiments will be conducted to test the safe-by-design hypotheses.
5 MODERN activities

The global modelling-related challenges to be addressed within the current proposal are: (i) the development of nanoparticle categories based on their physicochemical, structural and toxicological properties, including their environmental and human health impacts, and (ii) the development of computational approaches for nanostructure characterization (nanodescriptors) and in silico models to assess nanoparticle effects.

The specific objectives to attain the main research goals are:

Build a well-characterized library of eNPs with a comprehensive description of their structural, molecular and physicochemical properties.

This will be accomplished by gathering available data with nanoinformatics tools and implementing physicochemical computational models to: (i) determine the relevant physicochemical properties of eNPs and characterize their molecular level structure by using specific descriptors suitable for nanoparticles (i.e., geometrical, topological, electronic, 3D, hydrophobic); and (ii) perform targeted synthesis and characterization of eNPs to validate QNPRs and to test hypothesis for safe-by-design strategies based on doping and/or surface functionalization.

Develop and validate in silico models of biological activity of eNPs in organisms and in the environment from in vitro/in vivo profiling data.

To attain this objective a project database system, interoperable with existing repositories, will be generated from literature information, collaboration with other NMP ongoing projects, and targeted new experimental findings for additional training and validation of in silico approaches to predict relevant physicochemical properties of eNPs from their structural information (QNPR) and their nano(eco)toxicity (QNAR). In addition, preliminary data management and analysis strategies that could be suitable to discover and study molecular signatures (e.g., genetic mapping) representative of sub-cellular process triggered at low concentrations will be screened from any available HTS data since these exposures that are more realistic in terms of emission rates could ultimately lead to observable long term adverse (e.g., toxicity) effects.

Define and implement a categorization and hazard ranking methodology for eNPs based on structural similarity principles and toxicological profiles.

This will be achieved by (i) using data mining algorithms to identify categories of nanoparticles with common signatures (i.e., structural, physicochemical and toxicological profiles); (ii) integrating QNPRs and QNARs into a hazard ranking/decision framework, which will be developed with weight factor approaches or multicriteria decision analysis (MDA).

6 Directory

Table 1 Directory of people involved in the MODERN project as beneficiaries*.

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ModNanoTox
Modelling nanoparticle toxicity: principles, methods, novel approaches

Contract Agreement: NMP4-SL-2011-266712
Website: http://www.birmingham.ac.uk/generic/modnanotox/index.aspx
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Contents
1 Summary ................................................................. 81
2 Background ........................................................... 81
3 What is ModNanoTox ................................................ 82
4 Organisation of ModNanoTox ..................................... 82
5 ModNanoTox: progress to date .................................. 83
6 Directory ................................................................... 83
7 Copyright ................................................................... 84

Project Duration: 1 November 2011 – 31 October 2013
Project Funding: 1 Mio. EUR

1 Summary
ModNanoTox is developing a number of well-documented and technically advanced models describing the behaviour of engineered nanoparticles in organisms and the environment. Background to these models will be the construction of 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 will be evaluated for a number of quality criteria and internal consistency as a condition for inclusion. The evaluation stage will be 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 will be integrated in mechanistic models to predict the long term risks of engineered nanoparticles for populations under realistic environmental conditions. The risk assessment models will be developed in close collaboration with appropriate stakeholders and end users to ensure their suitability for practical use in relevant legislative contexts.

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 idea that a number of projects are currently generating large datasets of experimental results. Global nanosafety research would benefit greatly by harmonizing, rationalizing and converging these datasets and then use as the basis for robust large scale models of toxicity. Such models can best be developed by teams with experience in collecting the data, who also have a deep understanding of their limitations and relative quality and can influence the progress of ongoing experimental work. ModNanoTox will be focusing on in silico methodology, to complement and support research on and regulation of the environmental and human implications of exposure to engineered metal nanoparticles. There has been a relative explosion of interest in nanotoxicology and growing concerns that the assessments of the risks of nanotechnology is not keeping pace with
developments of the technology. As a result, in recent years, a number of nanotoxicology projects have been funded, notably under the FP7 programme of the European Commission, which are currently in progress and generating nanosafety data. A significant research effort is also under way in the US. ModNanoTox will aim to evaluate and synthesise the best available datasets from these sources, and fit them into new models. Such models, whether statistical or mechanistic need to take into consideration the novel properties of nanomaterials (NMs) and their potentially unpredictable behaviour, and thus models need to acquire a level of sophistication to accommodate that. Both statistical and mechanistic models are needed. Statistical models are necessary when mechanistic understanding is lacking and to capture uncertainties in relationships between nanoparticle properties and their behaviour. However, mechanistic models are more appropriate for extrapolating beyond existing data sets and exploring different scenarios.

3 What is ModNanoTox

ModNanoTox is a small project, designed to develop a number of well-documented integrated and technically advanced models describing the behaviour of engineered nanoparticles in an environmental or biological context to comprehensively address the following key hypotheses:

1. Toxicity of nanoparticles is the result of physicochemical properties and this has been documented reliably in completed/ongoing studies. Properties found to be relevant include size, surface area, structure and composition (WP1, 2)
2. Nanoparticle reactivity can be modelled computationally and can be linked to toxicity (WP1).
3. Toxic responses from cell culture studies and whole organisms can be correlated and rationalised and can be translated into tools useful for model development (WP2).
4. Bioaccumulation into cells or whole organisms can be characterized and modelled using biodynamic principles (i.e. by characterizing uptake rate constants from food and water as well as loss rate constants) (WP3).
5. Toxic responses from cell culture studies and whole organisms can be modelled reliably by QSAR type approaches (WP4).
6. Exposure concentrations can be assessed reliably and incorporated in appropriate models (WP5).
7. Mechanistic effect models can be developed by extrapolation from ecological and (eco)toxicological observations and can be built into risk assessment models (WP6).

4 Organisation of ModNanoTox

ModNanoTox consists of six RTD and one management workpackage. The workpackages and their interdependence are shown schematically in Figure 1. The specific objectives of each workpackage are as follows:

WP1: Physicochemical properties assessment
1) To identify and select suitable physicochemical properties (size, shape, phase, concentration, composition, surface modification, method of synthesis) so that groups of structurally similar particles can be identified for toxicity models.
2) To carry out atomistic simulations of surface reactivity of a reference set of particles (Ag NPs) to support this selection.
3) To develop mechanistic understanding of nanoparticle reactivity based on atomistic models.

Figure 1. Workpackage structure of ModNanoTox.

WP2: Data evaluation
1) To characterise and classify existing toxicity data (data mining), including evaluation of datasets from completed and on-going projects, in order to prioritise data for modelling in other workpackages.
2) To evaluate differences in parameters due to contributing factors such as synthesis, storage, and throughput to testing.
3) To recognise data quality limitations.
4) To evaluate characterisation and classification techniques.

WP3: Bioaccumulation models
1) To model toxicity at the individual organism level using bioaccumulation based toxicokinetic- toxicodynamic models.
2) To generate models, based on individual organisms, capable of incorporating experimental nanoparticle uptake and toxicity data and identify links to the mechanism of toxic action.

WP4: QSAR models
1) To model toxicity at individual organism level using QSAR models, based on data mining and machine learning algorithms.
2) To adapt existing models and generate new models capable of linking toxicity to nanoparticle properties.
WP5: Exposure concentration models

1) To evaluate models to estimate environmental exposure, including the REACH procedures.

2) To parameterize existing models for nanoparticles by extracting data from the literature and by collecting results from ongoing FP7 projects.

3) To model environmental exposure concentrations of nanoparticles in water, air, soils and sediments from the local to the continental scale.

4) To validate the results by comparison with analytical results from published research.

WP6: Population models and risk assessment

1) To model effects on ecological systems (i.e. populations) and generate risk assessment models.

2) To assimilate the data from the previous objectives to scale up effects to ecologically relevant entities (i.e. populations) and to produce complete risk assessment models developed appropriately for end users.

WP7: Project management

1) To manage the consortium and its stipulated agreement between partners & EU.

2) To coordinate the flow of scientific knowledge, the decision making structures, the control of milestones and deliverables, the promotion of interchange and linkage between project components and partners, and the control and monitoring of administrative and financial issues.

3) To harmonise actions, to keep the various activities of the project convergent with the central aim of final integration.

4) To review and assess project progress.

5 ModNanoTox: progress to date

The project has reached mid-term. WP1 has produced a completed survey and selection of relevant physicochemical properties to use towards building a range of descriptors of metallic nanoparticles and their potential toxicity. This workpackage is currently constructing relatively complex simulation models describing solubility and aggregation. WP2 has produced a first compilation of data suitable for input in other WPs (WP5, 4, 5 and 6). WP3 has produced an initial evaluation of the WP2 database for use in bioaccumulation modelling and toxicodynamic analysis and identified large gaps in AgNP available data for the use of toxicokinetic/toxicodynamic models; the work has therefore continued by applying analysis as far as data allows. WP4 has started with preparing, analysing and testing initial QSAR models with given data; again data availability limitations will have an impact on the breadth and applicability of the models under construction. WP5 has so far evaluated the available models for predicting nanoparticle concentrations in the environment and has critically compared the model results to analytical measurements that might be used to validate the models. It is providing predictive environmental concentrations for other WPs (specifically 3 and 6). WP6 has produced, in collaboration with WP5, a set of realistic worst-case scenarios of exposure to selected metal NPs (i.e., TiO$_2$-NP and Ag-NP) in freshwater pelagic and sediment compartments as well as a presumed conservative worst-case estimate for the marine environment. An assessment of species groups most/least likely to be at risk from metal NPs has also been initiated. The selection of two aquatic invertebrate species (Daphnia magna and Capitella teleta) for which WP6 will deliver population models are based on this preliminary assessment. WP6 has also developed a semi-generic individual based population model, parameterized it for Daphnia magna and tested it against independent data. As with other WPs, WP6 identified major data gap in the published literature both with regard to long term toxicity testing and realistic exposure concentration for the sediment compartment in particular.

6 Directory

Table 1 Directory of people involved in this project.

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Nanodetector

Ultrasensitive plasmonic detection of single nanoparticles

Contract Agreement: 280478    Website: http://www.nanodetector.eu
Coordinator: Vladimir Mirsky, Lausitz University of Applied Sciences, Senftenberg, Germany

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Contents

1 Summary ..................................................................................... 85
2 Background ................................................................................ 85
  2.1. Plasmonic detection of single nanoparticles .......................... 85
  2.2 Quantification of nanoparticles ........................................... 86
  2.3 Identification of nanoparticles .......................................... 87
3 Objectives of the project ........................................................ 87
4 Workplan .................................................................................... 88
5 Directory ..................................................................................... 89
6 Copyright .................................................................................... 89

1 Summary

Project Duration: 1 June 2012 – 30 November 2015

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.

Project Funding: 2.968 Mio. EUR

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 an until now unnoticed 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 Eigen-modes,
the surface waves 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, this shift 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.

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surface processes simultaneously.

Limitations in the lateral resolution of SPR microscopy have been
discussed since work in this area began. The large length of
plasmon propagation is usually seen as an obstacle, limiting optical
resolution to approximately 5 - 20 µm, preventing the observation
of individual nanoparticles by this technique. However, it has been
recently demonstrated by the project participant 2 that the
plasmon penetration 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 also particles of the size of few tens of
nanometers can be observed. Nanoparticles are detected by SPR
microscopy using CCD-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 ranging between 40
and 100nm to be observed.

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.

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 200 nm polystyrene nanoparticles and protein particles. The
particles were negatively charged; while the gold surface was
coated by positively charged aluminium 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

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

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.

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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.

![Graph](image)

**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 biotinylated nanoparticles by streptavidin coated gold layer. 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.

The new technology proposed 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.

<table>
<thead>
<tr>
<th>Expected feature</th>
<th>Method of its realization</th>
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<tr>
<td>Extremely high levels of sensitivity, due to large sensor area, up to tens mm²</td>
<td>The method is based on detection of single nanoparticles bound to the sensor surface</td>
</tr>
<tr>
<td>Quantitative information on nanoparticle concentrations (if material is known)</td>
<td>Large number of nanoparticles can be detected, which enables a statistical analysis of the frequency of binding</td>
</tr>
<tr>
<td>Information on the size of nanoparticles</td>
<td>Preliminary results demonstrate that it can be obtained from the intensity of image</td>
</tr>
<tr>
<td>Information on the refractive index of the material (if the size is known)</td>
<td>Preliminary tests demonstrate that the image intensity depends on the refractive index of the material</td>
</tr>
<tr>
<td>Information on chemical content of the surface of nanoparticles</td>
<td>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.</td>
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<tr>
<td>Possibility of on-line detection in native surrounding</td>
<td>On-line detection is possible with appropriate software.</td>
</tr>
<tr>
<td>Continuous or (quasi)continuous operation mode</td>
<td>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.</td>
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### 3 Objectives of the project

The main objectives of the project are (1) the development and validation of technologies for the detection and analysis of ultra-low concentrations 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 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.

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

The realization of the project concept is divided into 11 work packages (WP). The first work package (WP1) is focused on the project management while the last (WP11) describes dissemination and exploitation of the project results.

The goal of WP2 is the development of theory of the recently observed phenomenon which will form a scientific base of the present proposal and formulation of ideas for new experiments. The most reliable theory will be a direct solution of Maxwell's equations of electrodynamics with a spherical particle located at the interface of the thin-metal film and semi-infinite dielectric in the classic Kretschmann ATR geometry. Once developed, this theory will be applicable to any material of the nanoparticle, described through its frequency dependent dielectric permittivity $\varepsilon(\omega)$. Systematic investigations will focus on: (i) the effect of the material of nanoparticles (incorporated through its complex permittivity), their size and shape; (ii) the effect of the surface concentration of nanoparticles; (iii) the effect of the structure of the nanoparticles layer (lattice symmetry, clustering and such). Additionally the thickness of the metal resonant layer and an influence of electrochemical conditions for the experiments in electrolytes will be tested.

The WP3 and WPs 5 - 9 form a block of work that will be focused on the development of the initial and subsequently the final laboratory prototype of the device for ultrasensitive detection, quantification and identification of nanoparticles. The completion of these WP's will result in the fabrication of 8 pieces of the final laboratory prototype device.

The WP3 includes optimization of optics, resonant layers and measurement approach. The concept of bimetallic layers, suggested few years ago by one project partner, will also be tested. The resonance quality depends on the exactness the layer thickness as well as on roughness and homogeneity of the gold layer. This concept will be improved by optimization of metal deposition conditions. The results obtained from the theoretical analysis will be used to further improve the measurement technique. It can also be expected that analysis of angle or wavelength dependencies will enable the uncoupling of the influence of the size and refractive index on the image of nanoparticles.

The WP4 focuses on the development of coatings with affinity to defined types of nanoparticles. The coating will be based on self-assembly of omega-modified alkylthiols on the gold surface and on the use of photografting. These omega-groups will operate as receptors for nanoparticles. As an alternative, immobilization of carboxy-modified alkylthiols and subsequent immobilization of receptor groups on the carboxy-groups will also be used.

If a stronger binding is required, rough surfaces formed by immobilization of polymers on the gold will be considered; the receptor groups will be subsequently immobilized on this polymer or pre-synthesized polymers with corresponding receptors will be applied.

An improvement of selectivity will be reached by combination of sensors into array and by application of algorithms for pattern recognition (WP7). It is expected that an application of sensor array would give an effective mechanism for chemical analysis of main classes of inorganic nanoparticles. Selective detection of protein-based nanoparticles will be performed using corresponding antibodies as receptors.

The system optimized within WP3 as initial laboratory prototype will be further developed to form the "final laboratory prototype". This will be done within WP5 (optomechanical components), WP6 (electronics, fluids and controlling software) and WP9 (integration). The final laboratory prototype will be designed, optimized and produced as 8 devices.

The final laboratory prototype should be close to the next version of the device – it will be a prototype of the commercial device, however the development of a commercial device will demand significant resources and its development is not included in the project goals.

An improvement of measurement technology requires the development of image analysis software and this will be done within WP7. Different approaches for signal filtration and for recognition of images of nanoparticles will be tested. In addition to a simple statistical approach, background subtraction and filtrations, mathematical approaches with the aim of isolation of defined diffraction images (calculated in the WP2) in the stream of information from CCD- (or CMOS) camera will also be used. The software will also be used to count the adsorbed nanoparticles and for quantitative characterization of their images. This data can subsequently be used to generate information on the nanoparticle size and refractive index. Another goal of the WP7 is the development of software for identification of nanoparticles by sensor arrays. It will be performed using known approaches for pattern recognition, such as principal component analysis or neuronal networks.

The detection performance depends strongly on the quality of resonance layer. The goal of the WP8 is to develop resonant layers providing minimal rest signal in resonance, possible sharp resonance and minimal surface roughness. Success in WP8 will result in the simplification of the detection technique, making it easier for the operator to handle and reducing the requirement for operator training. Success in WP8 will be also important for the ability of the detector to detect/measure dangerous nanomaterials (e.g. highly toxic, radioactive, viruses, etc.) that will require disposable components within the measurement probes.

Although one can foresee a vast number of applications of the new technology, its complete potential is yet to be explored. The utility of the technology will be evaluated within WP10. Measurements will be performed using final laboratory prototypes in the laboratories and production facilities of the end users. The work will include a validation of the system performance and its application for detection and analysis of nanoparticles in drinking water and in non-colloidal drinks, for investigation of aging of nanostructured materials, for monitoring of work places during production of nanoparticles, for analysis and quality control of produced nanoparticles.

88 Compendium of Projects in the European NanoSafety Cluster
## Directory

Table 1 Directory of people involved in this project.

<table>
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<tr>
<th>First Name</th>
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## NANODEVICE

**Novel Concepts, Methods, and Technologies for the Production of Portable, Easy-to-Use Devices for the Measurement and Analysis of Airborne Engineered Nanoparticles in Workplace Air**

Contract Agreement: CP-IP 211464-2  
Website: http://www.nano-device.eu

Coordinator: Professor Kai Savolainen, Finnish Institute of Occupational Health

<table>
<thead>
<tr>
<th>No.</th>
<th>Beneficiary name</th>
<th>Short name</th>
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<td>FIOH</td>
<td>Finland</td>
</tr>
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<td>HEALTH AND SAFETY EXECUTIVE</td>
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<td>4</td>
<td>NEDERLANDSE ORGANISATIE VOOR TOEGEPAST NATIONUWETENSCHAPPELIJK ONDERZOEK - TNO</td>
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1 Introduction

The motive of the NANODEVICE project is based on the lack of knowledge of the health effects of the widely used engineered nanoparticles (ENP) and on the shortage of field-worthy, cost-effective ways - especially in real time - for reliable assessment of exposure levels to ENP in workplace air.

2 Project summary

Due to their unique properties, engineered nanoparticles (ENP) are now used for a myriad of novel applications with great economic and technological importance. However, some of these properties, especially their surface reactivity, have raised health concerns, which have prompted scientists, regulators, and industry to seek consensus protocols for the safe production and use of the different forms of ENP.

There is currently a shortage of field-worthy, cost-effective ways - especially in real time - for reliable assessment of exposure levels to ENP in workplace air. In addition to the problems with the size distribution, a major uncertainty in the safety assessment of airborne ENP arises from the lack of knowledge of their physical and chemical properties, and the levels of exposure. A special challenge of ENP monitoring is to separate ubiquitous background nanoparticles from different sources of the ENP.

Here the main project goal is to develop innovative concepts and reliable methods for characterizing ENP in workplace air with novel, portable and easy-to-use devices suitable for workplaces.

Additional research objectives are:
1) identification of relevant physico-chemical properties and metrics of airborne ENP, establishment of reference materials
2) exploring the association between physico-chemical and toxicological properties of ENP
3) analyzing industrial processes as a source of ENP in workplace air
4) developing methods for calibration and testing of the novel devices in real and simulated exposure situations 
5) dissemination of the research results to promote the safe use of ENP through guidance, standards and education, implementing of safety objectives in ENP production and handling, and promotion of safety related collaborations through an international nanosafety forum.

3 Scientific and technological objectives of the NANODEVICE project

Engineered nanoparticles (ENP), defined as having at least one dimension ≤100 nm, have attracted a great deal of interest during recent years, due to their many technologically interesting properties. The unique properties of ENP and their applications have given birth to immense technological and economic expectations for industries using ENP. However, some of these properties have given rise to concern that they may be harmful to humans. This has prompted scientists, regulators, and the industrial representatives to investigate the features of ENP in order to be sure of their safe use in nanotechnologies (NT), i.e. technologies utilizing ENP. The European Commission has also explored in-depth the characteristics of ENP and issued a document on ways to assure the safety of ENP.

Overall objectives of the research: New and innovative concepts and methods for measuring and characterizing airborne ENP with novel, portable and easy-to-use device(s) for workplaces.

4 Summary preliminary results

4.1 Devices

17 new devices in 4 device families and 3 feasibility studies will be developed in the project. The 4 families are:

1. Total or size specific N-S-M concentration in real time
2. Material specific monitors in real-time or quasi-real-time
3. Samplers for off-line particle analysis
4. Preseparator modules for size fractions relevant to the human respiratory tract (modular components)

Progress beyond the state of the art include:

1. Simpler device concepts
2. Reduced weight / portability
3. Ease of use
4. On-line, real-time capability
5. Progress in sampler technology
6. New / material specific particle information
7. Versatile system solutions

4.1.1 Total or size specific N-S-M concentration in real time

Specimens of the devices in the first family are presented in figures. 1-3.

4.1.2 Material specific monitors in real-time or quasi-real-time

Specimens of the devices in the second family are presented in Figure 4 and Figure 5.
4.1.3 Samplers for off-line particle analysis

Specimens of devices in the third family are presented in Figure 6 and Figure 7.

4.1.4 Preseparator modules for size fractions relevant to the human respiratory tract (modular components)

Specimen of device in the fourth family is presented in Figure 8.

4.2 Identification of relevant physico-chemical properties and metrics of airborne ENP, establishment of reference materials

SWCNT floating catalyst synthesis reactor has been designed, built and used to generate fresh SWCNT aerosols for the project’s needs (Figure 9).

The metal oxide flame reactor for nanoparticle generation was finalized and now operational, the generated powder is collected and the particles are being analysed.

Figure 4. CNT detector developed by NANEUM

Figure 5. The Catalytic Activity Aerosol Monitor (CAAM) developed by Karlsruhe Institute of Technology

Figure 6. Gas-exchange region (GE modular) pre-separator developed by University of Lund

Figure 7. Extra-thoracic region (ET1) modular pre-separator developed by University of Lund

Figure 8. Pre-prototype CNT specific sampler by Fraunhofer IPA.

Figure 9. TEM image of a CNT tube sample
4.3 Exploring the association between physico-chemical and toxicological properties of ENP

The Cytotoxicity (cell death, ATP-level, Cell cycle delay and oxidative stress), genotoxicity (DNA damage, micronuclei), and immunotoxicity (Pro-inflammatory reactions in cell models) of engineered nanoparticles have been studied and a databank is created for the results and it is available. The results have been published in journals and in scientific meetings. TEM image of human primary macrophage engulfing long, tangled CNT is presented in Figure 11.

4.4 Analyzing industrial processes as a source of ENP in workplace air

Experimental studies of source characterization, agglomeration and ventilation have been conducted and a data analysis on them has been completed. Computational Fluid Dynamic (CFD) modelling has been achieved with scenario building completed and simulation runs conducted.

4.5 Developing methods for calibration and testing of the novel devices in real and simulated exposure situations

A calibration tool "Characterization of Aerosol Instrumentation devoted for Measuring Aerosols of Nanoparticles (CAIMAN)" has been extensively used by the device developers in the project. A Nanotest facility has been validated with established aerosol measurement technology concerning ranges of particle sizes and concentrations as well as mixtures of particles. The first pre-prototypes have been tested with defined aerosols. This was done in the scope of a second, larger round robin test ("stress test") under real conditions compared to those expected in the further course of the NANODEVICE project. The Nanotest facility has been extensively used by device developers and a third, and final; round robin tests are yet expected. A pilot study shall be provided for the device developers on the test results.

4.6 Dissemination of the results

The project's website http://www.nano-device.eu is a communication channel to communities. A brochure introducing the project in a nutshell has been carefully developed and distributed (Figure 12). Multiple poster presentations have been held in several conferences and scientific meetings on the results of the project (Figure 13). The NANODEVICE project peaked at the SENN2012 Congress Oct. 28-31, 2012 in Helsinki, Finland (Figure 14).
5 Conclusions

NANODEVICE will provide new information on the physicochemical properties of engineered nanoparticles (ENP) and information about their toxicology. Also a novel measuring device will be developed to assess the exposure to ENP’s from workplace air. The purpose of the project is also to promote the safe use of ENP through guidance, standards and education, implementing of safety objectives in ENP production and handling, and promotion of safety related collaborations through an international nanosafety forum.

At the Industrial Technologies 2012 event held in Aarhus, Denmark June 19-21, 2012, NANODEVICE project was one of the ten finalists selected from 63 projects at the Best Project Award, which had had the greatest economic and societal impact, boosting European competitiveness by creating new products and processes (Figure 15).

The project’s stakeholder group consisted of representatives various European organizations: European Government: Zsuzsanna Mokry (National Innovation Office, Hungarian Government), Business Europe: Thomas Brock, European Technology Platform on Industrial (ETPIS): Olivier Salvi, Olli Ikkala (AALTO University). The stakeholder group stated that the benefits of the NANODEVICE project for the European countries (competitiveness) and European citizen are: Impact on the acceptance to work with nano technologies by bringing clearer understanding on the exposure to NMs: most of the persons are not aware / do not see a threat from the use of NMs and thus the project might bring some evidences for exposure and industry will be better prepared if there are questions by the public. The project enables to be pro-active and inform safety decisions.
### 6 Directory

Table 1 Directory of people involved in this project.

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NanoFATE
Nanoparticle Fate Assessment and Toxicity in the Environment

Contract Agreement: NMP4-SL-2010-247739  
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5    Faust & Backhaus                                                                 | F+B        | Germany
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Contents
1 Summary........................................................................................................ 99
2 The NanoFATE aim and focus ......................................................................... 100
3 How NanoFATE will improve the State-of-the-art for environmental fate and effects of ENPs ...................................................... 100
3.1 Background .............................................................................................. 100
3.2 Current baseline of knowledge and points where NanoFATE will progress beyond the state-of-the-art in meeting project objectives ...................................................... 101
3.3 Overall project structure ......................................................................... 107
4 Description of the work performed since the beginning of the project and the main results achieved ..................................................... 108
5 Citations ....................................................................................................... 109
6 Directory ...................................................................................................... 111
7 Copyright....................................................................................................... 114

1 Summary

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 CeO₂, 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 The NanoFATE aim and focus

NanoFATE focuses on developing a systematic understanding of fate and mechanisms of effects in a core set of ENPs and addressing how these may affect the application of current tools for ecological risk assessment. The fact that the ENPs we will study are associated with commonly and widely used products provides environmental and economic relevance to our work. Furthermore, the selected ENPs will each have different core and surface chemistry and physical properties. This will allow us to elaborate on current understanding of how ENP properties influence fate and behaviour in the environment, and their potential toxicity. This will be achieved by systematically studying aspects that are related to fate and toxicity, and seeking to refine risk assessment practices for use with ENPs, leading to the nine NanoFATE objectives detailed below (3.2)

3 How NanoFATE will improve the State-of-the-art for environmental fate and effects of ENPs

3.1 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, CELLMNATOX, 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 Wylie 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.
3.2 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 IPCCNANONET 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.

![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).](image)

**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 CeO$_2$ 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 CeO$_2$ 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 be 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
NanoFATE are as follows: The major techniques that will be used in the studies in within prokaryotic and eukaryotic organisms will also be utilised. Also allow determination of the uptake and localisation of ENPs important determinants of particle bioavailability. Methods that colloidal and particulate matter, since these interactions are available and bespoke manufactured and doped ENPs will be 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 (FFFFFF-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 (IHP, 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 interactions of ENP with particulate matter and possibly also imagine of tissues for metal ENPs inclusions (UOXF.DJ, UNIPMN).
floculation during wastewater treatment (UOXF.DJ). Also, IHPP has excellent field emission scanning microscope Leol530 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 on 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 CeO2 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 CeO2 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 Klapa 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).

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**Fig. 2. Example analysis of the impact of ionic copper on oxidative phosphorylation (A) and on glycolysis/glucogenesis (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; Vlarenco 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 interspecies 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 CeO₂ 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.

**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 ENP's 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.

**3.3 Overall project structure**

The NanoFATE PERT diagram (Fig. 4) below shows the relation of the nine work packages, each of which is embedded into the three main project components.
4 Description of the work performed since the beginning of the project and the main results achieved

While work has begun on all nine of the main NanoFATE S&T objectives (see above), obviously some are targeted for early delivery (i.e. Obj. 1 and 2) while full delivery for the others will not come till 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.

The working objectives addressed in the first 36 months of NanoFATE to ensure timely progress towards the overall objectives can be summarised as follows:

I. Source, produce and fully characterise the commercial ENPs and match the tagged version of ZnO as closely as possible. (Delivering Main Obj. 1)

II. Establish particle behaviour in the pure ecotox media to be used and at higher than environmental concentrations (to enable hazard assessment studies). (Working towards Main Obj. 3, 4 & 5)

III. Establish acute and chronic toxicity (to enable selection of relevant doses for the progression into the work in environmentally relevant media conditions. (Working towards Main Obj. 5, 6, 7 & 8)

IV. Develop initial simple assumption based fate models and estimate worst case environmental concentrations (Working towards Main Obj. 2 & 8)

V. Train all staff cross discipline, and disseminate our early findings and planned directions to other EU projects and stakeholders (Main Obj. 9)

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

In the first 36 months 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 are:

- The main ZnO particles are 30nm Nanosun from micronisers in Australia, with matching tagged ZnO ENP by IHP, with some work on BASF z-cote and z-cote HP1 ZnO
- Amepox 3-8nm Ag ENP and a 50nm Ag NP from NanoTrade
- CeO2 will be the Envirox or Antaria fuel additive and most likely a polishing agent from Unimicore.

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 the initial basic 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, serving to inform decision making and exposure design in WP 3 & 4 (M 2.1, M 2.2, D 2.2, and D 2.3).

WP 3 ENP Ecotoxicology

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 (except for CeO2). The chronic testing phase (D 3.2 and D 3.3) and the work to deliver the data for WP4 on bioavailability drivers is near completion. Samples have been archive for use in WP 5 (M 3.2) and data has been collated for use in PNEC assessment (M3-3). Work has also started on co-exposure of CeO2 and organic contaminants. also.

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 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 ongoing. A database of results for ENP exposure across soil and water types has been completed (D 4.2) and research reports on ENP property-property-effect relationships to address confounder effects on bioavailability (D 4.3 and M 4.3) are near completion.

WP 5 ENP toxicokinetics and toxicodynamics

The WP5 workshop session in Portugal (Jan 2011) developed a practical and workable sample handling and preservation system to enable the success of NanoFATE tissue banking. A well thought through tired approach to the tracking of ENPs in tissues was also developed, 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) although further in situ characterisation of ENP uptake is required. A few samples have been run on the UK synchrotron at Diamond Light Source and further samples have been run on the CARs facility at Exeter University (via QNano TA), to provide a highly characterised test bed for development of other more accessible techniques. Extensive use of the QNano facilities will be sort to complete these studies (D 5.5 and D 5.8).

WP 6 Integrated risk assessment

The initial milestone for WP 6 was to assess ENP production and product incorporation estimation engaging industry directly through a survey. With over 560 companies and associations involved in ENP production being contacted this represented an important attempt to gain intelligence on market size and growth for ENPs in Europe. However, a devastating lack of industry willingness to interact was suffered, as was the case for a similar effort by the NanoSustain project. Therefore the report was 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. The compilation of usage scenarios were thus completed without direct data from industry and initial fate and distribution models (local to EU region scale) developed. The resulting predicted environmental concentration maps are very informative albeit obviously conservative (high estimates) due to the assumptions currently surrounding production size and distribution. 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 will be further refined using a species sensitivity distribution approach informed by data generated through WP3 by month 36.

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 direct engagement with stakeholders NanoFATE organized with the fellow NanoSafety Cluster projects NanoRetox and Ennsatox an EU Cluster meeting in London September 2011 aiming to link Environmental fate and Ecotoxicology aspects within EU NanoSafety Cluster projects. Representatives from many EU projects, industry and regulatory bodies. Additionally, there have of course been numerous presentations of the NanoFATE work to international conferences and workshops, and the first peer reviewed papers are starting to come out. Furthermore, NanoFATE participated in a NanoRETOX regulator and industry focused workshop on the 11th of Sept 2012 in London. Recently we co-organised a well attended “Café Scientifique Workshop on nanotechnology” with ENNSATOX in Leeds December 13th 2012.

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 the 1st NanoFATE open PhD training workshop was held in Jan. 2011 at UVAR (Aveiro, Portugal), brought together 60 participants (24 from NanoFATE) and representing 14 nationalities. In January 2012 NanoFATE jointly with Ennsatox, NanoImpactNet & Marina ran a NSC training course at Plymouth University in the UK.

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. Dissemination is done through a wealth of channels and activities, but centred around interactive e-Newsletters and the project website www.nanofate.eu.

5 Citations


of aqueous suspensions of aggregates of nano-C-60. Aquatic Toxicology, 86, 379-381.


Modeling effects of mixtures of endocrine disrupting chemicals at the river catchment scale. Environmental Science & Technology, 40, 5478-5489.


6 Directory

Table 1 Directory of people involved in this project.

<table>
<thead>
<tr>
<th>First Name</th>
<th>Last Name</th>
<th>Affiliation</th>
<th>Address</th>
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NanoHouse

Cycle of Nanoparticle-based Products used in House Coating

Contract Agreement: NMP-2009-247810
Website: http://www.nanohouse.cea.fr
Coordinator: François Tardif Commissariat à l’Energie Atomique et aux Energies Alternatives, Grenoble, France

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Contents

1 Summary ..................................................................................... 116
2 Background ................................................................................ 116
3 What is NanoHouse .................................................................... 117
  3.1 Summary of NanoHouse’s key strengths ............................... 117
4 Organisation of NanoHouse ...................................................... 117
5 NanoHouse Reports and Events ............................................... 119
  5.1 Progress to date .............................................................. 119
  5.2 Events ............................................................................. 119
    5.2.1 Month-18, 24 meeting ............................................. 119
    5.2.2 Other workshops ..................................................... 119
  5.3 Collaboration .................................................................... 119
6 Directory .................................................................................... 120
7 Copyright .................................................................................... 121
1 Summary

NanoHouse project in some words:

NanoHouse collaborative project is founded by the European Commission in the frame of FP7 programs: NMP-2009-1-3-1 & ENV2009-3-1-3-2 “Activities towards the development of appropriate solutions for the use, recycling and/or final treatment of nanotechnology-based products.

This project started January 2010 for a duration of 42 months (until 06/2013) and a total budget of 3.1 M€.

The current and projected applications of Engineered NanoParticles (ENPs) span a very wide range of industrial and consumer sectors such as: biomedicine, pharmaceuticals, cosmetics, new sources of energy, environmental analysis and remediation, material science. At the same time, the potential impact of these new materials their production and their life-cycle implications on the places where people live on Environmental Health and Safety (EHS) is a key issue regarding the future acceptability and sustainability of nanoproducts. In this perspective, buildings and individual houses are critical in that they constitute the major surrounding of people in developed countries.

The NanoHOUSE project concentrate on this issue and aims at promoting a responsible and sustainable development of nanomaterials in building industry through a Life Cycle Thinking approach.

The NanoHOUSE project focuses on the most commonly used ENPs in construction materials: nano-Ag, nano-TiO₂, and nano-SiO₂ comprised in large amounts in paints and coatings for indoor and outdoor applications.

The goal of this project is to gather and to generate, when missing, reliable scientific information and analysis, using appropriate methodologies to understand the potential EHS impacts of nanoproducts used in building (coatings and paints).

2 Background

Nanosciences and Nanotechnologies (N&N) provide many opportunities to significantly improve materials properties and sustainability. The current and projected applications of Engineered NanoParticles (ENPs) span a very wide range of industrial and consumer sectors such as: biomedicine, pharmaceuticals, cosmetics, new sources of energy, environmental analysis and remediation, material sciences.

At the same time, the potential impact of these new materials their production and their life-cycle implications on the places where people live on human health and the environment is viewed with apprehension by citizens. A growing body of scientific evidence indicates that exposure to some ENPs can lead to harmful effects. In the Nanotechnology Action Plan 2004 (COM(2004) 338), the European Commission highlighted that “R&D need to take into account the impacts of nanotechnology throughout the whole of their life-cycle”. In the “Nanosciences and nanotechnologies: An action plan for Europe 2005-2009 (2005)” it is emphasized as well: “Health, safety and environmental risks that may be associated with products and applications of N&N need to be addressed upfront and throughout their life cycle”. Therefore, the environmental and health consequences of these materials, their production and the life-cycle implications of the products deserve attention now, during the early stages of development. This is a key issue regarding the future acceptability and sustainability of nanoproducts.

As far as human chronic exposure is concerned, addressing the issue of safety, and consequently of acceptability of nanoproducts calls for a focus on the places where people live. In this perspective, buildings and individual houses are critical in that they constitute the major surrounding of people in developed countries. The NanoHOUSE project will concentrate on this issue.

Therefore, the NanoHOUSE project covers not only a group of a specific population, but all the population and addresses a complementary issue of FP6-Nanosafe2 project that was focused on the human exposure at the working place.

Indeed, through the use of many different types of ENPs such as silica (hardener, antirefraction effect), zinc oxide, titanium dioxide, cerium oxide (anti-UV) and silver (biocide), nanotechnologies have been introduced in construction materials: concrete, glass window, coatings for metallic pieces, anti-scratch floor coatings, concrete or wooden façade coatings, decorative paints, and anti-microbial coatings and plastics in hospital.
In the context of the trend to increase energy efficiency of buildings by thermal insulation, the demand for protecting outside façades with functional façade paints could increase. Façade paints products containing ENPs could for example be an alternative solution for façade paints containing hazardous biocides. Nanotechnologies also are expected to hold potential for example for antibacterial or air-purifying inside paints containing ENPs.

The NanoHOUSE project focuses on the most commonly used ENPs in construction materials nano-Ag, nano-TiO₂ and nano-SiO₂ comprised in large amounts in paints and coatings for indoor and outdoor applications.

The scope of the project is circumscribed to the release of ENPs during the post-production stages in the life cycle of both indoor and outdoor paints and coatings for housing.

3 What is NanoHouse

The goal of this project is to gather and to generate, when missing, reliable scientific information and analysis, using appropriate methodologies to understand the potential EHS impacts of nanoproducts used in building (coatings and paints).

A life cycle approach prospectively gathers information about the EHS aspects throughout all the life cycle stages of these products and identifies the data gaps and drives the precise needs of experimental work. Firstly, experimental work focuses on the quantification of the actual sources of ENPs during the use and ageing of indoor and outdoor coatings, during renovation and demolition operations and during their final disposal.

The main innovative aspects of the NanoHOUSE project are: (i) to consider the whole product life cycle in regard to EHS and (ii) to study the environmental behaviour and the toxicological effects of the actually released ENPs (“aged” ENPs), and to compare them with the pristine ENPs.

As an important component of the environmental and ecological system, NanoHOUSE aims at quantifying the uptake of released ENPs by plants and determining the impact of ENPs on those organisms.

NanoHOUSE aims at identifying and quantifying the effects on human health along the pathways of exposure to human in urban or residential environment. The major goal is to gain insight into the influence of ENPs transformations (“aged” vs pristine), routes of intake, duration of exposure on the biokinetics throughout the entire organism (in vivo tests) and the mechanisms of toxicity at the cellular level (in vitro tests) and to develop a Physiologically Based Pharmacokinetik model (PBPK) with a pulmonary dispersion model to integrate different parts of human health effect measurement.

Finally, NanoHOUSE will improve end of life treatments regarding ENPs release in the environment, and will participate to the development of sustainable and competitive nanoproducts by decreasing their potential to release ENPs. NanoHOUSE project will thus contribute to the development of appropriate solutions for the use of safe, sustainable and competitive nanoproducts in housing through their whole life cycle.

3.1 Summary of NanoHouse’s key strengths

The main outcomes of the project are:

- evaluate the risks associated with the use of ENPs in materials for housing,
- improve the sustainability of ENPs containing paints and coatings for housing and other applications by decreasing their release-ability,
- propose a generic risk assessment methodology tested for a selected group of nanoproducts that takes into account the specificity of actually released ENPs,
- support the regulation concerning risk assessment by contributing recommendations specific to nanoproducts considering the whole life cycle of these products and elaborating a first attempt of LCA,
- participate to the normalisation of release tests for certification of nanoproducts in construction and other applications,
- improve the current technical solutions for end of life treatments of nanoproducts,
- propose a decision-making tool for sustainable and competitive innovation and for nanorisk management addressed to manufacturers,
- promote nanoproducts social acceptability.

4 Organisation of NanoHouse

The NanoHOUSE project is structured around five scientific work packages (WP1-WP5) whose the previous aims and the interdependency are described hereafter and in the Table 1.
Table 1  Workpackages (WP) of NanoHouse

<table>
<thead>
<tr>
<th>WP</th>
<th>Title</th>
<th>Topic</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Life Cycle Thinking</td>
<td>This workpackage aims at investigating the potential health, safety and environmental (EHS) impacts during all life cycle stages of façade coating products containing ENP. A first attempt of a comparative Life Cycle Assessment (LCA) study in accordance with the ISO 14040 standards shall be established; supporting the comprehensive assessment of the EHS impacts along the complete life cycle and allowing a comparison with traditional façade coatings, not containing ENPs. This WP is designed to systematically combine the quantitative risk assessment data on toxicology (WP4) and environmental fate (WP3) with other relevant knowledge on EHS impacts during the life cycle stages of façade coatings such as exposure situations (WP2) during the use phase among others. The combination of Life Cycle Thinking with the current knowledge on risk assessment may provide a basis for informed decision making by the industry and regulators.</td>
</tr>
<tr>
<td>2</td>
<td>Source Identification</td>
<td>WP2 aims at simulating ENPs releases in conditions representative of ageing of the paints but also during critical renovation operations, to quantify the flux of ENPs released and to gain insight into the mechanisms of ENPs released. The physical-chemical properties of the ENPs released will be characterised. The so called “aged ENPs” produced will be then be delivered to partners of WP3 and WP4 for environmental fate and toxicological studies.</td>
</tr>
<tr>
<td>3</td>
<td>Environmental fate</td>
<td>Nanoparticles released from the outside of buildings directly into the environment are under the influence of physical, chemical and biological processes that determine their fate and behaviour and ultimately their influence on biota and possibly also humans. Aggregation and dissolution reactions may strongly affect the longevity and transport of the ENPs and thus their persistency and distribution to environmental compartments. This WP aims to investigate the reactions of released ENPs in water (WP3-2, in the soil (WP3-3) and in plants (WP3-4) then modelling the fate of the released ENP in the soil-water-plant-human system (WP3-5).</td>
</tr>
<tr>
<td>4</td>
<td>Hazard characterization</td>
<td>The mechanisms of action of fine and ultrafine particles on the human health are not yet fully understood. this WP focuses on processes such as translocation, changes in the cell-layer, oxidative stress, pulmonary (and systemic) inflammation. First in ENPs translocation, biokinetics and bioavailability studies in cellular and animal models (WP4-1) then in Specific citotoxicity study of aged ENPs compared to pristine ENPs in order to highlight health effects (allergy and asthma) to be expected of ENP being present in coating (WP4-2). Hazard of the original (pristine) material, but also the product in combination with its solvents (the whole commercial product), and the “aged” product after use and disposal, have to be considered.</td>
</tr>
</tbody>
</table>
| 5    | Safer use and waste management | This workpackage The assessment and properly management of end-of-life of nano-based products are key aspects to be investigated for successfully applying life cycle concepts and for developing eco-friendly products. The main rationale behind this WP is :  
1) to estimate the impacts caused by the end-of-life treatment based on a description of state of the art concerning waste management of nano-based applications (WP5-1) and on a determination of geomembrane permeability for ENPs (WP5-3)  
2) to provide appropriate solutions for the end-of-life treatment (WP5-2) and propose safety improvement of selected nanoproducts (WP5-4) |

Project dissemination, ethics and nanorisks issues (WP6)

The dissemination of the results takes place step by step. Firstly the reports will be the base for dialogues with industries and regulators. Secondly the project relevant information for industrial and private consumers will be disseminated on the NanoHOUSE website. Furthermore, short dissemination reports for the public at large will be broadcasted. An additional part on the risk management will be built up based on the results of the project and made available through the e-learning interactive software Nanosmile1 already developed in the frame of NanoSafe2 project and available on the Internet. Finally, the project will give recommendations for the transferability of the methods that have been validated to other application domains where nanomaterials are increasingly used such as cosmetics, automotive, aeronautics and space.
5 NanoHouse Reports and Events

5.1 Progress to date

A report on the potential EHS impacts during the life cycle of façade coatings nanomaterials is achieved and published. This study is disseminated to a large public through the EC DG Environment public web site. New strategies in order to overcome the existing gaps on the life cycle assessment for engineered nanomaterials are being established. A precautionary matrix is established in order to estimate the degree of respect of nanosafety issues. A life cycle model including production, use and end of life is under development. In order to be able to identify emission sources, estimation of ENPs release via the dry and wet route from paint panels provided by the industrial partners in WP2 were performed. Coated panels were exposed to indoor and outdoor aging as UV light, heat and humidity cycles. A Taber standard method for investigating wear resistance to abrasion by "dry route" was chosen. The Taber abrasion device was started and coupled to an aerosol measurement system in order to measure dust released. Estimation of ENPs release induced by abrasion via the dry route before and after UV aging and weathering was achieved for different paints. Experimental and theoretical study of the release of ENPs just deposited from the surfaces and nanoparticles from the product matrix were performed. The particles < 50 nm are trapped on the surfaces by Van der Waals forces. The release of free nanoparticles is seen only when nanoparticles are not perfectly dispersed within the matrix. A new bench for measuring airborne ENPs released by sanding from paint panels was set-up. The sanding device was coupled to an aerosol measurement system in order to measure dust released. Estimation of ENPs release by sanding is in progress. Collected information and data were used to define a specific protocol for leaching tests for the nano-based products considered in the NanoHouse project. A new bench for measuring ENPs released by leaching was set-up. Leaching tests on painted panels, weathered and unweathered coated panels are in progress. Radio labelled Ag-nanoparticles and TiO2 fluorescent nanoparticles have been synthesized. Behaviour of these ENPs in soils was performed. The penetration of TiO2 and Ag nanoparticles through the sand columns is determined using gamma ray and fluorescent detection. The penetration of TiO2 and Ag nanoparticles inside the plants and the transfer of NPs in plants was estimated by different techniques. In vitro citotoxicity using Ag, TiO2 and SiO2 nanoparticles was determined. The effect of these Nanoparticles on human bronchial epithelial cells was estimated as well. “Aged” ENP’s (TiO2, SiO2) in high quantities (few grams) for environmental fate experiments (WP3) and toxicological tests (both in vivo and in vitro) (WP4) were supplied. The size and distribution of the particles/agglomerates obtained were evaluated through SEM, DLS and Laser Granulometer. The aged ENP behaviour will be compared with the pristine ENP in the environment and toxicology. A detailed literature research concerning release of nanoparticles from the end of life of nano-product was performed. End of life treatment solutions for waste paints containing ENP are currently under development. A protocol for leaching through waste containing nanoparticles was established. Leaching tests are in progress from paint waste containing nanoparticles.

Finally, two websites (internal and external) have been designed (http://www.nanosmile.org, http://www.nanohouse.cea.fr/scripts/home/publigen/content/templates/show.asp?P=55&L=EN&ITEMID=2).

5.2 Events

5.2.1 Month-18, 24 meeting

The Month-18 meeting was held in Paris France the 27th-28th June 2011. The Month-24 meeting was held in Leuven Belgium the 24th-25th November 2011.

5.2.2 Other workshops

The NanoHOUSE project has been represented in the "Nanosafety Cluster" in Lausanne 03/2011 (Peter Wick), Barcelona 05/2011 (Roland Hischter), Budapest 05/2011 (Peter Hoet), Rome 11/2011 (L.Golanski, P. Hoet). Nanohouse has been represented at the 21st SETAC EUROPE ANNUAL MEETING, in Milan (Italy) May 2011, at the 5th International Symposium on Nanotechnology-Occupational and Environmental Health, August 9-12, 2011, Boston, USA. The midterm dissemination workshop will be organized at Dublin within the QNano conference end of February, 2012.

5.3 Collaboration

NanoHOUSE and NanoSustain projects have established a collaboration which aims to promote a responsible and sustainable development of nanomaterials in the building industry through a Life Cycle Thinking approach. The two projects have agreed to collaborate on weathering and abrasion tests. Anne Thoustrup Saber (DK) from the NanoSustain project sent samples and Dario Cervellati (GFC) carried out weathering tests. This samples will be abraded at CEA by Arnaud Guiot, L.Golanski and release will be estimated before and after weathering.
## 6 Directory

*Table 2 Directory of people involved in this project.*

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NanoSafetyCluster - Compendium 2013

NanoLyse
Nanoparticles in food: Analytical methods for detection and characterisation

Call identifier FP7-KBBE-2009-3, Grant Agreement: 245162  Website: http://www.nanolyse.eu
Coordinator: Stefan Weigel, RIKILT- Institute of Food Safety; Stichting Dienst Landbouwkundig Onderzoek, part of Wageningen UR, The Netherlands

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<tr>
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<td>3</td>
<td>Danmarks Tekniske Universitet</td>
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<td>4</td>
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Contents
1 Summary .................................................................................... 123
2 Objectives .................................................................................. 124
3 Concept and structure ............................................................... 124
4 Project tasks and results ........................................................... 125
  4.1 WP1: Reference Materials ...................................................... 125
  4.2 WP2: Rapid imaging and screening methods ........................ 126
  4.3 WP3: Coupled separation / characterisation methods for inorganic nanoparticles ................................................ 128
  4.4 WP4: Coupled separation / characterisation methods for organic and functionalised ENPs ........................................... 129
  4.5 WP5: Dissemination and training ........................................... 129
5 NanoLyse Project outcomes .................................................... 130
  5.1 Scientific publications ............................................................ 130
  5.2 Open days ............................................................................... 131
  5.3 Training workshops ................................................................ 131
6 Directory..................................................................................... 131
7 Copyright.................................................................................... 132

1 Summary

The NanoLyse project focuses on the development of validated methods and reference materials for the analysis of engineered nano-particles (ENP) in food and beverages. The developed methods aim to cover all relevant classes of ENP with reported or expected food and food contact material applications, i.e. metal, metal oxide/silicate, carbon and organic (encapsulate, carrier) ENP. Priority ENPs have been selected out of each class as model particles to demonstrate the applicability of the developed approaches, e.g. nano-silver, nano-silica, fullerenes and organic nano-carriers systems. Priority is given to methods which can be implemented in existing food analysis laboratories. A dual approach is followed. Rapid imaging and screening methods will allow the distinction between samples which contain ENP and those that do not. These methods are characterised by minimal sample preparation, cost-efficiency, high throughput and will be achieved by the application of automated smart electron
microscopy imaging and screening techniques in sensor and ELISA formats. More sophisticated, hyphenated methods will allow the unambiguous characterisation and quantification of ENP. These will include elaborate sample preparation techniques, separation by flow field fractionation and chromatographic techniques as well as mass spectrometric and electron microscopic characterisation techniques. The developed methods will be validated using the well characterised food matrix reference materials that are produced within the project. Small-scale interlaboratory method performance studies and the analysis of a few commercially available products claiming or suspect to contain ENP will demonstrate the applicability and soundness of the developed methods.

The project has a duration of 45 months (2010 – 2013).

2 Objectives

“The Scientific Committee makes a series of recommendations; in particular, actions should be taken to develop methods to detect and measure ENMs [engineered nanomaterials] in food/feed and biological tissues, to survey the use of ENMs in the food/feed area, to assess the exposure in consumers and livestock, and to generate information on the toxicity of different ENMs.” (EFSA, Scientific Opinion of the Scientific Committee on the Potential Risks Arising from Nanoscience and Nanotechnologies on Food and Feed Safety, The EFSA Journal (2009) 958,1-39)

In addition to research institutes busy with development and toxicological evaluation of nanomaterials, there is an urgent need both for official food control entities and industry for analytical methods that allow the routine detection of engineered nanoparticles in food, as well as for reference materials for the validation of analytical methods and for proficiency testing of laboratories. The NanoLyse project addresses these needs by the following objectives.

- Development of reference materials for the analysis of nanoparticles in food and beverages matrices
- Development of sample preparation methods for the detection of nanoparticles in food
- Development of rapid imaging and screening methods for nanoparticles in food
- Development of analytical methods for the identification, characterisation and quantification of inorganic and organic nanoparticles from the food matrix
- Dissemination and training of the new methods to relevant stakeholders

PRODUCTS

According to the objectives of the project NanoLyse will deliver a number of different products to the general public, the scientific community and in particular to specific stakeholders involved in the risk assessment and legislation for the use of nanoparticles in food as well as in the analysis of engineered nanoparticles in these matrices for exposure assessment, monitoring and quality control purposes. The deliverables include:

- Protocols for the analysis of different engineered nanoparticles in food and beverages (WP2, WP3, WP4), including sample preparation methods
- Reference materials for nanoparticles in food matrix (for use within the project, available to external laboratories which participate in the interlaboratory method performance studies) (WP1)
- Protocols for the reproducible production of such reference materials (WP1)
- Method validation concept for engineered nanoparticles in food (WP1)
- Publications in international journals on the scientific results of the project (WP1, WP2, WP3, WP4)
- Open days for stakeholders and the interested public (WP5)
- Training workshops for the transfer of the developed methods to stakeholder laboratories (WP5)

3 Concept and structure

CONCEPT

Basically the concept of NanoLyse is to merge the technologies which are available for engineered nanoparticles (ENP) analysis in other disciplines, e.g. materials and environmental sciences, into the analytical strategies and procedures characteristic for the food safety area, taking into account the very specific physico-chemical properties of nanoparticles as compared to their macro-scale or dissolved analogues.

A current analytical strategy in food safety monitoring is a dual approach comprising screening (fast, qualitative) and confirmatory (high standard, quantitative) methods. Screening methods are designed to sort out negative samples in a fast, cost-efficient, automated high-throughput approach. The samples identified as suspect positives are subjected to a more sophisticated method which allows the unambiguous identification and quantification of the target analytes.

NanoLyse adopts this approach by the development of two levels of methods:

(i) imaging and screening methods for a rapid decision on the presence of ENP in food samples and

(ii) methods for the full identification, characterisation and quantification of ENP in food.

Rapid analysis will be achieved in WP2 by electron microscopy (EM) imaging as well as by two screening assays: a sensor assay for automated high-throughput analysis and an immunoassay in ELISA format for direct implementation of ENP analysis even in basic food laboratories. For the precise characterisation and quantification the most suitable separation (i.e. flow field fractionation, hydrodynamic and size exclusion chromatography) and detection techniques (e.g. EM and mass spectrometry) will be coupled into hyphenated methods in WP3 (inorganic ENP) and WP4 (organic ENP). The developed methods will be validated using
the well characterised food matrix reference materials that will be produced within the project (WP1).

STRUCTURE

The project is structured into four RTD and two supporting work packages (fig. 1). All WPs are closely linked with each other to ensure maximum synergies. WP1 supplies all method developers with characterised ENP dispersions and test and reference materials for method development and validation. All method development WPs (2-4) collaborate very closely on the sample preparation via the respective inter-WP working group (WG), especially in the first phase of the project. In the same way WP 3 and WP4 collaborate on the analytical separation techniques. All RTD WPs (1-4) contribute to the dissemination and training activities which are organised by WP5. WP6 supplies all information necessary for the successful execution of the project to all WPs and collects all data needed for the regular reporting and the monitoring of the progress of work.

Figure 1: Interrelations between Work packages (WP) of NanoLyse

4 Project tasks and results

4.1 WP1: Reference Materials

OBJECTIVES

Reference materials are essential to calibrate analytical instruments, develop and validate test methods and assess the performance of individual laboratories. Up to date only few reference materials are available (as aqueous suspensions) for nanoparticles (most of them not relevant for food related applications). Therefore, Work package 1 has three main objectives, namely the production of reference materials for method development and method validation, the development of a solid and sound approach for method validation and compilation of the knowledge gained in the project into a document outlining a reproducible preparation of ENP containing food reference materials, including information on processing, homogeneity and stability.

- Supply of well defined and characterised engineered nanoparticles and labelled analogues

- Production and characterisation of engineered nanoparticles reference materials

- Development of a metrologically robust method validation approach for engineered nanoparticles in food

ACTIVITIES

WP1 supports WP2, WP3 and WP4 by preparing and supplying suspensions of labelled and non-labelled engineered nanoparticles (ENP) and food materials spiked with suspensions of ENPs. The suspensions will be characterised for their purity and ENP concentration, and the ENP size distribution.

A number of relevant ENPs has been purchased or produced, and processed into appropriately characterised ENP suspensions. Four different types of model particles have been chosen, namely metal nanoparticles, metal-oxide nanoparticles, fullerenes and organic nanoparticles. These particles have been sourced and, in the first stage of the project, aqueous suspensions of these four types of particles have been prepared. These materials are used by the other project partners for spiking experiments during method development. The aqueous suspensions are tested for homogeneity and stability, thus ensuring that the materials fulfil all requirements for reference materials.

In the second phase of the project, food materials will be spiked with the same materials. Also these spiked food materials will be tested for homogeneity and stability. These food reference materials will finally undergo characterisation by intercomparison, thus allowing assessment of trueness of the methods involved.

A metrologically robust method validation approach for the analysis of ENP in food will be developed in WP1 with input from the advisory board on validation and standardisation requirements. The protocols and reports on the production of reference materials will be available for future use in e.g. proficiency testing of laboratories for the detection of ENPs in food.

RESULTS

Nanoparticle dispersions

Stability testing of silica and Ag nanoparticle dispersions continued and no significant change in particle diameter was observed. The silica and silver solutions have been characterised by dynamic light scattering (DLS), centrifugal liquid sedimentation (CLS) and electron microscopy (EM).

Organic nanoparticles made of cross-linked gelatine were obtained. Two suspensions of different mass-fractions were purchased. These particles are of high scientific interest, as they clearly highlight the differences between various methods: CLS and DLS mainly "see" the large particles, whereas GEMMA measures mainly the more numerous small particles. The suspensions were tested for homogeneity and distributed.

Finally, suspensions of C60 fullerenes in toluene were produced, tested for homogeneity and stability and were distributed to the project partners.

Nanoparticle-doped matrix materials

After preparation of suspension, work focused on the preparation of candidate reference materials that contain nanoparticles in food matrices.
Tomato soup was prepared and doped with silica nanoparticles at two mass fractions. As methods for the particles themselves are still being developed, homogeneity and stability was tested via the total silicon and ash content. The materials were found sufficiently homogeneous and were distributed.

Chicken meat was homogenised and doped with Ag particles, mimicking migration from Ag-integrated cutting boards, cling films etc. Several modes of preservation were tested to identify a method that not only preserves the integrity of the meat, but also the integrity of the nanoparticles in the meat matrix. Finally, the experiments succeeded and materials were distributed.

Finally, a sports drink was doped with cross-linked gelatine nanoparticles at two different mass fractions. Different ways of preservations were used to prevent bacterial degradation of the gelatine particles. Homogeneity was tested and the materials were distributed.

Validation of methods for the detection and quantification of engineered nanoparticles in food

The potential impact of nanomaterials on the environment and on human health has already triggered legislation requiring labelling of products containing nanoparticles. However, so far, no validated analytical methods for the implementation of this legislation exist. A generic approach for the validation of methods for detection and quantification of nanoparticles in food samples has been developed in NanoLyse and published in Food Chemistry. It proposes validation of identity, selectivity, precision, working range, limit of detection and robustness, bearing in mind that each "result" must include information about the chemical identity, particle size and mass or particle number concentration. This has an impact on testing for selectivity and trueness, which also must take these aspects into consideration. Selectivity must not only be tested against matrix constituents and other nanoparticles, but it shall also be tested whether the methods apply equally well to particles of different suppliers. In trueness testing, information whether the particle size distribution has changed during analysis is required. Results are largely expected to follow normal distributions due to the expected high number of particles. An approach of estimating measurement uncertainties from the validation data is given.

Towards a harmonised validation guideline

Given the rapid progress in the development of next generation nanoparticles as well as of respective analytical techniques and instruments the standardisation of measurement methods may not always be the most suitable and up-to-date approach. Therefore, NanoLyse proposes to work also on the standardisation of the quality of analytical results. This can best be achieved via harmonised validation guidelines for respective methods. This will assure a high level of reliability of the results obtained with methods validated according to the guidelines. The NanoLyse validation approach should be seen as the starting point towards harmonised validation guidelines for nanoparticle analysis. In an iterative process this first document will be further developed upon the experiences gained in the NanoLyse project as well as by taking into account the experience of the broader nano-analytical community. To this end, we invite comments and suggestions to further refine the present version (via www.NanoLyse.eu).

4.2 WP2: Rapid imaging and screening methods

OBJECTIVES

Presumably, many foods will not contain any engineered nanoparticles. Applying rapid, cost-efficient and robust methods to distinguish the samples which actually contain engineered nanoparticles from the majority which doesn't, would allow to focus more laborious quantitative methods on those samples. The objective of WP2 is to develop such rapid analytical methods, based on imaging and screening techniques, for providing qualitative and semi-quantitative data on engineered nanoparticles in different food matrices. The developed methods should enable a rapid decision if any target particles are present or absent in a food sample.

- Sample preparation methodology tailored to imaging and screening methods for engineered nanoparticles in foods
- A simplified electron microscopic imaging tool with automated smart image analysis for the rapid detection of engineered nanoparticles presence in different food matrices
- Screening assays for engineered nanoparticles in sensor and ELISA format

ACTIVITIES

In order that the developed techniques can be applied broadly in the future, the work package will explore a range of engineered nanoparticle types that are relevant to food such as: metal-based (Ag), metal oxides (SiO2), and organic nano-carrier systems.

Electron microscopy: A limited number of imaging methodologies (SEM, TEM, including inherent characterisation of elemental composition by EDX, EELS) will be compared initially for aqueous engineered nanoparticles dispersions. The most suited will be selected for further method development. A major challenge will be the preparation of the food materials for analysis. The work package will therefore explore sample preparation techniques for a range of matrices starting with non-complex systems (i.e. water), and finally moving to more complex matrices. Work will be focused on easy to use and low-cost techniques e.g. resin embedding. For more complex samples a range of more sophisticated techniques will be available, e.g. capsules to enable imaging under fully liquid conditions. Finally the most successful sample preparation and detection methods for different engineered nanoparticle types will be combined into fully validated methods. In order to achieve automation and high throughput automated object-based image analysis will be explored and further developed.
Screening assays: Two approaches will be followed:
(i) an ELISA approach for engineered nanoparticles for direct implementation in basic food labs,
(ii) a sensor approach based either on bio- or physico-chemical recognition of functionalised, encapsulate or metal(oxide) engineered nanoparticles, respectively, for automated high throughput analysis. Both methods will be validated according to the standards for screening methods.

The validation will include the analysis of a limited number of real samples from the market, claiming or suspect to contain engineered nanoparticles.

RESULTS

The electron microscopy (EM) based techniques applied in the project allow the detection and measurement of nanoparticles (NPs) within the food matrices using simple and time efficient sample preparation protocols. This makes EM a suitable tool for rapid screening of NPs in food. For solid food samples, transmission electron microscopy approach (TEM) yields optimal results, since the technique allows detailed characterisation of NPs embedded in the nanometric food layer. For liquid food samples, scanning electron microscopy (SEM) allows very good contrast for NPs. Good contrast means that images can be taken at relatively low magnification, and therefore:

- Contain more number of measurable NPs in a single image,
- Increase the volume of analysed sample,
- Decrease the time required for analysis,
- Cover relatively broad particle size range (difference between size of the smallest and the largest particle approximately 20 fold),
- Distinguish NPs from the matrix elements by contrast.

Both approaches (TEM and SEM) allow the measurement of mean diameter of NPs in food with a similar relative intermediate precision standard deviation of 15-17%.

The image data are further analysed for particle size/shape parameters by specially designed object based image analysis software based on eCognition platform for measurement of NPs in food application (Trimble, Germany). Using this approach, it is possible to measure NPs of complex morphology and variable contrast, as well as to distinguish the primary particles from clusters. The use of object based image analysis results in more precise particle size measurement and enumeration than obtainable by pixel based image analysis and thus presents a major advancement for reliable measurement of particle size distribution.

Figure 3: Particle size measurement by pixel based image analysis leads to a) particle size overestimation and object merging or b) particle size underestimation; whereas object based image analysis c) outlines and measures the particle sizes more accurately

Two screening assays based on two different approaches for determination of nanoparticles in food were developed.

A test kit for screening of soft drink for the presence of organic nanoparticles by ELISA methods has been developed. Cross-linked gelatine nanoparticles (gelatine NP) were chosen as reference material for organic NPs. After production and characterization of specific polyclonal antibodies against this organic NP, ELISA method was developed and further optimized. A prototype test kit was produced and was evaluated for its performance and practicability under routine conditions. After the prototype testing, a full validation of the newly developed method was performed according to the European Decision 2002/657/EC and the validation approach produced by WP1 for a screening method.

Surface Plasmon Resonance (SPR) biosensor assays for rapid detection of silver nanoparticles in food and surface water as well as for organic nanoparticles in beverages have been developed. The assays for organic NPs target two different types of particles: cross-linked gelatine as well as β-lactoglobulin-epigallocatechin gallate (β-Lg-EGCG). The recognition of the target nanoparticles is achieved via specific antibodies. In the case of β-Lg-EGCG a size specific sample preparation step was included to distinguish the engineered particles from native β-lactoglobulin naturally present in e.g. dairy products. The assays have been optimised and validated for soft drinks which are expected to be enriched with nano-formulated supplements such as the antioxidant EGCG. This extends the applicability of the SPR sensor to the rapid screening for silver and organic nanoparticles in food and beverages.

Figure 4: Test kit for screening of soft drink for the presence of cross-linked gelatine by ELISA methods

Figure 5: The SPR biosensor for silver nanoparticles; a: principle, b: analysis cycles for different Ag NP concentrations
4.3 WP3: Coupled separation / characterisation methods for inorganic nanoparticles

OBJECTIVES

If engineered inorganic nanoparticles are present in foods their identity and quantity needs to be determined, e.g. for proper exposure assessments or the testing for any (future) legal limits. The goal of WP3 is to develop methods for the unambiguous characterisation and quantification of inorganic nanoparticles in food, including sampling, sample preparation, analytical separation and instrumental detection. Separation and detection will be coupled on-line into reliable quantitative methods.

- Sampling and sample preparation methodologies tailored to the quantitative detection of inorganic engineered nanoparticles in foods
- Validated methods for the determination of inorganic engineered nanoparticles in food extracts, based on size separation (HDC, FFF), size determination (light scattering) and specific detection (ICP-MS)

ACTIVITIES

For quantification purposes most inorganic engineered nanoparticles (ENPs) will require a sample preparation step to isolate them from the matrix. Potential sample preparation techniques include physical separations, wet digestion or thermal treatments. Due to the presence of residual matrix components in sample extracts an additional analytical separation of the engineered nanoparticles will be inevitable and field-flow fractionation (FFF) and hydrodynamic chromatography (HDC) have already been recognized as highly suitable for this. In cases of very small particles which do not aggregate, size exclusion chromatography (SEC) may be a third option. Light scattering techniques and spectrometric techniques (ICP-MS, ICP-OES, UV-DAD and fluorescence) will be used for particle sizing and detection.

A protocol for representative sampling of engineered nanoparticles containing food will be developed on a statistical basis taking into account the size distribution and number density of ENPs at a range of concentrations above the detection limits. The sample preparation methodologies will follow the track of

1) reducing complexity of the sample matrices with minimum alteration of the virgin engineered nanoparticles, including chemical or enzymatic matrix digestion, followed or accompanied by
2) a physical separation step as ultracentrifugation, ultrafiltration, density separation, liquid/liquid extraction, preparative SEC and the split-flow thin cell (SPLITT) technique to finally
3) transfer the engineered nanoparticles into a state compatible with FFF and HDC.

Analytical fractionation methods for ENPs isolated from the food matrix will be established based on HDC and FFF. Suitable detection methods will be selected (e.g. UV-DAD, static/dynamic light scattering and ICP-MS or-OES) and further optimized for the ENPs received from WP1 in simple matrices. Performance characteristics will be determined. The following criteria will be used for further consideration of a given methodology: recovery of ENPs, fractionation efficiency and detection selectivity, repeatability, sensitivity. The selected hyphenated methods will be fully validated. The validation will include the analysis of a limited number of real samples from the market, claiming or suspect to contain ENP.

RESULTS

The main task of WP3 is to systematically develop and optimize Field Flow Fractionation (FFF) separation methods coupled to UV-Vis, dynamic light scattering (DLS), multi angle light scattering (MALLS) and Inductively Coupled Plasma Mass Spectrometry (ICP-MS) for the detection, sizing and quantification of nanoparticles in food, namely silver (AgNPs) in chicken meat and silica (SiO2-NPs) in tomato soup, and further develop suitable sample preparation techniques taking into account the stability of the nanoparticles which can be endangered during the sample preparation or by the sample handling.

Applying the systematic and stepwise approach fully quantitative methods for characterization of SiO2-NP in tomato soup and Ag NP in meat were developed. Isolation of NP from the food matrix was achieved by acid digestion followed by particle stabilization (SiO2) and by enzymatic digestion (Ag). The stable particle suspensions were suitable for further size characterization by FFF and online coupled ICP-MS analysis. Standard operating procedures (SOP) are established for the Ag and SiO2 nanoparticles methods. Based on the SOP, an inter-laboratory validation study is scheduled.

Figure 6: AF4 fractogram of 100 nm SiO2 with light scattering detection with different injected volumes (blue 100µL, red 50µL, green 10µL), and respective rms radius fit (triangles). The retention time of the 50 and 10 µL were artificially shifted for better readability.

The FFF approach is complemented by single particle ICP-MS. spICP-MS is carried out on a conventional ICP-MS instrument in time resolved mode which allows to measure individual particles in an element specific way. This approach addresses the EC recommendation for the definition of a nanomaterial in the best possible way as it is capable of measuring real number based concentrations in a chemically specific way. A method for Ag particles in meat is currently optimised and validated.

Interlaboratory method performance study for the determination of Ag nanoparticles by single particle ICP-MS

For a broader application of the spICP-MS method it is essential to proof its transferability to other laboratories. To that end, RIKILT has organised an interlaboratory method performance study for the developed spICP-MS method, in collaboration with JRC IHCP & IRMM and partners of the NanoLyse project. The goal of this study is to evaluate the precision and robustness of the method under interlaboratory conditions. 23 high level laboratories from Europe, the US and Canada participated in the study, including Universities.
research centres and instrument manufacturers. The first round was dedicated to the analysis of Ag nanoparticles in migration simulants. First results indicate a reasonable interlaboratory reproducibility of the method. A second round will address Ag NP in a food matrix.

4.4 WP4: Coupled separation / characterisation methods for organic and functionalised ENPs

OBJECTIVES

In the case that engineered organic nanoparticles are present in foods their identity and quantity needs to be determined, e.g. for proper exposure assessments or the testing for any (future) legal limits. The aim of WP4 is to develop respective methods for the detection and characterisation of organic nanoparticles in food, including sampling, sample preparation, analytical separation and instrumental detection.

- Sampling and sample preparation methods for organic and surface functionalised engineered nanoparticles in food matrices
- Validated combined separation and detection methods for organic/functionalised engineered nanoparticles in food matrices based on flow separation techniques and mass spectrometry

ACTIVITIES

The detection and identification of organic, and functionalized, engineered nanoparticles (ENPs) in food items is difficult since the shell of organic engineered nanoparticles is often of a similar nature as that of many food constituents (e.g. proteins, lipids, carbohydrates). A first necessity is therefore the availability of a technique capable of discriminating between organic engineered nanoparticles and residues of matrix constituents. Techniques with that potential will be selected and sampling, sample preparation and separation/fractionation methods for organic engineered nanoparticles from food will be developed from there. Finally, a separation and detection technique will be combined and validated as a complete method for the detection and characterization of organic engineered nanoparticles in food.

RESULTS

Sampling, sample preparation and chromatographic separation and detection methods for organic and carbon ENPs in food were developed and validated. Protein-, carbohydrate-, lipid-based and full-carbon particles are used as model nanoparticles.

Filtration and size exclusion chromatography have been combined with hydrodynamic chromatography (HDC) and off-line MALDI-TOFMS to form a complete procedure for the detection and characterisation of organic nanoparticles. A standard operating procedure was written and the method was fully validated for the determination of liposome-type nanoparticles in beverages.

Ultra-high-performance liquid chromatography combined with time-of-flight mass spectrometry (UHPLC-TOFMS) is used for the screening for ENPs based on polysorbates. This high sensitive method focuses on specific in-source fragments of the shell of the nanoparticle or the active substance in the nanoparticle. Another method uses direct analysis in real time (DART) combined with MS detection. With this technique it is possible to screen polysorbate-based nanoparticles in beverages in a time frame of 60 seconds. Both techniques allow the detection of organic nanoparticle components in fruit juices at levels below 1%. Both techniques were validated and standard operating procedures were written.

Liquid chromatography combined with mass spectrometry (LC/MS) is used for the separation of carbon-based nanoparticles, in this case C60-fullerenes. A method was developed for the determination of C60-fullerenes in olive- and rapeseed oil. As far as we know this is the first method for determination of this compound in fatty food matrices. The method is validated for olive oil and is now being scaled-up for the detection of C60-fullerenes in fish.

A parallel differential mobility analysis (pDMA) method has been developed for the separation of organic nanoparticles in liquid suspensions. The method has been validated using a condensation particle counter (CPC) for detection. In parallel collection of nanoparticles using an electro nanoparticle sampler allows further analysis with techniques like electron microscopy (EM), atomic force microscopy (AFM) or matrix assisted laser desorption ionization mass spectrometry (MALDI-TOFMS).

4.5 WP5: Dissemination and training

OBJECTIVES

The NanoLyse project intends to exploit the knowledge which is generated within the project in the most beneficial way in various aspects. This includes consumer food safety, competitiveness of the European economy as well as scientific progress. Main goal of WP5 is to ensure that the knowledge and methods which are developed within NanoLyse are distributed to stakeholders and exploited in a proper way.
• Active dissemination of results to stakeholders and scientists via website, newsletter, publications and presentations at stakeholder and scientific events
• Technology transfer and training to consortium members and external end-users (governmental, education, industry)

ACTIVITIES

WP5 addresses the key knowledge transfer activities: transfer to the scientific community, to risk assessors and policy makers (e.g. EFSA, DG Sanco, national Food Safety Authorities) and potential users of the developed analytical tools (statutory laboratories, food analysis contract laboratories, food industry, SMEs), but also within the project consortium.

Core elements of the dissemination strategy are

(i) The public NanoLyse website (www.NanoLyse.eu)
The website is a main portal for the dissemination of the results dedicated to general public, official authorities, food and feed sectors and scientists.

(ii) The e-Newsletter
Bi-annual e-newsletter is distributed actively to stakeholders and other interested parties. The newsletter informs on the scientific progress in the analysis of engineered nanoparticles in food and beverages within NanoLyse, as well as on upcoming events relevant for this field.

(iii) Presentation of results
The scientific outcome of the project will be published in peer reviewed international journals and presented at international scientific conferences, after careful consideration of IPR issues. In addition, two “NanoLyse Open Days” are organised to present the approach and the outcome of the project to a wider public, focusing on potential stakeholders of the developed methods.

(iv) Training workshops
will be organised at the end of the project. Goal of these hands-on workshops will be the technology transfer of the developed tools to laboratories which have or will have the need to analyse food and beverages for presence and levels of engineered nanoparticles. In the first place laboratories involved in the risk assessment and in the (future) monitoring of food for engineered nanoparticles will be addressed, but interested parties from food industry and private contract food laboratories will also be invited.

5 NanoLyse Project outcomes

Representation of the project at various events

Upcoming regulations on nanoparticles in consumer products and food trigger an urgent need for analytical methods to reliably determine engineered nanoparticles in these complex matrices. The JRC (IHCP) and NanoLyse recognized this need and organised an expert workshop in Brussels on 23−24 April 2012, on “Development and validation of analytical methods for nanoparticles in food and consumer products (in support of the implementation of the EC definition of a nanomaterial)“. On the first day international experts in this field, including academia, research centres and instrument manufacturers, presented and discussed different approaches to tackle this issue. On the second day, the state of the art was presented to European regulatory and risk assessment stakeholders. Representatives from the European Commission (DG RTD, DG SANCO, DG Enterprise) as well as from the European Food Safety Authority (EFSA) discussed with the present experts the current and future possibilities and indicated their needs and requirements. This exchange was considered to be very valuable for both sides and made this workshop a very successful event.

NanoLyse is also actively involved in the EU NanoSafetyCluster, a DG RTD NMP initiative, to maximise the synergies between the existing FP6 and FP7 projects addressing all aspects of nanosafety including toxicology, ecotoxicology, exposure assessment, mechanisms of interaction, risk assessment and standardisation. The approaches developed in NanoLyse to reliably characterise nanoparticles in complex matrices were presented to the participants of the SIINN ERANet workshop in Grenoble on 29−30 May 2012 as well as during a workshop of the three recently launched projects INSTANT, SMART-NANO and NANODETECTOR to foster cooperation, learn from each other and share materials and best practices.

Furthermore, NanoLyse is constantly busy with the dissemination of its results to potential users of the developed methods. Governmental stakeholders from the EU member states were addressed during the annual meeting of the EFSA Scientific Network for Risk Assessment of Nanotechnologies in Food and Feed in April 2012, in Parma while the NanoLyse contribution to FoodDrinkEurope’s “Fifth Years of Nanotechnology Dialogue” on 19th October 2012 event was more directed to private stakeholders from the food and beverage industry.

Training program

The intra consortium training programme, aimed at the exchange of expertise among the project participants and supporting young scientists in the development of their careers, continued. Training for two trainees was held at UVIE, Vienna, Austria, during March 2012. Other training for one trainee was organised by DTU, Copenhagen, Denmark, in May 2012. In addition, study stay of one PhD student from ICT Prague at DTU, was organised within the ERASMUS program during period of July-Sep 2012.

The NanoLyse consortium members represented the project at several scientific events with the aim to disseminate information both about the project goals, its activities and knowledge generated within the project.

5.1 Scientific publications

Thomas P.J. Linsinger, Gert Roebben, Conxita Solans, Roland Ramsch (2011), Reference materials for measuring the size of nanomaterials in food by electron microscopy, Trends in Analytical Chemistry 30:18-27


Ruud Peters, Guillaume ten Dam, Hans Bouwmeester, Hans Helsper, Günter Allmaier, Frank vd Kammer, Roland Ramsch,
Conxita Solans, Monika Tomaniová, Jana Hajslova, Stefan Weigel (2011), Identification and characterization of organic nanoparticles in food, Trends in Analytical Chemistry 30:100-112

Frank Von der Kammer, Samuel Legros (2011), Separation and characterization of nanoparticles in complex samples (food/environment) by FFF, Trends in Analytical Chemistry 30:425-436


V. Dehalu, S. Weigel, S. Rebe, R. Grombe, R. Löbenberg, P. Delahaut (2012). Production and characterization of antibodies against crosslinked gelatin nanoparticles and first steps toward developing an ELISA screening kit, Analytical and Bioanalytical Chemistry 403:2851–2857


5.2 Open days

The first NanoLyse Open Day had been organised on the 2nd November 2011, during the 5th International Symposium on Recent Advances in Food Analysis (RAFA 2011), held from the 1st to the 4th November 2011 in Prague, Czech Republic.

The Open day was attended by more than 50 participants from various countries and different sectors as well as from national and European authorities. They actively engaged in discussions with the present NanoLyse scientists. The level of interaction was remarkably high and there was an intense exchange not only on the presented goals and results of the project, but also about future needs and possible collaborations.

The 2nd NanoLyse Open day will be organised as satellite event of the EUROFOODCHEM XVII conference, which is held in Istanbul, Turkey, 9 May, 2013. The progress of the project will be presented through posters, (video) demonstration activities, hand-outs and oral explanations. Opportunities for discussions on the presented project activities with the involved scientists will be available.

Figure 9: Impression of the first NanoLyse Open Day

5.3 Training workshops

Training workshops are scheduled for the first half of 2013 for the technology transfer of the developed methods to potential end-users of the new methods for the analysis of engineered nanoparticles in food and other complex matrices.

The NanoLyse workshop on „Single particle ICP-MS for Ag NP analysis“ will be organised on 25–27 March 2013 at RIKILT Wageningen UR, The Netherlands.

NanoLyse workshops on „Field Flow Fractionation“ are scheduled on 17–19 April 2013 at DTU, Copenhagen, Denmark and UVIE, Vienna, Austria.

Both workshops will provide introduction in respective field and overview of individual procedures, followed by hands-on training.
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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

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Contents

NANOMICEX .................................................................................... 133
1 Summary .................................................................................... 133
2 Background ............................................................................... 134
3 Concept and Objectives ............................................................ 135
   3.1 Project Concept .................................................................. 135
   3.2 Project Objectives ............................................................ 135
4 Overall view of the Workplan .................................................... 135
5 Advances over the state of the art ........................................... 136
6 Progress to date ........................................................................ 137
7 Conclusion To-Date & Plan for the Future ................................ 139
8 Directory .................................................................................... 139
9 Copyright .................................................................................. 140

Project Duration: 1 April 2012 – 30 March 2015
Project Funding: 3.5 Mio. EUR

1 Summary

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 or 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 determine 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 an uncertain behaviour.

2 Background

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 as 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, TiO2, Al2O3 and Fe3O4), 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 Overall view 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

<table>
<thead>
<tr>
<th>WP nº</th>
<th>WP Title</th>
<th>WP Leader</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Characterization of engineered nanoparticles</td>
<td>HU</td>
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<tr>
<td>2</td>
<td>Development and selection of functional modified nanoparticles</td>
<td>YU</td>
</tr>
<tr>
<td>3</td>
<td>Hazard Assessment</td>
<td>HWU</td>
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<tr>
<td>4</td>
<td>Exposure Assessment</td>
<td>IOM</td>
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<tr>
<td>5</td>
<td>Risk Management and Control Measures</td>
<td>ITENE</td>
</tr>
<tr>
<td>6</td>
<td>Nano SLCRA: Adaptive Streamlined Life Cycle / Risk Assessment of nanoparticle-based inks and pigments</td>
<td>LEITAT</td>
</tr>
<tr>
<td>7</td>
<td>Industrial Case Studies</td>
<td>ARDEJE</td>
</tr>
<tr>
<td>8</td>
<td>Project Coordination and Management</td>
<td>ITENE</td>
</tr>
<tr>
<td>9</td>
<td>Project dissemination and training</td>
<td>NIA</td>
</tr>
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</table>

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

<table>
<thead>
<tr>
<th>WP</th>
<th>Title</th>
<th>Description</th>
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<tbody>
<tr>
<td>1</td>
<td>Characterization of engineered nanoparticles</td>
<td>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 CoAl2O4 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.</td>
</tr>
<tr>
<td>2</td>
<td>Development and selection of functional modified nanoparticles</td>
<td>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 newly developed within the project. The new NPs synthesized will be characterized by imaging techniques such as SEM and AFM along with other characterization techniques.</td>
</tr>
<tr>
<td>3</td>
<td>Hazard Assessment</td>
<td>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.</td>
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<td>4</td>
<td>Exposure Assessment</td>
<td>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.</td>
</tr>
<tr>
<td>5</td>
<td>Risk Management and Control Measures</td>
<td>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 determine the most effective techniques to reduce and mitigate the hazard and exposure, and therefore minimize the risk, focusing on safe use.</td>
</tr>
<tr>
<td>6</td>
<td>Nano SLCRA: Adaptive Streamlined Life Cycle / Risk Assessment of nanoparticle-based inks and pigments</td>
<td>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.</td>
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The workpackages and their interdependence are shown schematically below:
5 Advances over 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 personnel. In this sense, the surface modifications of metal oxide nanoparticles, AgNPs, CdSe quantum dots (QDs) and mixed-metal oxides (CoAl2O4) 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 LC50/EC5 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 ENMs with imaging and spectroscopic techniques such as SEM, AFM, DLS, UV/Vis Spectroscopy, IR, Raman spectroscopy and surface-enhanced Raman scattering (WP 1).
2. Exploration of the surface chemistry alteration strategies for Al2O3, TiO2, Fe2O3, CoAl2O4 and ZnO nanoparticles. A variety of ligands with biological origin such as carbohydrates and oligonucleotides has been tested (WP 2).
3. Cytotoxicity screening for all modified NPs, including the preparation of a complete literature review of biological effects of the nanoparticles involved in the project (WP 2- WP3).
4. A complete review of literature evaluating the possible biological effects and mode-of-action, environmental fate and behaviour of the particles researched in Nanomicex (WP 3).
5. First meeting to define activities under WP 4 and 5, aimed at Identifying the main strategies to collect information on exposure and the experimental set up to assess the effectiveness of PPEs.
6. Participation in several international events related to the research topic of the project, mainly workshops and conferences.
7. 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:

**WP 1. Characterization of engineered nanoparticles**

The panel of nanoparticles to be studied within the project was defined during the first months of the project. The decision was taken on the basis of the interest showed by the industrial partners included in the project, as well as in view of the potential application of the ENMS to enhance the properties of pigments and ink/paint formulations. Deliverable 1.1 includes a complete review of the applications of the nanotechnology in the reference sectors of the project, including a brief description of the main improvements that can be gained using ENNs as pigments or fillers in ink and paint formulations. The report concludes that ZnO is probably the material of highest interest, being mentioned by all the partners that have proposed nanoparticles candidates. ZnO has been extensively employed in industry for disparate technological applications, such as UV-radiation shield material and pigments. It is also used as a pigment in inks and paints for their final formulation. In addition, among other oxide materials proposed, cobalt aluminate (CoAl2O4), titanium dioxide (TiO2) and aluminium oxide (Al2O3) are also of high interest.

Secondly, the research teams of professors Nicola Pinna (HU) and Mustafa Culha (YU) have completed the physicochemical characterization of the target NPs.

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.

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

**WP 2. Development and selection of functional modified nanoparticles**

A full literature search was undertaken to access the knowledge of the current surface modifiers used for coating the ENMs. The toxicity of the possible coating materials and the NMs prepared from these coating 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.

**WP 3. Hazard Assessment**

The work has focused on the literature review and initiating the toxicological and environmental practical work. The ENRHES project was used to get an initial set of publications for some of the particles, and a number of searches on Pubmed, Web of Science and other databases was conducted.

Regarding the experimental work, the research group of the Heriot-Watt University have conducted a number of experiments, including ecotoxicity test with algal exposures to TiO2 and ZnO and human toxicology, where WST-1 and LDH assays (cell viability and cytotoxicity) have been completed for J774, C3A and A549 cells (macrophages, hepatocytes and alveolar epithelial cells).

**WP 4. Exposure Assessment / WP 5. Risk Management and Control Measures**

Both WPs are starting to work. A meeting to define the activities to be conducted was held in Edinburg on January 16, 2013, where Martie Van Tongeren and Carlos Fito, WPs leader of WP 4 and WP 5 respectively defined the key priorities and next steps.


WP 5 will start next march 2013. It will be led by the research group of LEITAT, being focused on the characterization of the risks posed by the use of the ENMs along their life cycle.

**WP 7. Industrial case studies**

The activities within WP 7 are related to the validation of the risk management strategies by industrial partners. At present only the surface modifiers can be validated by the industrial end-users in order to avoid changes on the propertied of finished products.

Dr. Culha’s team is searching for the optimal amount of coating without altering the color of the nanoparticles. Recent results demonstrate the possibility of changing the color of the nanoparticles but also other physicochemical properties such as conductivity.

**WP 8. Project Dissemination**

A number of activities have been conducted, including the preparation and publication of the project web site, the publication of the first project brochure and the presentation of the project in two relevant conferences:

- Safe implementation of nanotechnologies: Common challenges. May 2012
- Safety issues and Regulatory Challenges of Nanomaterials Symposium. May 2012
7 Conclusion To-Date & Plan for the Future

The project is an early stage at the moment, but the Partners are working in the selection of the better surface modifiers able to reduce the toxicological and ecotoxicological profile of the selected ENMs. Current results show how the toxicity can be reduced, however the properties of the ENMs are also modified, being necessary new studies in coordination with the industrial partners to avoid changes in the properties and functions of the ink and paint formulations.

Regarding the hazard assessment, the consortium will continue with the experimental work to determine the potential hazards posed by the nanoparticle panel to human health and the environment. In terms of human toxicity, a range of cell lines that represent significant exposure and target organs in the human body will be conducted. The approach to the ecotoxicological work will be similar to the one adopted for the toxicity studies. The practical work will focus on three well-established OECD test models, to cover different ecological strata and guilds.

The exposure assessment will be conducted through the study of peer-reviewed literature and feedback from industrial users related to the current industrial conditions, as well as the characterization of airborne NPs by means of consistent exposure measurements.

Regarding the evaluation of the effectiveness of current risk management measures, workplace controls as personnel protective equipment, ventilation, filtration and other controls will be checked.

A complete critical analysis of the current standard methods to evaluate the effectiveness of the risk management measures will be conducted.

The experimental work will start in March 2013, where a complete battery of test will be conducted to characterize performance factors such as the barrier efficiency for skin protective equipment, particle penetration potential for protective clothing and filtration, as well as leakage efficacy for respirators and gloves.

The activities within WP 6 will start working on the definition of hot spots along the ENMs life cycle. A Streamlined LCA will be used to evaluate the potential environmental impacts of the nanoparticles panel along its entire life cycle, considering relevant modeling approaches in combination with relevant impact categories.

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 mid-term report at the end of the year.

8 Directory

Table 3 Directory of people involved in this project.

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# 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](http://www.nanomile.eu)  
Coordinator: Eugenia (Éva) Valsami-Jones, University of Birmingham, Edgbaston, Birmingham B15 2TT, United Kingdom

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## Contents

1. Summary .................................................................................... 142
2. Project Objectives and Organisation ........................................ 142
3. Key Challenges being addressed by NanoMILE .......................... 143
4. NanoMILE’s Expected Impacts ................................................. 147
5. References ................................................................................. 147
6. Directory ................................................................................... 148
7. Copyright ................................................................................... 150
1 Summary


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 is a unique partnership of the highest calibre European and US institutes in nanosafety, offering the full complement of expertise required to understand the mechanisms of interactions of engineered nanomaterials with living systems and the environment.

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, capable of contributing to or advancing the innovations of NanoMILE to industrial applications.

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

2 Project Objectives and Organisation

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 the classification of nanomaterial based on their potential toxicity and to create a universally applicable framework for nanosafety.

The specific objectives, placed here in the chronological order of their 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 the 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/organisms/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**

### 3 Consortium Description

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.

### 4 Key Challenges being addressed by NanoMILE

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 [1], 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 as 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, synthesise/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 project QNano) protocols, thus avoiding problems of unreliable cross referencing of experimental results. The MNM characterisation data will be integrated into the extensive database of nanoparticle information contained within the NANOhub database, which is
hosted and maintained by JRC, thus utilising and expanding this resource.

**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 and predictions of MNM behaviour are currently 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 form or in an 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 TiO₂ 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 highlighted [1], 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 biota. 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. In addition, the availability of in vivo repeated dose toxicity studies is limited. 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 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
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 toxicants. 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), 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 range of sizes. Carbon based materials will form a separate class of materials to test, although similarities in issues related to surface modifications apply across all MNM 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 recommend 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

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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.
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 intend to link the state of knowledge in research with the innovation processes in industries in order 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 will have a WP and 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.

5 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 uncertainty 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.

6 References

12. Auffan et al., 2009, Towards a definition of inorganic nanoparticles from an environmental, health and safety perspective, Nature Nano, 4: 634-641
### Table 1. NanoMILE Stakeholder groups and dissemination approaches for each

<table>
<thead>
<tr>
<th>Who</th>
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<tr>
<td><strong>Academic peers</strong></td>
<td>- Scientific data regarding different classes of NMs&lt;br&gt;- Scientific data / descriptions of methods / models / assays etc.&lt;br&gt;- Assay / method protocols</td>
<td>Conference presentations&lt;br&gt;Publications&lt;br&gt;Training Schools&lt;br&gt;Best practice documents&lt;br&gt;Assay / method protocols</td>
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<td><strong>Industry</strong></td>
<td>- Design rules for safer MNMs&lt;br&gt;- QSAR/QPAR tools&lt;br&gt;- Methods / assays suitable for standardization / generation of data for regulatory dossiers</td>
<td>Trade shows&lt;br&gt;Demonstration models Brokerage event&lt;br&gt;IP offer via exploitation plan</td>
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<td><strong>Risk managers</strong></td>
<td>- Ranking of MNMs on basis of single / correlated physico-chemical or biological descriptors, from HT/HC screening &amp; later from “omics” evaluation&lt;br&gt;- Tools for QSARs/QPARs</td>
<td>Best practice guidance&lt;br&gt;Short guides to the QS/PARs tools&lt;br&gt;Workshops targeted to risk managers Short targeted brochure</td>
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<td><strong>Regulators</strong></td>
<td>- Ranking of MNMs on basis of single / correlated physico-chemical or biological descriptors&lt;br&gt;- Tools for QSARs/QPARs&lt;br&gt;- Assays / platforms for validation as suitable for generation of regulatory dossier data</td>
<td>Summary of decision &amp; ranking criteria from regulatory viewpoint&lt;br&gt;Description of application of QSAR/QPAR tools for regulatory evaluation&lt;br&gt;Presentation of outputs at stakeholder event, e.g. with Nanosafety cluster&lt;br&gt;Recommendations around methods for use in generation of regulatory dossiers for MNMs</td>
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<td><strong>Policy makers</strong></td>
<td>- Cost-benefit tools (e.g. QPARs / QSARs to identify optimal trade-off between MNM functionality and safety)</td>
<td>White paper on the ranking of MNMs and the approach&lt;br&gt;Recommendations on appropriate / inappropriate used of MNMs based on their safety ranking</td>
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<tr>
<td><strong>Standardisation organisations</strong></td>
<td>- Assay protocols&lt;br&gt;- Methodology descriptions&lt;br&gt;- Round Robin results&lt;br&gt;- Data platforms</td>
<td>CEN Liaison&lt;br&gt;Contribution to OECD / ISO / CEN&lt;br&gt;Workshop Agreements</td>
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<tr>
<td><strong>NGOs and society</strong></td>
<td>- Need for the project&lt;br&gt;- Planned benefits from the project&lt;br&gt;- Nanotechnology in society&lt;br&gt;- Women in science</td>
<td>Project flyer Project website Popular press articles High school debates&lt;br&gt;“Open day” as part of Annual meetings</td>
</tr>
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### Directory

**Table 2. Directory of people involved in the NanoMILE project**

<table>
<thead>
<tr>
<th>First Name</th>
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NanoPolyTox

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

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</table>

Contents

Summary ...........................................................................................................151
1 Concept and Objectives ...........................................................................152
  1.1 Background .........................................................................................152
  1.2 Objectives .........................................................................................152
    1.2.1 Specific Objectives ......................................................................152
2 Methodology and Associated Work Plan ....................................................153
  2.1 Synthesis and characterization of raw NM (WP1) .................................153
  2.2 Development of polymer nanocomposites (WP2) .................................153
  2.3 Weathering of polymer nanocomposites (WP3) .....................................153
  2.4 Development of non-destructive separation techniques: Proof of concept of NM recycling and disposal techniques (WP4) .........................153
  2.5 Toxicological and ecotoxicological evaluation of NM at different stages of their life cycle (WP5) .................................................................153
  2.6 Theoretical studies and LCA analysis (WP6) ..........................................154
  2.7 Technological solutions for the recycling and disposal of NM included in polymeric matrices (WP7) .................................................................154
  2.8 Dissemination and exploitation activities (WP8) ....................................154
  2.9 Project Management (WP9) ..................................................................155
  3 Current status of the project ....................................................................155
    3.1 Synthesis and characterization of NM ...............................................155
    3.2 Development of polymer nanocomposites .........................................155
    3.3 Weathering of polymer nanocomposites ...........................................155
    3.4 Development of non-destructive separation techniques: Proof of concept of NM recycling and disposal techniques ........................................156
    3.5 Toxicological and ecotoxicological evaluation of NM at different stages of their life cycle .................................................................156
    3.6 Theoretical studies and LCA analysis ..................................................157
    3.7 Mechanical and Chemical recycling ...................................................157
  4 Directory ..................................................................................................158
  5 Copyright ..................................................................................................158

Summary

NanoPolyTox main objective consists of monitoring the evolution (nanomaterials properties and toxicity) of three families of nanomaterials (nanotubes, nanoclays, metal oxide nanoparticles) during their life cycle as nanofillers in polymeric hosts. This project will include monitoring of the chemical and physical properties of the nanomaterials and their toxicity from the synthesis, during processing, aging (use) and recycling to their end of life (disposal) quantifying their migration and/or release to the environment...
during their aging (use). The biological and environmental fate of these nanomaterials will be studied monitoring their physical-chemical and toxicological properties. The theoretical analysis of the data obtained during the project will lead to the development of predictive models for the impact of nanomaterials on human health and environment. These studies will include the LCA analysis of nanomaterials included in polymeric host to determine their global environmental impact. Additionally, three recycling strategies will be considered in order to give solutions for the recovery of innocuous nanomaterials toxic. For this purpose, exhaustive evaluations for the selection of adequate dissolving and extraction methods to separate the nanomaterials from the polymeric matrix will be carried out. The strategies proposed for the recycling process will be the following: The direct mechanical recycling of nanocomposites, the recycling of nanomaterials and polymers obtained by novel chemical separation techniques based on nanofiltration using tailored nanofiber-based filters, and the recycling of polymers and immobilization of toxic nanomaterials in inert matrices.

1 Concept and Objectives

NANOPOLYTOX is 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).

1.1 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 the 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 proposes to study the evolution of nanomaterials physicochemical and toxicological properties during their life cycle to evaluate their global environmental impact. Furthermore, NanoPolyTox studies will include the development of innovative strategies for the recycling and disposal of nanomaterials that are included in polymeric matrices.

1.2 Objectives

The main goal of NanoPolyTox is 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 project is focused on after-production stages and will address 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 will provide, at laboratory scale, technological solutions for recycling and final treatment of nanotechnology-based products.

1.2.1 Specific Objectives

- 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 further 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 physicochemical 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 and toxic nanomaterials including the development of a new, efficient and cost effective chemical recycling technology based on specific nanofiber filters
- Development of new solutions for the disposal of toxic nanomaterials based on the inclusion of specific nanofibers filters containing the toxic nanomaterials, into xerogel matrices by sol-gel processes and sintering
- 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

NanoPolyTox will provide important information on a general concern regarding the degradability of polymer nanocomposites and their direct impact on human health and environment. It is expected that these results can prevent or minimize the exposure
of workers and consumers, and releases to environment of hazardous nanomaterials.

2 Methodology and Associated Work Plan

NanoPolyTox work plan is divided in seven technical work packages (WP), which are described below.

2.1 Synthesis and characterization of raw NM (WP1)

The nanomaterials selected for NanoPolyTox studies are the following: Carbon nanotubes (MWCNT), metal oxide nanoparticles (TiO₂, ZnO and SiO₂) and nanoclays (small and large). The selection was based on the analysis of the list of nanofillers used in polymer nanocomposite industry. The nanomaterials selected are the most use in the plastic industry for a variety of applications.

In this WP1, the selected nanomaterials will be prepared using different methods of synthesis (depending on the type of nanomaterial) and tailored with different functional groups to match their surface properties (polarities, chemical functionalities) with three different types of polymeric hosts (selected and described in WP2).

The studies carried out in this work package will include the full characterization of the nanomaterials synthesized. Physical and chemical data of the different nanomaterials will be described in different technical cards. The technical cards will consist of ID cards of the nanomaterial during the project containing all the relevant data about their properties.

2.2 Development of polymer nanocomposites (WP2)

The objective of WP2 is to generate polymeric nanocomposites including the nanomaterials obtained in WP1 using the typical industrial processes such as extrusion and injection techniques. The selection of the polymeric matrices will be based mainly on the polymer industrial uses and in their chemical nature, covering several fields of applications and presenting different chemical properties.

The polymeric matrices will be loaded with a 3% of the nanomaterial, in each case. The nanocomposite pellets obtained from the extrusion processes will be injected to obtain the polymeric demonstrators that will be further studied in the project. The distribution of the nanomaterials in the polymeric matrix and the physical properties of the nanocomposites will be determined by different analytical techniques.

2.3 Weathering of polymer nanocomposites (WP3)

In WP3 the main objective is to simulate the outdoor use of polymeric nanocomposites, evaluating the degradation of the polymers under external conditions (sun-light and climate simulations). Nanomaterials, unmodified polymers and polymer nanocomposites will be aged under the selected conditions. All these materials will be submitted to weathering cycles (hours of ageing in a climatic chamber under the selected conditions) that could then be extrapolated to real aging time based on the results obtained doing aging studies in real time conditions. The materials will be aged in climatic chambers specifically tailored for the collection of any potential releases of nanomaterials from the polymeric matrices during weathering.

The weathered nanomaterials and nanocomposites will be characterized using the same techniques as in WP1 and WP2 in order to obtain comparable data. The potential released nanomaterials collected during the aging process will be characterized by ICP-MS in order to quantify the released nanomaterials.

2.4 Development of non-destructive separation techniques: Proof of concept of NM recycling and disposal techniques (WP4)

The conventional recycling processes used in the polymer industry are based on mechanical processes obtaining polymers with different properties and consequently will be used in other applications. In the case of polymer nanocomposites, in which the additives are nanomaterials (expensive additives), the main interest will be to recover these additives and reuse them maintaining their properties.

Therefore, in this project the recovery of nanomaterials included in polymeric matrices will be carried out in two steps: First, polymeric nanocomposites will be dissolved by non-destructive techniques and then, the colloidal solution will be filtered to recover the nanomaterials. The chemical nature of the polymer will determine the dissolving method and in the case of high resistant polymers, those will be recycled using mechanical methods.

In a first approach, the nanomaterials will be separated from the polymeric matrices by optimized conventional methods: Centrifugation and membrane nanofiltration. In a second approach, novel organic/inorganic filters based on nanofibers will be generated using electrospinning technology. The filtration capability of these novel filters will be compared with the conventional filters used for nanofiltration. The two techniques will be evaluated in terms of costs and industrial viability.

The properties of the nanomaterials collected after filtration will be evaluated by the analytical methods described in WP1. These data will provide information on the evolution of the physicochemical properties of the nanomaterials along their life cycle.

2.5 Toxicological and ecotoxicological evaluation of NM at different stages of their life cycle (WP5)

The toxicological studies proposed in NanoPolyTox will cover the in vitro human health acute toxicity and ecotoxicity in aquatic and terrestrial environments. A preliminary in vitro screening will be carried out with the following samples: The raw nanomaterials, the nanomaterials separated from the processed polymeric nanocomposites, the nanomaterials separated from the aged polymeric matrices and the nanomaterials separated from mechanical recycled polymeric nanocomposites. The more toxic nanomaterials obtained from the in vitro screening (number determined during the course of the project) will be evaluated by in vivo assays to determine their biological and environmental fate.
These studies require the dispersion of nanomaterials in an aqueous medium which it is not toxic itself for the biological systems studied. The dispersant cannot be unified due to the high diverse nature of the nanomaterials studied. Therefore, protocols of dispersion will be developed for all the nanomaterials studied within the project.

The rational to select the set of cell lines, for in vitro human health studies, is based on the way nanomaterials enter inside the body and to represent the main target organs (liver, kidney, skin, gastrointestinal tract, lung and lymphocytes) that could be affected by nanomaterials. All these cell lines will be tested on the following assays: Viability assay, proliferation assay and apoptosis assay. Additional in vitro test will be carried out with specific cell lines: The absorption assessment with Caco-2 cells and the evaluation of biodistribution mediated toxicity with a hepatic cell line. In the in vivo assays, the nanomaterials will be administered intravenously setting up at least three treatment groups and a control group.

The environmental fate and ecotoxicity of nanomaterials will be investigated using six different assays (in vitro and in vivo). The investigations will be performed with aquatic and terrestrial organisms. The following studies are anticipated: Fish embryo toxicity (FET) test to evaluate the early life stages of Zebra fish larvae, a fish dietary bioaccumulation study, studying the effect of nanomaterials on soil-dwelling organisms (Collembolan) in vivo, distribution of the nanomaterials in soil compartments (adsorption-desorption in soil), aerobic transformation of nanomaterials in water/sediment compartment and aerobic and anaerobic transformation of nanomaterials in soil.

The outputs from these studies will be the inputs in the LCIA analysis of nanomaterials.

### 2.6 Theoretical studies and LCA analysis (WP6)

In this work package the main objective is to analyze all the data obtained on the physical, chemical and toxicological properties of the nanomaterials over their life cycle and use it for the development of theoretical models to predict the human health and environmental impact of nanomaterials. This theoretical analysis of the data will allow developing predictive models to evaluate the impact of nanomaterials along their life cycle. All the studies will be combined to determine the critical factors influencing: the structural changes, migration and toxicity of the nanomaterials.

Additionally, LCA will be performed in accordance with the ISO standards, which establishes four interrelated basic stages for LCA: the goal and scope, the inventory analysis, the impact assessment, and the interpretation. A comprehensive framework describing the impact caused by engineered nanomaterials included in polymeric matrices (nanocomposites) over their entire life cycle will be obtained. The inputs and outputs collected over the life cycle will be then converted into the corresponding potential environmental impacts. The sum of such environmental impacts will represent the overall environmental effect of the nanomaterial along their life cycle. This will enable a quantitative and qualitative assessment of the overall impacts and trade-offs for nanomaterials. New algorithms will be postulated to obtain the impact indicators for the new characterization factors that have been included in the inventory specific to nanomaterials. These algorithms will be proposed taking into account all the theoretical studies done in this work package.

### 2.7 Technological solutions for the recycling and disposal of NM included in polymeric matrices (WP7)

NanoPolyTox project proposes two strategies for the recycling and one strategy for the disposal of nanomaterials included in polymeric nanocomposites:

- Direct mechanical recycling of the nanocomposites for new applications. The samples obtained after recycling will be fully analyzed.
- Filtration and separation of the innocuous nanomaterials from the polymeric host using nanofiber-based filters specially designed for nanomaterials filtration in WP4.
- Filtration, separation and inertization of the toxic nanomaterials in glass matrices: Filtration of the nanomaterials with metal oxide nanofiber-based filters able to react/interact strongly with the toxic nanomaterials, then introduction of the charged metal oxide nanofiber filters in a xerogel by sol-gel processes and final sintering.

### 2.8 Dissemination and exploitation activities (WP8)

The dissemination plan will be the divulgation of the main innovative aspects evolving during the development of the project, in accordance with IPR restrictions. All partners will be involved in the definition of the dissemination strategy, which will be included in the detailed dissemination plan.

On a scientific level, the dissemination activities will be carried out through publications in specialized journals in the areas of nanotechnology, toxicology, polymers and material science. Wider dissemination will be achieved via a more general strategy for attaining a broad coverage of the project to a wide range of public.

The results of the project will be presented at different events (workshops, technical conferences, fairs and exhibitions) organized by the members of the consortium and in other potentially interesting events that could be planned by other organizations.

Additionally, and to promote the dissemination and collaboration of NanoPolyTox with the four projects financed in 2009 in the area of nanosafety, the active participation on the Nanosafety Cluster by the coordinator and by the members of the consortium is expected. It will allow efforts to be joined on direction of establishing guidelines and providing data about the safety of nanoparticles and nanomaterials within the EU territory.

The partners will analyze and validate the primary and secondary market potential for the developments of the project activities, and structure a market penetration & development plan accordingly.
2.9 Project Management (WP9)

This work package covers the management and coordination of the project. All planned activities will be closely monitored and if necessary, corrections will be performed. The coordinator will be responsible of coordinating the overall running of the whole project and, with the help of the Project Management Team, will ensure that all planned activities are pursued.

3 Current status of the project

Nanopolytox is a 3-year project which started in May 2010; the advances on the project for the last 18 months will be described below.

3.1 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 whole 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 the flame spray pyrolysis process 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). The physicochemical characterization of all the nanomaterials synthesized was carried out using the following analytical techniques: Transmission Electron Microscopy (TEM), Scanning Electron Microscopy (SEM) analyses and X-Ray Scattering techniques (XRS and XRD) and to determine the size crystallography and geometry of the nanomaterials, Dynamic Light Scattering (DLS) measurements to determine the hydrodynamic radius in solution, and BET analyses for the porosity and surface area determination. The chemical characterization included determination of the chemical composition of nanomaterials, their surface functionalities and their stability using Inductively Coupled Plasma Mass Spectrometry (ICP-MS), Fourier Transform Infrared Spectroscopy (FTIR), Ultraviolet-visible Spectroscopy (UV-vis) and ζ-potential, respectively. The data collected was included in the technical card which will be the ID of the nanomaterials through all their life cycle.

3.2 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 compatibilization and dispersibility of the nanomaterials in the polymeric matrices were evaluated by microscopy analysis (SEM). ICP-MS and TGA analysis were used to determine the concentration of nanomaterial in the polymeric matrices after each step of processing (extrusion and injection). Furthermore, physical characterization of the polymer nanocomposite demonstrators was focused on mechanical properties and thermal resistance determination (DMA, Dynamometric tests, HDT and VICAT, DSC).

The results obtained showed that most 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 ºC higher) and 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 have been introduced in the technical cards.

3.3 Weathering of polymer nanocomposites

The climatic chambers were tailored for the weathering tests to be done within the NanoPolyTox project. The demonstrators developed in WP2, raw nanomaterials and unmodified polymers were subjected to the selected ageing conditions (combination of climate and sunlight radiation during 1000 h, under the modified normative ISO 4892-2) and the potential releases of nanomaterials from the polymeric matrices during ageing processes were collected and quantified (some quantification still in progress).

Aged nanomaterials in the powder form were dried and analyzed after being submitted to the whole process of ageing. All the nanomaterials but nanoclays showed alterations compared to the corresponding non-aged nanomaterial. The main difference is the hydration of all the nanomaterials. Furthermore, in some cases, FT-IR indicates the modification of nanomaterial surface chemistry. FT-IR results were supported by the results obtained by BET analyses, which show a significant change in the surface area, pore volume and/or pore diameter of the material. 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. TGA confirmed these results and also showed a thermal destabilization of carbon nanotubes. ICP-MS analyses showed important metal leaching in SiO$_2$ and 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 (e.g. PP with MWCNT or PA6 with TiO₂), but in other cases there is no protection or even embrittle the material and do them more thermally unstable (e.g. PA6 with MWCNT).

To evaluate the migration of nanomaterial from the polymeric matrices, nanocomposites are being analyzed by TEM and SEM and compare with those before ageing. These results will be complemented with the data obtained for the nanomaterial recovered from the simulated rain during aging. Furthermore, analysis by ICP-MS is in progress to allow nanomaterial quantification in the nanocomposite after ageing.

Furthermore, the materials released from the polymeric matrices during ageing have been recovered from the collected water by freeze-drying (lyophilisation). Quantification of released materials and determination of material composition using different analytical techniques (BET, FTIR, TGA, ICP-MS, DSC) is in progress. First results showed that only degradation products from the polymer are released from MWCNT nanocomposites, while for metal oxide nanocomposites there is a high presence of nanomaterial in the released materials. The release was low in all cases (< 0.7 %), but their hazard and health risk will depend on their NM content and toxicity.

For comparison, it was designed an additional experiment outdoors with the nanocomposites samples. In this case, 5 specimens of each nanocomposite have been exposed to external outdoors with the nanocomposites samples. In this case, 5 specimens of each nanocomposite have been exposed to external conditions during a year. These materials are being analyzed and the data obtained will be used to determine the equivalence of the accelerated aging in the weathering chamber with a real ageing process in a Mediterranean climate.

### 3.4 Development of non-destructive separation techniques: Proof of concept of NM recycling and disposal techniques

The main objective of this WP is 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 physicochemical properties of the nanomaterials. From the different methodologies found, were selected those ones with the mildest conditions and then tested with raw nanomaterials. The results indicated that the most affected nanomaterials were the metal oxide nanoparticles changing their surface chemistry or their degree of functionalization. Even though, these results cannot be totally extrapolated to the effects on the nanofillers of the nanocomposites, because the polymeric matrix can protect the nanomaterial.

These methodologies have been applied to the polymeric nanocomposites and the colloidal solution obtained will be then filtered to separate the nanomaterials from the polymeric matrices. Different methodologies have been applied for each polymer to their different dissolving behavior.

Dissolution of PP nanocomposites resulted very difficult (only aggressive treatments led to the dissolution of the nanocomposite materials), therefore calcination was chosen as separation technique to recover nanomaterials from these nanocomposites. Calcinations were carried out at 430 °C to not affect nanomaterials properties (TEM showed no sintering occurred) and almost complete recovering of NM could be achieved.

EVA nanocomposites could only be dissolved in hot toluene and recovery of nanomaterials from these solutions was carried out by filtration with nanofiber-based filters. Therefore, ceramic nanofiber filters have been developed by means of electrospinning technique and characterized for their morphology and separation efficiency. These filters even though they are useful for the filtration of other nanoparticles disperse in low density media; they were not useful for the recovery of nanofillers from EVA nanocomposites. The high density of the solution and the amount of solvent needed for separation of small quantities of nanofillers, made the method not applicable. The alternative method for recovery of NM from EVA matrix was calcination of the nanocomposites.

Furthermore, PA6 nanocomposites could be easily dissolved and up to 60% of metal oxide nanoparticles and MWCNT could be recovered after centrifugation processes, with the exception of ZnO nanoparticles that could not be recovered due to the degradation of ZnO NP under the dissolving conditions. Only 11-18% of nanoclays were recovered using this method, characterization of the recovered material showed that the functionalization was lost during the process.

### 3.5 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 have been carried out
case by case and the dispersion protocols have been described for each nanomaterial. The dispersants selected for the studies were Bovine Serum Albumin (BSA), Fetal Bovine Serum (FBS), Tween 20, Sodium Citrate and MilliQ H₂O. The control over the stability of nanomaterials in the dispersion medium was studied with different analytical techniques: UV-Vis spectroscopy, ζ-potential analysis and DLS. The data collected were analyzed and the best dispersion medium was selected for each nanomaterial.

The toxicity of raw nanomaterials, both aged and non-aged, and nanomaterials extracted from aged and non-aged nanocomposites have been 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, have also been 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. The toxic potential of zinc oxide nanoparticles was higher in human cell lines than in the standard parameters of the fish embryo test, although they did affect embryo hatching. This suggests that zinc nanoparticles may not be able to cross the yolk sac.

Several studies have been carried out for investigating the toxicity and the environmental fate of the nanomaterials. These studies include in vitro and in vivo ADME studies, terrestrial toxicity, bioaccumulation in fish, and adsorption-desorption studies. Analyses of the samples (ICP-MS, TGA, TEM, etc.) are ongoing.

Moreover, a model for predicting environmental fate of nanomaterials and their potential ecotoxicological and human health effects has been developed. Theoretical fate, ecotoxicological and human health factors have been obtained for MWCNTs (input data obtained from this project and the literature). The data generated has been used to perform a LCA study of this the MWCNT-polymeric nanocomposite. This and the remaining LCA that will be performed will include all life stages of each nanomaterial, and the data will be presented as a global aggregate and by life stages, to facilitate transparency and future use of the data.

3.7 Mechanical and Chemical recycling

Recycling (mechanical or chemical) of the nanomaterials included in nanocomposites of different nature and their final disposal is envisaged in WP7 of the project. Furthermore, the data obtained will be used as input parameters in the LCA (WP6). The main technique used in the plastic industry for recycling is mechanical recycling. Therefore in this WP PP and EVA nanocomposites were recycled using this method to obtain composite materials with novel properties. Chemical recycling techniques combined with centrifugation were applied to PA nanocomposites in order to recover the nanofillers and reuse it. The physical properties of the nanocomposites obtained after recycling processes were analyzed to compare with non-recycled nanocomposites. Ageing of the new nanocomposites is ongoing and their properties monitored. Furthermore, solutions to disposal of nanomaterials recovered from nanocomposites are given for identified toxic NPs (ZnO NPs). ZnO NPs recovered after calcination processes were encapsulated in ceramic composites by vitrification technique. Finally, studies are ongoing to evaluate the potential releases of toxic NM from the ceramic nanocomposites generated. The composites will be submitted to adverse conditions and NM release will be quantified.
4  Directory

Table Directory of people involved in this project.

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NanoPUZZLES

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

Contract Agreement: Grant agreement 309837
Website: http://www.nanopuzzles.eu
Coordinator: Dr Tomasz Puzyn, University of Gdańsk, Faculty of Chemistry, Gdańsk, PL

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Contents

1 Summary .................................................................................... 159
2 Background .............................................................................. 160
3 What is NanoPUZZLES ............................................................ 160
3.1 Expected impact of NanoPUZZLES ........................................ 160
4 Organisation of NanoPUZZLES .................................................. 161
4.1 WP 1: NanoDATA .................................................................. 161
4.2 WP 2: NanoDESC ............................................................... 161
4.3 WP 3: NanoINTER .............................................................. 162
4.4 WP 4: NanoQSAR ............................................................... 162
4.5 WP 5 and 6: Management and dissemination .......................... 162
5 Directory .................................................................................. 163
6 Copyright .................................................................................. 164

Project Duration: 1 February 2013 – 31 December 2015
Project Budget: 1.2 Mio. EUR (EU contribution: 980’000 EUR)

1 Summary

The main objective of the NanoPuzzles project is to develop, within two years, a package of computational algorithms for the 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 use of computational techniques and (ii) will define a basis for development of further modelling techniques for a large variety of nanoparticles.

The project will focus on two groups of compounds: (i) inorganic engineered nanoparticles (metal nanooxides) and (ii) carbon nanoparticles (carbon nanotubes (single-walled and multiwalled), fullerenes and fullerene derivatives). That choice was dictated by the wide application of these nanoparticles in everyday household products, and by the fact that these compounds are commercially available in the market which eliminates the necessity for their synthesis (reduction of 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).

Application of the methods developed within the four thematic areas will allow for predicting toxicity and the behaviour of novel nanoparticles from their structure and/or physicochemical properties without the necessity of performing extensive empirical testing (reduction of costs and need for animal testing). Moreover, it will result in a framework being established to categorise nanoparticles according to the potential for exposure, as well as physicochemical, structural and toxicological properties (based on available empirical data and computationally predicted results).
This, in the longer perspective, should lead to designing and engineering nanomaterials that are of low risk for human and the environment.

2 Background

Recent contributions report evident toxicity and/or ecotoxicity of selected nanoparticles and highlight the potential risk related to the development of nanoengineering.

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.

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, encoded by so-called molecular descriptors, and the variance in a given physicochemical property in a set of compounds. In practice, this means, that if one has experimentally measured substituent constants, other physicochemical properties or 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) have to be utilized 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. 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. These have been shown to be able to predict skin sensitization potency as well as effects of respiratory sensitisation. In addition, it has been shown that structural similarity can be used to predict complex endpoints such as teratogenicity and reproductive toxicity. These grouping techniques have also been shown to be of use for environmental effects such as acute fish toxicity. 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 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 a controlled exposure to the materials..

3 What is NanoPUZZLES

NanoPUZZLES is a project developed by five partners from four countries. The specific objectives of the project are:

- Developing a framework for classified engineered nanoparticles based on the existing data utilising pattern recognition methods
- Developing 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
- Developing methods to predict and explain interactions of engineered nanoparticles with biological systems and small molecules
- Developing scientifically justifies and technically viable methods of quantitative 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.

3.1 Expected impact of NanoPUZZLES

The merit of the NanoPuzzles is to deliver a package of algorithms enabling to model relationships between the structure, properties, molecular interactions and toxicity of engineered nanoparticles, that can be simply applied by the industry to design safe and environmentally friendly nanomaterials. This includes:
4.1 WP 1: **NanoDATA**

The main objective of the first thematic area is to develop a framework for classified 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 physico/chemical and toxicity data with pattern recognition techniques (the characterisation and classification techniques) to identify classes of similar properties/toxicity.
- Launching a publicly available database with high quality (evaluated) empirical data.

Along with the work programme requirements, all 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 experimental prof. Leszczynski’s 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, 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 variance in data is related greater than the different measuring/testing protocols preformed by different laboratories and/or the errors existing in the data than to the real variability in the nanoparticles’ properties, an application of any classifying and modelling techniques has no sense. In such a case, simply saying: poor quality in the input results in poor quality in the output of a model. Therefore, NanoDATA will develop an algorithm for the experimental data evaluation on the basis of statistics and good laboratory practice. The evaluation will also include the analysis of the meta-data and experimental conditions are also potential source of information (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 a publicly available database. It is worth mentioning that those databases could be utilized by other consortia competing in this call. Finally, we will use those 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 emission and the properties responsible for environmental transport (behaviour).

4.2 **WP 2: NanoDESC**

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 characterization of NPs.
- Development of simplified molecular models sufficient to characterize 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 the prof. Leszczynski’s group (Interdisciplinary Center for Nanotoxicity, Jackson, (MS, US)).

4.3 WP 3: NanoINTER

The objective of the third thematic area is to develop methods to predict and explain interactions of engineered nanoparticles with biological systems and small molecules. This includes:

- 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 (NP) /[biological molecule].

There are several important factors related with 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) functionalization. It is understood that functionalization may affect the toxicity of the NP as well as its solubility. We shall look for 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: NP/[biological molecule]. Moreover, engineered nanoparticles exposed to environment participate in reactions of other environmental pollutants (oxidation reactions etc.) and can change as reaction rates of degradation (oxidation) processes of those pollutants, as well as to change reaction pathways and produce new metabolites.

4.4 WP 4: NanoQSAR

The objective of this thematic area is to develop scientifically justified and technically viable methods of quantitative 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:

- Investigating the impact of size on the physico/chemical properties of NPs at the appropriate level of the quantum-mechanical theory.
- Developing NanoQSAR models of toxicity and environmentally relevant physico/chemical 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 physico/chemical 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).
- Update of the database (including 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 brings together all findings and summarizes 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 utilized 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.

4.5 WP 5 and 6: Management and dissemination

This part of the project covers all project management issues, including: reporting, quality control, progress meetings, conferences, deadlines, contracts with the EU, financial and administrative coordination of cost control and dissemination.
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6 Copyright

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NanoReTox
The Reactivity and Toxicity of Engineered Nanoparticles: Risks to the Environment and Human Health

Contract Agreement: NMP4-SL-2008-214478  Website: http://www.nanoretox.eu
Coordinator: Dr Eugenia Valsami-Jones, Natural History Museum, London, UK*

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Contents
1  Summary ................................................................. 165
2  Background ............................................................ 166
3  What is NanoReTox .................................................. 166
3.1 The three principles of NanoReTox.............................. 167
4  Individual components of NanoReTox............................ 167
4.1 WP 1: Synthesis and characterisation......................... 167
4.2 WP 2: Abiotic reactivity............................................. 168
4.3 WP 3: In vivo exposure, aquatic organisms.................... 168
4.4 WP 4: In vitro exposure, aquatic species primary cells..... 169
4.5 WP 5: Cellular/molecular mechanisms of action; human..... 169
4.6 WP 6: Cellular/molecular mechanisms of action; human .... 169
4.7 WP 7: Interpretative comparison and interlinkage of reactivity, bioavailability and health effects .............. 170
4.8 WP 8: Risk model and communication.......................... 170
4.9 WP 9: Project management ......................................... 170
5  Directory .................................................................... 171
6  Copyright ................................................................... 174

Project Duration: 1 December 2008 – 30 November 2012

1  Summary
NanoReTox has investigated the potential risks of engineered nanomaterials to the environment and human health by comprehensively addressing five key questions:

[1] How does the environment affect the physicochemical properties and the bioreactivity of nanoparticles?
[2] How does this impact on their ability to interact with and/or penetrate organisms and cells and will bioavailability result in toxicity?

[3] Is there a pattern of cellular reactivity and/or toxicity related to physicochemical properties?
[4] What combination of conditions are most likely to pose a risk to human health and the environment?
[5] How can this information be incorporated in a risk assessment model?
A team of experts from across the EU and the US have worked together to address these questions in depth. A description of each group involved can be found at www.nanoretox.eu.

The specific scientific and technical objectives are:

[1] To synthesise and fully characterise a set of engineered nanoparticles with a range of physicochemical properties using industrial and laboratory methods.

[2] To study the abiotic reactivity (transformations) of the synthesised nanoparticles in simulated environmental and biological media.

[3] To investigate in vivo uptake of nanoparticles by aquatic species and study mechanisms and paths of internalisation.


[5] To consider the genotoxicity and carcinogenicity of metal nanoparticles.

[6] To determine whether cellular responses between human cells, mammalian cells, cell lines and invertebrate cells or whole organisms are comparable or different with relevance to screening models.


2 Background

The physicochemical properties of nano-sized particles are distinct from the properties of equivalent bulk substances. As the use of nanomaterials increases, the research into any potential risks of adverse effects on the environment and/or health must be intensified. Concerns on free engineered nanoparticles have been highlighted in several reports, including those by the Royal Society and the Royal Academy of Engineering in the UK (2004), the EPA’s Nanotechnology White Paper (2005), the European Commission’s Action Plan for Nanotechnology (2006), and the Royal Commission on Environmental Pollution (2008). All expressed unease over the apparent lack of urgency (at the time of the reports) in identifying the extent of the potential risks.

Indications of the potential toxicity of engineered nanoparticles can be drawn from epidemiological studies of inhaled environmental particulate matter in humans. These show that one of the primary target organs, in this case the lung, cannot necessarily defend other body systems from the effects of inhaling nanosized material. Consequently, the cardiovasculature is also affected. Indications from these human studies are that particles may enter the circulation and translocate to other organs and/or that there are “knock-on” systemic effects due to locally produced pro-inflammatory and pro-thrombotic mediators. Clearly, other mechanisms may exist. Studies in experimental animals suggest that inhaled nano-sized particles relocate to the brain, vasculature, liver, kidney and spleen. Similarly, intravenous nanoparticles can access multiple organs including the foetus. Other important portals of entry of exogenous nano-substances are the skin and gastrointestinal tract. Furthermore, there is in vivo evidence that activation of specific body compartments by some nanoparticles can initiate both local and systemic reactivity. All these findings may have serious implications for human health. What is not known is which engineered nanoparticle(s) induce cellular reactivity, how and where this might occur.

The rapid expansion of nanotechnology means there is a vast array of nanomaterials, many of which are already in industrial production. Because of the wide variety in physicochemical properties amongst different nanomaterials it is not possible at present to predict which elicit human and/or environmental harm. However, until the mechanistic associations between nanomaterial characteristics and putative toxicity are understood, determination of nanorisks will not move forward. Many recent toxicological studies have fallen short of this; furthermore many studies have led to contrasting results and interpretations about risks, possibly reflecting the diverse sources and nature of the test materials. This illustrates the importance of studying commercial and designer particles that have been fully characterised before, during and after toxicity studies. Of the many different types of engineered nanoparticles currently produced, industrially or in the laboratory, environmental risks from non-carbon-based nanoparticles are the least studied. This is despite the rapidly growing use of particles such as TiO₂, ZnO, SiO₂, Ag, CuO and CdS. The chemical composition of metal nanoparticles may contribute to their having significant additional toxicity, but few studies address this.

Ecotoxicological studies with engineered nanomaterials are currently limited. Most of the studies so far undertaken are simple “proof of principle” tests evaluating the possibility of either toxicity, under high concentration exposures, and/or the visual penetration of cells. There is a clear need for a more systematic approach to evaluating the processes that determine hazard, exposure and risk and for validated models predicting the release, transport, transformation, accumulation and uptake of nanoparticles in the environment and the human body.

3 What is NanoReTox

The overriding objective of NanoReTox was to contribute new knowledge to the global endeavour in addressing the scientific uncertainties related to the health and environmental effects of engineered nanoparticles and to provide a body of new information and a new tool that industry and governments can use to begin to assess the risks of these nanomaterials. Thus, NanoReTox attempted to identify the potential risks of free engineered metal nanoparticles to the environment and human health, and address a great deal of the issues described earlier.

More specifically:

NanoReTox examined the molecular and cellular reactivity of well characterised nanoparticles on a panel of primary human/mammalian cells and human cell lines originating from different target organs and exposure routes as an indicator of in vivo toxicity. The aim was to discover which features of nanoparticles confer reactivity with which cell types/target organs.

NanoReTox comprehensively addressed all physicochemical properties of industrially important metal based nanoparticles with a potential to induce toxicity (particle size, size distribution, shape, agglomeration state, crystal phase, chemical composition, surface area, surface chemistry and surface charge). By using labeled particles (with either stable isotopes or fluorescent dyes),
NanoReTox quantitatively determined the accumulated NPs in in vivo systems.

NanoReTox compared animal models that are likely to be sensitive to nanomaterials: animals that pump vast quantities of water across their gills (bivalves) or ingest plants or sediments where nanomaterials are likely to accumulate. NanoReTox used determinations of biodynamic characteristics, including rate of uptake from water, assimilation efficiency and rate of uptake from food, as well as retention (rate constant of loss), across several species and a range of particles. This screening approach (equivalent to a biological bioavailability probe) allowed a substantial number of particle formulations and characteristics to be compared. The biodynamic studies were be complemented by longer term experiments with fewer particle types to verify probe results.

By quantifying nanoparticle uptake using labelling, verifying the presence of particles in cells with visual or light scattering approaches, and observing responses of the organism at the cellular and whole organism level, the project assembled the lines of evidence necessary to determine if bioavailability and toxicity are feasible expectations for the metal nanoparticles.

NanoReTox focused on comparing data between mammalian and non-mammalian systems; this provided a unique set of data regarding the relationship between particle physicochemistry, bioavailability, cellular uptake and reactivity across a range of relevant target cell systems.

Finally, NanoReTox employed the extensive experience within the partnership, and a specific work package devoted to risk assessment and risk communication to develop appropriate criteria, considering factors that have, historically, generated surprising risks in the past.

### 3.1 The three principles of NanoReTox

- Focus on engineered metal nanoparticles, synthesised and fully characterised by project partners (both research & industry), to provide a coherent well defined study material with controllable properties.
- Use of organisms that are not currently included in standard toxicity tests, but which have (a) greater potential to be affected by nanoparticle toxicity due to the environment where they live or their biology and (b) are potentially valuable indicators.
- Minimal use of animal models in keeping with the 7th Amendment to the EU Cosmetics Directive (European Commission, 2003; Council Directive 76/768/EEC) which aims to reduce the use of experimental animal research.

### 4 Individual components of NanoReTox

NanoReTox comprised of six inter-related and interconnected work packages (WPs, see graph below):

1. **WP1: Synthesis and characterisation**
   - This workpackage produced sets of well-characterized nanoparticles using different methods of synthesis. The nanoparticle sets were tailored to display a range of physicochemical properties of interest. In order to establish that the nanoparticles tested by NanoReTox were representative of what is currently and in the future released in the environment, both top down (i.e. nanoparticles produced from bulk materials by milling) as well as bottom up (wet chemical, plasma and microfluidic synthesis), so that many important routes of synthesis were represented. This “in-house” “tailored” synthesis was essential for materials of this nature, because unlike conventional chemical toxins (where a solution of a particular substance has the same properties regardless of the way it was produced or its source) nanoparticle properties can vary substantially depending on the method of synthesis and subsequent functionalisation. This approach was complementary to that of the OECD Working Party on Manufactured Nanomaterials, by placing emphasis on the controlled variation of properties. The particles tested were: TiO₂, SiO₂, ZnO, CuO, CdS, Ag (Figure 1) and Au.

   - The nanomaterials studied in NanoReTox were extensively characterised using analytical and biochemical techniques such as Inductively Coupled Plasma Mass Spectrometry (ICP-MS), Dynamic Light Scattering (DLS) and Zeta Potential Analysis (ZPA), Single Particle Tracing (SPT), Gel Filtration (GF), Fast Protein Liquid Chromatography (FPLC), Scanning Electron Microscopy (SEM), Transmitted Electron Microscopy (TEM), Atomic Force Microscopy (AFM) in both wet and dry mode, X-ray Diffraction (XRD) and surface area analysis (BET). Multicollector ICP-MS was used for analyses of labelled nanoparticle isotopic composition. Focused Ion Beam Scanning Electron Microscopy for visualising the nanomaterials produced and their inner structure. X-ray Photoelectron Spectroscopy (XPS) and Time-of-Flight Secondary Ion Mass Spectrometry (ToF SIMS) for nanoparticle surface composition.
4.2 WP 2: Abiotic reactivity

Using the nanomaterials synthesised in WP1, this work package had the role of assessing nanoparticle behaviour (i.e. abiotic reactivity and potential transformations) in a variety of media, in order to: (1) select the optimum form and dose for in vivo and in vitro experiments; (2) prioritise which sets of the synthesised nanoparticles to study; and (3) elucidate nanoparticle behaviour in biological and environmental matrices. Physicochemical properties that were specifically monitored included: solubility, surface charge, particle size and size distribution, agglomeration/dispersion, surface area and other surface characteristics (roughness, porosity, and appearance), crystallinity and crystal structure.

Behaviour of nanoparticles in biological or environmental media was also monitored. It was anticipated that nanoparticles in some situations (particularly when present in concentrated suspensions) will tend to aggregate; however it is not clear whether aggregates, even when formed, behave like larger particles. Another important parameter under investigation was the stability (in terms of solubility and physical/chemical degradation) of the nanoparticles, to establish how their properties evolve in different media with time. Most physicochemical properties of the nanoparticles, notably size, composition, surface modification and even, in some cases, structure, may evolve with time. Abiotic reactivity studies of the nanoparticles were therefore carried out in media simulating environmental (hard/soft freshwater, seawater) and biological (simulated body fluid, lung fluid, gastric fluid) matrices. In these series of experiments factors such as pH, ionic strength and the presence of organic ligands (of biological, e.g. proteins, or chemical, e.g. humic acids relevance) of the model media are investigated.

4.3 WP 3: In vivo exposure, aquatic organisms

It is unclear to what extent nanoparticulate metals are accessible for uptake into the tissues and cells of organisms. The goal of this work package was to quantify the bioavailability of different types of nanoparticles and determine if bioavailable nanoparticles exert an adverse response within organisms.

Bioavailability was addressed using particles from WP1, occasionally labelled with artificially enriched stable isotopes and fluorescent labels to quantify biodynamic uptake and loss characteristics. Bioaccumulation was modelled from biodynamics for a variety of particle formulations, characteristics and compositions. The biodynamic predictions were verified by longer-term experiments on fewer particle types. The distribution pattern of metal nanoparticles were compared with that of metals themselves, identifying target cells and tissues for the toxic action of metal nanoparticles.

These experiments accompanied studies of adverse responses at both whole organism and cellular levels. Partners experimented with different organisms (Figure 2) in order to compare implications of different biological traits. Bivalve molluscs were compared that filter at different rates and consume different food (Mytilus galloprovincialis, Scrobicularia plana, Macoma balthica). Freshwater and marine snails (Potamopyrgus antipodarum, Lymnaea stagnalis, Peringia ulvae) that ingest plant material where nanoparticles might deposit were compared to animals that ingest sediments (polychaetes Nereis diversicolor and Capitella capitata). Zebrafish (Danio rerio) was studied as representative model vertebrate aquatic organism. Microscopy techniques and subcellular fractionation of metals within organisms were used to assess the internal uptake and distribution of nanoparticles. Oxidative stress, genotoxicity, metallothionein induction, DNA damage, lysosomal membrane destabilization histopathology and behaviour (burrowing, feeding rate) were used to represent important indicators of stress from metals. Nanomaterials themselves produce similar type responses, in vitro. If organisms show such responses to bioavailable nanomaterials, in vivo, it is unequivocal evidence that nanomaterial uptake causes the organisms to respond. Visual evidence of nanomaterials present internally, evaluation of internal dissolution and rigorous experimental design was used to determine if responses are due to internal dissolution of the metal oxide particle or due to disruption by the nanoparticle itself.
4.4 **WP 4: In vitro exposure, aquatic species primary cells**

This workpackage addressed the question whether nanoparticles induce responses that are indicative of a bioactive or potentially toxic material after the particle is taken into the cell of an organism. For example, nanomaterials could possibly be inert within cells, or detoxified by mechanisms in place to fend off foreign particles. In such a case no response would be expected by mechanisms that defend the cell against toxins. However, when a response was observed this was taken as evidence the nanoparticle represented a potential threat. Furthermore, responses known to be associated with metals or nanoparticles were considered. Understanding whether cells recognized and responded to nanoparticles, and how (the exact mechanisms of response) could be efficiently and effectively addressed with in vitro cell cultures both in humans and in other animals was a major part of the work in this WP. Thus in vitro studies in this WP were complementary of the in vivo approaches in WP3 and their combination aimed to avoid false conclusions about risks from nanoparticles. Most importantly, in vivo (WP3) and in vitro (WP4) approaches were co-ordinated using the same aquatic organisms (mussels) and similar endpoints, thus linking interpretation of in vitro and in vivo responses.

In close connection to WP3, WP4 determined the in vitro effects of nanoparticles in primary cell cultures of mussel haemocytes and gill cells. Haemocytes or immunocytes comprise the main internal defence system in mussels. Effects on this cell type could reflect damage on the immune system, which could have consequences at higher levels of biological organisation, ie, individuals and communities. The in vitro experiments with mussel haemocytes and gill cells used the same selected set of particles as in in vivo bio-response studies (WP3). In addition to general toxicity tests (cell viability), the emphasis was on surveying a broad range of biological targets that could be damaged by nanoparticle exposure. The goal was to cover as many potential effects as possible in order to identify the most relevant biological targets. These included oxidative stress (superoxide dismutase SOD, catalase CAT, superoxide anion and hydrogen peroxide), apoptosis (tunel assay) and genotoxicity (Comet assay, micronucleus test, oxidative DNA damage). Further, specific tests for haemocytes (tunel assay) and genotoxicity (Comet assay, micronucleus test, catalase CAT, superoxide anion and hydrogen peroxide), apoptosis (tunel assay) and genotoxicity (Comet assay, micronucleus test, oxidative DNA damage). Further, specific tests for haemocytes (tunel assay) and genotoxicity (Comet assay, micronucleus test, oxidative DNA damage). Further, specific tests for haemocytes (tunel assay) and genotoxicity (Comet assay, micronucleus test, oxidative DNA damage). Further, specific tests for haemocytes (tunel assay) and genotoxicity (Comet assay, micronucleus test, oxidative DNA damage). Further, specific tests for haemocytes (tunel assay) and genotoxicity (Comet assay, micronucleus test, oxidative DNA damage). Further, specific tests for haemocytes (tunel assay) and genotoxicity (Comet assay, micronucleus test, oxidative DNA damage).

4.5 **WP 5: Cellular/molecular mechanisms of action; human**

Using in vivo models, it is becoming apparent that particles delivered via one system (e.g. lung) can reach, and have detrimental effects on, other body systems/compartment (e.g. vasculature). However, these studies utilise significant numbers of animals, are labour-intensive and are impractical for examining the comparative effects and mechanism of action of a panel of compounds. In NanoReTox we used in vitro models to examine cellular responses to nanoparticles; this approach was also in line with the 7th amendment to the EU Cosmetics Directive (European Commission, 2003; Council Directive 76/768/EEC) to avoid excessive animal testing.

We hypothesised that the cellular reactivity of the particles will critically depend both on the target tissue and the function of the cell type within that tissue. Thus, whilst some nanoparticles may be overtly cytotoxic, even at low levels, others may not, but they may adversely affect cell function, for example stimulating inflammatory mediator release or compromising epithelial barrier integrity. Conversely, the magnitude and profile of the cellular response will depend on the physicochemical properties of each type and format of particle and its exposure dose. This WP concentrated on Ag, TiO₂, SiO₂, ZnO, Cds (and test all different sets of nanoparticles synthesised), which were expected to have a broad range of activity for comparative purposes. The work addressed the following questions: 1) Which cell types are most vulnerable to nanoparticle exposure? 2) Which cellular functions are affected? 3) Which mechanisms and cellular pathways are involved? 4) What is the cellular fate of nanoparticles? 5) Which physicochemical properties of nanoparticles render them more/less bio-reactive?

This WP aimed to investigate the cellular and molecular reactivity of the selected metal nanoparticles in a) primary mammalian and human cells and b) in a panel of established human cell lines. The chosen cells reflected likely nanoparticle exposure routes, and primary cell work was performed on lung and skin models.

4.6 **WP 6: Cellular/molecular mechanisms of action; human**

Occupational and environmental exposures to metals are associated with the development of various pathologies, including cancer; however, the mechanisms of action, especially at the molecular level, are still unclear. Recently, it was shown that exposure to toxic metals may be induced not only by absorption in micro-molecular form but also as nanoparticles. Although metal nanoparticles have been demonstrated to cause pathological responses, the mechanisms of toxicity remain explained. Metal-mediated formation of free radicals, reactive oxygen species (ROS) and reactive nitrogen species (RNS) can cause various modifications to DNA bases, enhanced lipid peroxidation, and changes in calcium and sulphhydryl homeostasis and evidence indicates that such ROS and RNS play an important role in the aetiology of a number of diseases, in particular neurodegenerative pathologies and cancer. Previous studies on human peripheral lymphocytes, show DNA damage and suggest that some metal nanoparticles might be genotoxic and therefore have carcinogenic potential; one important mechanism involves increased oxidative stress. This WP investigated whether metal nanoparticles possess genotoxic and carcinogenic potential; specifically: 1) Do nanoparticles induce cytogenetic changes and formation of micronuclei? 2) Do nanoparticles cause damage at the DNA level? 3) Do nanoparticles interfere with cell proliferation? 4) Do nanoparticles induce cell transformation? 5) Do the genotoxic effects of nanoparticles vary between individuals and between species? 6) Which physicochemical properties of nanoparticles render them more/less genotoxic?

The chosen cell models in this WP were fully characterised and were based on human leukocyte cultures (obtained from healthy volunteers), and on cell lines relevant to occupational and environmental exposure (Figure 3). The A549 (human lung...
epithelial) cells modelled the inhalation processes and the RAW264.7 murine macrophage cell line modelled the inflammatory process. In vivo studies concentrated in zebrafish liver as this small tropical fish species is a well-known model for hepatocarcinogenesis. In addition, possible carcinogenic effects were also studied in mussels, where haemic or haemocytic neoplasia and gonadal neoplasia have been reported.

Figure 3. Top: Genotoxicity endpoints evaluated by CBMN Cytome assay in primary human lymphocytes after treatment with nanoparticles. Bottom: damage evaluated by Comet assay in primary human lymphocytes after treatment with nanoparticles.

4.7 WP 7: Interpretative comparison and interlinkage of reactivity, bioavailability and health effects

Using data collected in all previous WPs, this WP compared species, particles of differing nature, as well as human and aquatic organism responses. All datasets were brought together into a unique “global table”. The WP dedicated specific effort to finding commonalitis among the different studies so as to maximize generalizations and applications to risk assessment. For example, many properties of cells are biologically conservative: that is, many similar mechanisms characterize the functioning of cells of all life forms. If there are commonalities in the way humans and other organisms react to nanoparticles then universal methods might be developed to both detect and better understand nanorisks. Specific questions addressed were: 1) Do organisms differ from humans or among species in their stress responses and/or sensitivity? 2) Can we use abiotic reactivity to predict toxicity? 3) Is in vitro dose response to metal nanoparticles indicative of in vivo responses? 4) Is there a pattern of cellular reactivity and/or toxicity related to physicochemical properties, i.e. a hierarchy of activity?

4.8 WP 8: Risk model and communication

Risk assessment: Ultimately a formal assessment of nanoparticle risks is essential. NanoReTox, in one study, addressed multiple nanoparticle formulations, in multiple media, using multiple species (including humans) and employed in vitro and in vivo approaches. The goal of WP8 was to incorporate this broad set of data from a single study into a risk assessment. Though there is increasing attention toward studying human health risks from nanoparticles, a common framework for conducting risk assessments is lacking. Information on environmental risks associated with nanoparticles, and particularly metallic nanoparticles, is scarce. An important outcome of the project was the development of a conceptual model to guide evaluation of hazards and risks from nanoparticles. The model was developed to be applicable to the body of evidence that will surely grow quickly as knowledge of nano-materials grows.

Risk communication: The profile of nanotechnology and any associated risk is high in the media; so inadvertent miscommunication is possible. Another goal of NanoReTox was to develop a risk communication strategy that guided project communications, and contributed in advising recipients of the project’s results (government, industry) communicate risks in a balanced, robust manner. It is essential to “get the risks from nanoparticles right” because the technology offers many potential benefits. The costs of over-stating or understating risks could be high. Although general risk assessment procedures are well known, there are many unique attributes of nanoparticles that required new or adjusted methodologies. Communicating new results in an unbiased, balanced and value free way is critical to public credibility. Communicating risks appropriately also requires a holistic view of the issues, as well as a careful, rational and transparent approach.

4.9 WP 9: Project management

All aspects of project management were covered by this WP, including reporting, quality control, progress meetings, financial/administrative coordination cost control, deadlines, contacts with the EU and dissemination.
# 5 Directory

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Compendium of Projects in the European NanoSafety Cluster
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NanosafePACK

Development of a best practices guide for the safe handling and use of nanoparticles in packaging industries

Contract Agreement: 286362    Website: http://www.nanosafepack.eu
Coordinator: Jose Luis Romero, Tecni-Plasper, S.L., Roca del Vallès (Barcelona), Spain

No.  Beneficiary name                  Short name        Country
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2    European Plastics Converters    EuPC              Belgium
3    The Portuguese Association of Plastic Industry  APIP     Portugal
4    Packaging, Transport and logistics research center ITENE  Spain
5    Institute of Occupational Medicine IOM    United Kingdom
6    Centro Español de Plásticos      C.E.P             Spain
7    Tec Star, s.r.l.                Tecstar           Italy

Contents
1  Summary................................................................. 175
2  Background .............................................................. 176
3  Concept and Objectives .............................................. 176
   3.1 Project Concept.................................................. 176
   3.2 Project Objectives ................................................ 176
4  Overall view of the Workplan ....................................... 177

Project Duration: 1 December 2011 – 31 December 2014
Project Funding: 1.6 Mio. EUR

The workpackages and their interdependence are shown schematically below: .............................................. 178
5  Advances over the state of the art................................. 179
6  Progress to date ......................................................... 179
7  Conclusion To-Date & Plan for the Future........................ 182
8  Directory ...................................................................... 182
9  Copyright .................................................................... 183

1  Summary

The use of these nanometer sized materials, traditionally known as nanofillers in the polymer industry, improve the volume properties, surface properties, dimensional stability, chemical stability, permeability and other functional properties of the reinforced polymers e.g. photocatalytic, optical, electrical, magnetic and thermal stability.

These new properties and the expected development in the near future results in a continuous growth of nanocomposites in the market. However a lack of information exists about the risk posed by the NPs used as nanofillers to human health and the environment. At the same time, while research on developing new nanocomposite materials has prolifer for more than a decade, research aiming to improve our understanding of the health and environmental impacts arising from the manufacture and commercialization of nanocomposites is far less advanced, with a particular concern regarding the exposure to NPs at all stages of nanocomposites production, use and disposal.

Besides the above, commercial compounding is typically achieved by feeding the NPs and polymer into a twin screw extruder, where the airborne particles released are of particular risk, as they can readily enter the body through inhalation.

Moreover, studies of existing nanometer-sized particles of many materials, including those typically used in the nanocomposite industry, have shown adverse health effects, and aerosol control methods have not been well-characterized for nanometer-sized particles, consequently, a number of concerns have raised relating to the safety of nanosized materials.

The aim of the NanoSafePACK consortium is to develop a best practices guide to allow the safe handling and use of nanomaterials in packaging industries, considering integrated strategies to control the exposure to NPs in industrial settings, and provide the SMEs with scientific data to minimize and control the nanoparticles release and migration from the polymer nanocomposites placed on the market.

To achieve this aim, a complete hazard and exposure assessment will be conducted to obtain new scientific data about the safety of polymer composites reinforced using nanometer-sized particles. The proposed work will focus on a selected set of nanometer-sized materials relevant to the packaging sector.
will be carried out, followed by an exposure measurement in order to identify and quantify any potential particle release in the production and processing activities.

A comprehensive hazard assessment will allow the evaluation of effects on human and environmental models, including the development of a nanoparticle migration and release index as a hazard indicator. Results from the exposure and hazard assessment studies will be used to compile a risk assessment of the use of nanoparticles in the packaging industry. An evaluation of the effectiveness of risk management measures will be undertaken in order to select and design practical and cost effective strategies, which will be easy to implement in the real operational conditions of industrial settings. In addition, as part of this assessment we will conduct a life cycle assessment of nanocomposites, by evaluating their impacts during the processes of manufacture, use and disposal.

2 Background

The further development of the nanotechnology applied to the packaging industry has enabled the production of functional nanocomposites that are already placed on the market. In this sense, nanoclays have been used as nanoreinforcements in several polymers such as nylon, polyolefins (polypropylene), polystyrene or poly(ethylene terephthalate). Similar aspects can be found in relation to the use of the metal oxide nanoparticles (Ag, TiO2, MgO, ZnO), used to develop high-conducting and low-leakage porous polymers.

On the other hand, the use of nanoparticles currently raises many questions and generates concerns, due to the fragmentary scientific knowledge of their health and safety risks. The uncertainties are great because such small particles exhibit novel properties that are distinctively different from their conventional forms and can affect their physical, chemical and biological behaviour. In general, the engineered nanoparticles are more toxic than equivalent larger-scale chemical substances. Simultaneously, the use of nanomaterials by workers presents new challenges to understanding, predicting, and managing potential health risks.

Primary routes of occupational exposure to nanoparticles include inhalation, trans-dermal absorption and ingestion, however the exposure to nanomaterials is likely to vary throughout the product life cycle (production, use and disposal). Surveys have indicated that nanotechnology – related industry workers have the potential to be exposed to nanoparticles and the degree of skin contamination could not be negligible. In this sense, several studies have determined representative values of airborne nanoparticles in the worker environment due to the ability of nanoparticles to be easily dispersed as a dust (e.g. a powder) or an airborne spray or droplets, resulting in greater worker exposure. In fact, large quantities of nanoparticles are released into the air during the extruder heating phase.

In terms of protection strategies, the current available information is only based on the implementation of engineering techniques, administrative controls and personal protective equipment, without considering specific operative conditions and the unpredictable behavior of the airborne nanoparticles. In this sense, the state of the art shows that the design quality and especially the verification of efficiency are essential factors to ensure adequate protection of workers and nowadays there are no studies in the scientific literature regarding evaluation of the performance of the ventilation equipment used in applications with engineered nanoparticles.

Finally, considering the consumer stage, several studies show a substantial release of nanoparticles from synthetic polymers. Furthermore, NPs may be released when nanocomposites are subjected to wear, such as UV radiation or abrasive uses in the case of packaging nanocomposites. In this sense, there is significant evidence that TiO2 NPs used in polymeric solutions are detached by natural weathering and the tribological studies on SiO2/acylate nanocomposites show that friction leads to the gradual loss of SiO2 NPs. Amongst these concerns, several strategies have been identified to reduce release and loss of nanoparticles during the service life of nanoproducts, for example the tribomechanical performance of nanoparticle filled polymer composites.

3 Concept and Objectives

3.1 Project Concept

The concept of NanosafePACK stems from the need to ensure the safety of workers dealing with nanoparticles and to guarantee the safety of the nanocomposites placed on the market, complying with the European regulation and avoiding endangering consumers’ health and the environment.

We need to find a cost effective solution to guarantee a safe working environment in the specific nanocomposites production process, as well as to predict and control the release of nanoparticles at all stages of the nanocomposites life cycle, avoiding the exposure of consumers to NPs. We must cover these needs in order to promote the manufacture of innovative products that can compete with the growing composites industry of China and India, improving the competitiveness of our members and the EU packaging industry in general. To achieve this, we must ensure the fulfillment of the current regulation in terms of worker safety and consumer health, avoiding workers exposure to nanoparticles and testing the potential release of the nanofillers across of the polymeric matrix in normal conditions of use.

Figure 1. Concept of the NanoSafePACK project

3.2 Project Objectives

The main objective of the NanoSafePACK project is to develop a best practices guide to allow the safe handling and use of nanomaterials in packaging industries, considering integrated...
strategies to control the exposure to nanoparticles in industrial settings, and provide the SMEs and industrial users with scientific data to minimize and control the nanoparticles release and migration from the polymer nanocomposites placed on the market.

The detailed objectives of NanoSafePACK are summarized below:

1. To characterize physicochemical properties, toxicological and ecotoxicological profile of the specific nanoclays and metal oxide particles employed in the packaging industry.
2. Hazard characterization of nanoreinforcements including functionalizer agents.
3. To Characterize the particle migration potential in terms of nanoparticle displacement
4. To assess the toxicity of the nanocomposites as such, considering the nanoparticles as a part of the polymeric matrix.
5. To characterize the exposure to nanoparticles through the development of specific exposure scenarios.
6. To characterize the potential release of nanoparticles on consumer products
7. To improve the effectiveness of the risk management measures according to the industrial settings of the packaging industry.
8. To identify the migration factors of nanoparticles into the consumer products
9. To define appropriate release factors of nanoclays and metal oxide nanoparticles
10. To characterize the most suitable waste management measures.
11. To solve problems with waste coming from nanocomposites, including carbon nanotubes (CNT).
12. To develop a cost effective strategy to improve the safety during nanocomposite production use and disposal.
13. To disseminate the research results for a large community of SMEs
14. To study the viability and benefits of the use of nanoreinforcements, taking into account the full product life cycle (production, use and disposal/recycling).
15. To characterize the regulatory aspects concerning the use of each kind of the nanoparticles studied in the project.

4 Overall view of the Workplan

NanoSafePACK consists of 8 complementary Work Packages (WP), summarised in Table 1. For each WP, a complete description is presented in the following page:

Table 1: Work Packages of NanoSafePACK

<table>
<thead>
<tr>
<th>WP nº</th>
<th>WP Title</th>
<th>WP Leader</th>
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<tbody>
<tr>
<td>1</td>
<td>Characterisation of Nanofillers</td>
<td>ITENE</td>
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<tr>
<td>2</td>
<td>Hazard Assessment</td>
<td>IOM</td>
</tr>
<tr>
<td>3</td>
<td>Development of Exposure Scenarios</td>
<td>IOM</td>
</tr>
<tr>
<td>4</td>
<td>Environmental impact of nanocomposites for packaging</td>
<td>ITENE</td>
</tr>
<tr>
<td>5</td>
<td>Development of the Best Practices Guide</td>
<td>IOM</td>
</tr>
<tr>
<td>6</td>
<td>Field Testing and Validation</td>
<td>ITENE</td>
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<tr>
<td>7</td>
<td>Project coordination and management</td>
<td>Plasper</td>
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<tr>
<td>8</td>
<td>Project dissemination</td>
<td>EuPC</td>
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</table>

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. 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 6, 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 Plasper.

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 NanoSafePACK project. These activities will be conducted within WP 8.
### Table 2 Technical & Scientific Workpackages (WP) of NanoSafePACK

<table>
<thead>
<tr>
<th>WP</th>
<th>Title</th>
<th>Description / Objectives</th>
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<tbody>
<tr>
<td>1</td>
<td>Characterisation of Nanofillers</td>
<td>The nanoclays and metal oxide nanoparticles employed in the packaging industry will be clearly identified, taking into account the list of endpoints published by the OCDE in relation to the nanomaterial identification and physicochemical properties.</td>
</tr>
<tr>
<td>2</td>
<td>Hazard Assessment</td>
<td>To characterize the toxicological and ecotoxicological profile of each nanofiller identified in the previous workpackage. To assess the changes induced by the functionalization of the nanofillers in relation to their toxicological and ecotoxicological profile. To assess the toxicity and ecotoxicity of the nanocomposites as such. To characterize a hazard indicator based on the particle migration potential.</td>
</tr>
<tr>
<td>3</td>
<td>Development of Exposure Scenarios</td>
<td>The exposure assessment work package will extend the experience-based understanding of appropriate practices for the safe production, processing and use of nanocomposites based on nanoclays and metal oxide nanoparticles. The objective is to carry out exposure characterisation for the production and processing of nanoclays and metal oxide nanoparticles and their release assessment as a part of the nanocomposites during the consumer stage.</td>
</tr>
<tr>
<td>4</td>
<td>Environmental impact of nanocomposites for packaging</td>
<td>Environmental impacts will be analysed applying Life Cycle Assessment (LCA) method for nanoclays and for metal oxide nanoparticles. The LCAs will be developed in accordance with the internationally accepted ISO standards 14040 and 14044. In addition, management alternatives for nanocomposites wastes will be analysed. Environmental information from this WP will be useful for researchers to choose the best options for future development of nanocomposites.</td>
</tr>
<tr>
<td>5</td>
<td>Development of the Best Practices Guide</td>
<td>The purpose of this workpackage will be principally the development of the best practices guide thought the integration of the project results. To develop that best practices guide, the following objectives must be covered within this workpackage: Design of the best practice structure an contents, Integration of the project results, Redaction and validation of the guide.</td>
</tr>
<tr>
<td>6</td>
<td>Field Testing and Validation</td>
<td>To validate the applicability of the best practices guide in the SMEs (Demonstration). To prove the viability of the solutions proposed in the industrials settings. To evaluate the effectiveness of the risk management measures proposed. To evaluate the improvement of the nanocomposite’s safety once applied the best practices referenced on the guide.</td>
</tr>
<tr>
<td>7</td>
<td>Project coordination and management</td>
<td>The overall objective is to ensure that the objectives of the different Work Packages are reached and activities complete in accordance with the time schedule and the deliverables and milestones are reported.</td>
</tr>
<tr>
<td>8</td>
<td>Project dissemination</td>
<td>The objective of WP8 is to ensure right development and impact of project dissemination and training activities.</td>
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The workpackages and their interdependence are shown schematically below:
5 Advances over the state of the art

Due to the variability of the properties of the nanoreinforcements considered and the evident lack of information about the potential effects and impacts of nano-sized materials on human health and environment, we have not been able to identify any manual or guide that can provide valuable advice about the use of layered organoclays and metal oxide particles. This is what the NanoSafePACK project will provide, a specific guide to improve the use of nanoparticles in order to develop functional materials, controlling the exposure to nanoparticles in the worker environment and minimizing the release potential during the service life of the polymer.

Besides the above, the NanoSafePACK project will work to progress beyond the current knowledge related to applicability of nanoparticles as nanofillers, as well as the enhancement of knowledge about the health and environmental impacts of the target nanoparticles and nanocomposites. In this sense, the project will take into account the “baseline data” in order to measure our progress, considering also the current research activities related to the field of work of NanoSafePACK, avoiding the duplicity of efforts.

The main baseline data to be considered will be:
- Studies and research concerning the applicability of nanoparticles as nanofillers.
- Available data on toxicological and ecotoxicological properties of the target NPs and nanocomposites
- Available data on migration studies
- Available data on exposure assessment methodologies
- Current risk management strategies recommended
- Existing manuals and handbooks

In relation to the existing references, several studies on polymer nanocomposites have been identified in the bibliography, however, none of them represent a direct guide to be applied at all stages of nanocomposites production, use and disposal, and do not take into account the concerns related to workers and consumers safety. In addition, we have not been able to identify any study specifically relating to the potential release of nanoparticles from the polymeric matrix, and even fewer in relation to the methodologies to be employed in migration studies involving nanoparticles.

In relation to the emerging research, we have identified several projects working on aspects related with some of the objectives pursued by our project, e.g. safe handling of nanoparticles, risks associated with the production of polymer nanocomposites and toxicological impact of nanomaterials derived from processing, weathering and recycling of polymer nanocomposites, nevertheless, these projects are not specifically aimed at SME trade moulders who will not probably be able to readily get hold of this information.

In addition, methodologies to assess the exposure and hazard aren’t standardized, so that the results of projects like NanoSafe are difficult to interpret and apply to other conditions. Thus, we aim to develop a best practices guide directly applicable in the nanocomposite industry.

The current handbooks, guides and reports of research projects are not focused on specific nanoparticles used in the current industrial setting of the packaging industry, which can differ enormously from another industrial process involving the use of polymers and nanoparticles. At this stage, our solution is innovative due to the direct application of our members in particular SMEs and industrial settings, as well as providing valuable information to characterize the toxicological profile and potential risk of the new manufactured nanocomposites and also those placed on the market in the recent years.

In relation to nanocomposite safety, the commercial manufacture of nanoparticles is relatively new, so that, there isn’t consensus in relation to the potential migration and release of the nanofillers employed on the nanocomposite industry. In this sense, taking into account physicochemical properties of the nanofillers employed, the possibility of migration is likely. Therefore, manufactures of nanocomposites, with special attention to those who manufacture food contact materials, must predict the potential migration in order to protect the consumer’s health and comply with the current regulation. At this stage, our proposal will study the aspects concerning the migration and release of nanoparticles in the polymeric matrix, providing the SMEs with scientific and valid data to select the less hazardous nanofillers or predict the potential release to the end user.

After studying the existing solutions and emerging research, the consortium has concluded that the submitted project has a high innovative character and will enhance significantly the state-of-the-art in the nanocomposite area. There is a demand that the current solutions are not able to meet, and the other research approaches are not focused in the specific field of the nanocomposite industry. Therefore we have an opportunity to exploit our innovative approach, including the achievement of the results in a reasonable period of time.

On the other hand, due to the current lack of data in the nanotechnology area, mainly in relation to the adequacy of testing models and exposure measurements, we have conducted a complete contingency plan, including a detailed risk analysis and the contingency actions to guaranty the progress beyond the state of the art, ensuring that the project results will be addressed.

6 Progress to date

The project started officially on December 1st 2011 and had its kick-off meeting at the CEP-Centro Español de Plásticos offices in Barcelona on January 12, 2012.

Much of the research has been focused on the definition of the most relevant NPs-polymer combinations, the physicochemical characterization of the target nano-fillers on the basis of the list of endpoints established by the OECD, migration studies and toxicological and ecotoxicological screening studies to identify the hazard profiles of both nanofillers and NPs-polymer composites.

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 ENMs with specific techniques such as Thermogravimetric analysis, Gas physical adsorption, as well as other imaging and spectroscopic techniques such as SEM, AFM, DLS, UV/Vis Spectroscopy, IR or Raman spectroscopy, (WP 1).
2. Migration studies based on current guidelines on testing conditions for articles in contact with foodstuffs (WP 2)

3. Evaluation of the effects of the nano-fillers in key aquatic species, including the water flea Daphnia magna and the unicellular alga Pseudokirchneriella subcapitata.

4. Characterization of a compendium of exposure scenarios based on the information retrieved from scientific literature, conferences talks and observation during the scoping visits (WP 3)

5. First studies on the airborne behavior of the nanofillers under controlled conditions at laboratory scale (WP 3)

6. Development of the first experimental set up to characterize the effectiveness of respirators against nanomaterials (WP 3)

7. Definition of the goal and scope of the Life Cycle Assessment and critical assessment of the possibilities and limits of the conventional Life Cycle Impact Assessment methodologies and tools when applied to nanomaterials (WP 4)

8. First proposal of the contents of the Best Practices Guide to be developed within the project (WP 5)

9. Participation in several international events related to the research topic of the project, mainly workshops and conferences (WP 8)

10. Concerning dissemination materials, the project web site and the first project brochure is available. A number of newsletters has been also published by partners (WP 8)

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

**WP 1. Characterization of nano-fillers**

The scheduled activities within WP 1 where totally completed on October 2012. Deliverable 1.1 was successfully submitted, providing a detailed explanation of the specific types of nanoparticles to be considered under the scope of the project on the basis of their applications and properties addressed by the use in composite materials.

The report concluded that the use of nanometer size materials as fillers for packaging materials enables the improvement of the volume properties (modulus, strength), surface properties (hardness, abrasion resistance, and surface energy), dimensional stability, chemical stability (UV resistance, flame retardancy), permeability and other functional properties of the reinforced polymers. In relation to the most relevant nanofillers on the basis of the scientific publications, market reports as well as the opinion of industrial end users, the organic layered nanoclays, metal NPs, metal oxide NPs and carbon nanotubes are the most used nanoparticles to develop packaging materials.

Ag, Au and Zn NPs are the most studied metal NPs with antimicrobial function, with Ag-NPs already found in several commercial applications. For mechanical and barrier properties, the polymers incorporating nano-clays are among the first nanocomposites to emerge on the market, being incorporated in a wide range of polymers.

On the other hand, the nanotechnology department of ITENE of completed the physicochemical characterization of the target nanofillers. These characterizations will continue during the project execution due to the need of conduct new characterization prior to the toxicological test under task 2.1, as well as before the assessment of the effects of the target nanofillers in the environment to be conducted under task 2.2.

**WP 2. Hazard Assessment**

The nanomaterials department of ITENE has been working on the determination of the overall migration of the target nanofillers into food simulants in order to determine the potential migration of nanosized fillers from polymer nanocomposite film.

On the other hand, ITENE and IOM are working have conducted a number of experiments, including the evaluation of adverse effects on different types of cell lines, as well as the evaluation of the toxicity of nanocomposites as such and nanofilms in aquatic (fish, crustacean Daphnia magna, microalga P. subcapitata) and terrestrial species (earthworm Eisenia fetida).

Finally, the research group of ITENE has performed a micro-mesocosm to study the fate and behavior of the nanofillers in the environment.

**WP 3. Development of Exposure Scenarios**

The activities within WP 3 have been focused on the compilation of information to develop the generic exposure scenarios under the scope of work package 3, including information on the conditions of use and risk management measures employed in the specific
conditions of the packaging industry, as well as information on the levels of exposure to nanoparticles identified in a number of scientific articles and other peer reviewed publications.

As a result of the information gathering conducted, the consortium has identified a number of exposure scenarios during different operations, including the main routes to produce the nano-fillers, and specially the incorporation of the nano-fillers into the polymer matrix.

On the basis of the information retrieved from peer reviewed publications, as well as the information collected during the scoping visits, we have defined four main generic exposure scenarios, considering all life cycle stages of a nanofiller used for developing nanocomposites materials within the packaging sector: synthesis, melt compounding, cleaning operations and recycling of nanocomposite based products.

In terms of emission sources and exposure, which are the main goals of the document, the following conclusions have been considered:

- Exposure and release during the synthesis
  The results show an increase in the number concentration during the synthesis, as well as during the cleaning of the systems, where the fillers are removed from the walls of the reactor, valves and tubing.

- Release during the weighing operation
  The handling of pure, non-consolidated nanoparticles could be one of the most critical operations in the process because it involves direct manipulation of the nano-fillers (powder) that can potentially disperse as airborne nano-objects in the workplace.

- Blending process
  The blending process results in the release of nanoparticles from the place of blending, generating peaks of nanosized particles in the workplace.

- Melt Compounding
  On the basis of the levels or airborne nanoparticles measured in the different studies published, it can be affirmed that during the compounding process, the high level of energy applied can led both to a thermo-mechanical degradation of the nanocomposite (especially the thermoplastic matrix) and off-gassing, thus enabling embedded nano-objects to go airborne.

On the other hand, a phase which seems to be critical is the discharging of the materials, including both nanofillers as such or the masterbatch granulates (1-4 mm). In the second option, the mixed material containing the nanofillers is exposed to temperatures close to those of the precessing conditions, meaning potential thermal degradation and emission of otherwise entrapped gases or particles.

- Release during the grinding process
  On the basis of the publications addressing this stage, the grinding process generates a large amount of airborne particles, mostly small pieces of polymer containing nano-fillers.

- Release during nanocomposite shredding
  The findings suggest that recycling of nanoclay-reinforced plastics does not have a strong potential to generate more airborne nanoparticles than recycling of conventional plastics. This information gathered indicates that few airborne particles larger than 300 nm were produced by the shredding.

WP 4. Environmental impact of nanocomposites for packaging

The activities within WP 4 have been focused on the critical evaluation of the LCIA methods for toxicity assessment when dealing with nanoparticles. The main methods that have been reviewed are: Eco-Indicator 99, CML 2001, IMPACT 2002+, USES-LCA 2.0 and USEtox.

On the other hand, a the sustainability department of ITENE is working in the construction of the Life Cycle Inventory, working closely with the rest of partner to collect valuable information on the toxicological and ecotoxicological endpoints that may be used to identify the main damage categories.


The contents of the best practices guide to be developed within the project are being discussed at the time of writing. A monthly meeting has been agreed between partners to clearly define the contents on the basis of the industrial needs.

No other tasks and activities have been conducted under the technical point of view during the first 12 months of the project.

Regarding the dissemination WP, a number of activities have been conducted, including the preparation and publication of the project web site, the publication of the first project brochure and the presentation of the project in relevant conferences.
7 Conclusion To-Date & Plan for the Future

The first results obtained within the project shown how the use of nano-fillers can adversely affect the human health and the environment. In this regard, significant exposures to nano-fillers has been observed across the nanocomposites life-cycle, and early results related to the migrations studies have revealed that typical nano-fillers such as CaCO\textsubscript{3} can migrate and enter in contact with the food chain.

The next steps to be conducted within the project will be focused on the characterization of the toxicological and ecotoxicological profile of fresh and weathered nanocomposites, which will provide us with relevant data regarding the potential adverse effects that nanocomposites may cause to the environment and human health, the characterization of the levels of exposure in real case studies and the evaluation of the effectiveness of the risk management measures against nanoparticles.

In summary, the following list of activities will be conducted in 2013:

1) Toxicity studies of micronized polymers and nanocomposites in different cell lines.
2) Ecotoxicity studies in soil organism (earthworm Eisenia fetida and/or Lumbricus terrestris) and freshwater organism to generate data on the adverse effects of the NMs on ecosystems (i.e. crustacean Daphnia magna and the microalga P. subcapitata)
3) Evaluation of the protection efficiency of the LEV systems, filtration, respiratory protective equipment (RPE), skin protective equipment (SPE), safety goggles and protective clothing when dealing with nanomaterials in the workplace.
4) Application of LCIA methodologies to study the impact of the nano-fillers in the environment. To this end the toxicological and ecotoxicological data generated within the project will be collected and analyzed, selecting those data that can be used with the models selected to calculate the impact of the nanofillers along their life cycle.
5) WP 5 will work on the development of the Best Practices Guidance, including:
   • Design of the best practice structure and contents
   • Integration of the project results
   • Redaction and validation of the guide

The dissemination related activities will continue with the scheduled tasks, 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 and submission of the mid-term report.

8 Directory

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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

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Contents

1  Summary ................................................................. 185
2  Background ............................................................. 186
3  What is nanoSTAIR? .................................................... 186
4  Objectives .............................................................. 186
5  The nanoSTAIR approach and organisation .......................... 187
6  Impact .................................................................. 188
7  Citations ............................................................... 189
8  Directory ............................................................... 190
9  Copyright ............................................................... 190

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 will boost the development of new documentary standards. A mechanism will be set up to identify, with a bottom-up approach, the opportunities for standardization from the results of research projects, co-funded by the European Commission or by National Research Programmes. This mechanism will be established using existing networks and initiatives such as NanoSafety Cluster or NANOfutures, as well as the network of the national standardization bodies in the various Member States.

Then, the expression of the needs for standards from various stakeholders will be collected and resources from consortia sharing similar standardization opportunities will be pooled together to launch New Work Items Proposals (NWIP). The nanoSTAIR approach will be verified during the project thanks to 2 NWIP initiated. The consortium will provide assistance to select the right standardization umbrella (Technical Committee and Working Group at CEN or ISO level).

As a result, nanoSTAIR will provide 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 will structure and ease the development of new documentary standards, and thus enable the European nanotechnology related industry to rapidly operate according to the state of the art and thus increase its competitiveness.
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 (STAnardization, 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 What is nanoSTAIR?

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 NANOftures 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 NANOftures 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), in the context of the STAIR approach promoted by CEN-CENELEC.

The aims of the project are definitely 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 will foster 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 will consider 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 The nanoSTAIR approach and organisation

The project is designed to 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.

It is organized around four work packages (supporting activities): 1) WP1: Screening and identification of standardization opportunities, 2) WP2: Pooling resources to launch standardization work items, 3) WP3: Verification of the approach with the preparation of standardization work item (Selecting standardization tools and launch of new work item) and 4) WP4: Tool box and dissemination.

The WP1 and WP2 are dedicated to the development of the procedures and tools that will support the implementation of the nanoSTAIR approach. In the WP3 Verification of the approach with the preparation of standardization work item, it is intended to start the preparation of 2 work items for demonstrating and verifying the feasibility. The procedures and tools developed in WP1 and WP2 will lead to ease the preparation of new standards work items in the field of nanotechnologies. In WP3, the concrete implementation will be performed on 2 work items. The WP4 Tool box and dissemination will assemble the outcomes produced in the previous WPs to construct tools and guidelines to promote and translate in a practical way European nano-research into documentary standards.

The most important outcomes of the project are the nanoSTAIR platform that will bring together the best experts to launch standardization work item proposals and the nanoSTAIR process to develop new work item proposal.

The nanoSTAIR process will be described in procedures, checklists and communication channels. The Figure 2 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.

Both nanoSTAIR process and platform will support the preparation of documentary standards work in the field of nanotechnologies, and will help structuring the emergence of new work items that will go then to the normal standardization process that is described in the CEN COMPASS. In addition, the nanoSTAIR process and platform will speed up the preparation of the proposal and increase the number of proposals.

In practical terms this will be achieved thanks to the development of a set of practical procedures and tools such as:

- A matrix with explicit criteria to identify the candidates for new work item proposals (NWIPs) in the field of nanotechnologies,
- A practical nanoSTAIR guideline to help the development of standards in the field of nanotechnologies using the nanoSTAIR process and platform.
- A nanoSTAIR network for standardization in the field of nanotechnologies gathering scientists involved in research projects or networks with national standardization bodies, with regulators and policy makers.

Knowledge developed during the project will be 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. To disseminate the results a project web-site will be regularly updated and all presentations and reports will be 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 been set up and will assist in the dissemination.
### Table 1 Workpackages (WP) of Scaffold

<table>
<thead>
<tr>
<th>WP</th>
<th>Title</th>
<th>Topic</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Screening and identification of standardization opportunities</td>
<td>WP1 will aim to define criteria to assess the results of research projects and select candidates for standardization and establish a process to continuously identify possible candidates.</td>
</tr>
<tr>
<td>2</td>
<td>Pooling resources to launch standardization work items</td>
<td>WP2 will aim 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.</td>
</tr>
<tr>
<td>3</td>
<td>Verification of the approach with the preparation of standardization work item (Selecting standardization tools and launch of new work item)</td>
<td>WP3 will aim to verify by proof of principle with 2 worked examples that the procedures and tools developed in the project will lead to ease the preparation of new standards work items in the field of nanotechnologies.</td>
</tr>
<tr>
<td>4</td>
<td>Tool box and dissemination</td>
<td>WP4 will aim 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.</td>
</tr>
<tr>
<td>5</td>
<td>Project management</td>
<td>WP5 will deal with coordinating and managing the project by covering technical, administrative, legal and financial issues and the relation with the EC.</td>
</tr>
</tbody>
</table>

### 6 Impact

The main expected impact of nanoSTAIR is the support to strengthen the position of European nanotechnology industry on the international scene because nanoSTAIR will: 1) Speed up the emergence of new standards based on research results, 2) Bring together the stakeholders and the content providers able to deliver documentary standards at once 3) Ease 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 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 wide diffusion of the outcomes of the project to any interested group of public and private researchers is the main goal of the project. The dissemination of the project findings will promote standardization in this field and thus help 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 by such as e.g. Enterprises, Research which drafts the European standardization mandates, rules, regulations, directives and FP VII.

Delivery of new standardization documents

In more concrete terms, during the run of the nanoSTAIR project, two new standard work item proposals will be prepared. These two examples will be 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 will 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 will contribute 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 annum. 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 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)
## Directory

Table 2 Directory of people involved in this project.

<table>
<thead>
<tr>
<th>First Name</th>
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*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, NordMiliö AB, Sunnemo, Sweden

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<td>NOMI</td>
<td>Sweden</td>
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<tr>
<td>2</td>
<td>The Institute of Nanotechnology</td>
<td>ION</td>
<td>United Kingdom</td>
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<tr>
<td>3</td>
<td>National Research Centre for the Working Environment</td>
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<td>6</td>
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<td>7</td>
<td>European Commission Joint Research Centre, Institute for Environment and Sustainability</td>
<td>EC-JRC</td>
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<td>National Institute for R&amp;D in Microtechnologies</td>
<td>IMT</td>
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Content

1 Summary .......................................................................................................................... 191
2 Scientific / regulatory / industry needs and problems addressed ............................. 192
3 Concept, scope and strategy ...................................................................................... 192
4 Technical approach and work description .................................................................. 193
5 Status of the project ................................................................................................. 193
   5.1 Project management and ST coordination (WP1) .................................................. 193
   5.2 Data gathering, generation, evaluation and validation (WP2) ............................... 194
   5.3 Hazard characterization and impact assessment (WP3) ......................................... 194
   5.4 Life Cycle Assessment (LCA) and preliminary Assessment (WP4) ........................... 195
5.5 Exploring technical solutions for recycling, treatment and disposal (WP5) .................... 196
5.6 Dissemination and exploitation of results (WP6) ..................................................... 197
6 Expected Impact ......................................................................................................... 198
7 Cooperation ................................................................................................................ 198
8 Directory ..................................................................................................................... 198
9 Copyright .................................................................................................................... 200

1 Summary

Project Duration: 1 May 2008 – 31 April 2013
Project Funding: 2.5 Mio. EUR

The ultimate goal of the NanoSustain project is to develop new technical solutions that foster the design, manufacture, use, reuse/recycling and final treatment and/or disposal of sustainable engineered nanomaterials (ENM). To reach this goal a comprehensive physicochemical (pc) and hazard characterization (toxicology, dose-response, no-effect levels) campaign has been started, including exposure (human, environment), risk (RA) and life cycle assessment (LCA) of selected ENM (nanocellulose, nano-TiO2, CNTs, nano-ZnO) and associated products, also in relation to their fate, transport and transformation.

The following specific objectives build the cornerstones of the project:

1 Hazard identification by finding the most important hazard characteristics, exposures and risks for selected ENM, dose-response and no-effect levels to humans and the environment for after-production life-cycle phases, such as application or use,
recycling, final treatment and disposal, to improve existing risk assessment (RA) methods applicable to selected ENM.

2 Life cycle assessment and preliminary assessment by applying leading edge methodology to identify potential environmental impacts throughout the whole life cycle of a material or product (from production and application/use to final recycling) and by further developing prospective and preliminary LCA into precautionary risk management.

3 Human health and environmental impact assessment by producing and assessing a set of well-defined ENM that represent the main properties and stages during their life cycle, by using critical endpoints, such as the inflammatory reaction as a key event following exposure to ENM in the lung or the comet assay to determine genotoxic effects by Reactive Oxygen Species (ROS) formation, and DNA damage in cells and animals. Also eco-toxicity tests are used and adapted, such as the luminescent bacteria (Vibrio fischeri) and white worm (Enchytraeus sp.) test, to assess the impact of nanoparticles in aquatic and terrestrial systems and to elucidate toxicity mechanisms that may arise during manufacturing, transport, application, recycling and disposal, or directly from used materials or indirectly via the environment, or by their accidental release.

4 Exploring technical sustainable solutions by testing the behavior of selected ENM during end-of-life phases (recycling by composting, melting), treatment (by incineration) and land-filling, also to redesign materials and products.

2 Scientific / regulatory / industry needs and problems addressed

The behavior and properties of nanomaterials can be quite different to bulk materials, a fact that drives considerable international research and development activities towards exploitation, innovation and commercial application, with a corresponding increase in the number of nanotechnology based products reaching the end of their life-cycle. At the same time, 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, 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 on wildlife and potential bioaccumulation in various organisms.

The rapidly increasing amount of nanomaterials produced worldwide raises in particular the question of 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.

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 tested and where necessary adapted or modified, and validated. NanoSustain will in particular evaluate the extent to which existing regulatory and risk management strategies and tools can be applied to after-production stages of nanomaterials, in particular to their recycling, final treatment and disposal.

3 Concept, scope and strategy

NanoSustain is based on the concept of “sustainability” and “scarce resources”, which means that the use of new innovative materials, like engineered nanomaterials, must not only consider human needs today but also of future generations, including all possible effects occurring along their life-cycle, and should ensure recyclability and avoidance of dissipative losses of contained nanomaterials. Both concepts are 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.

The following specific organic and inorganic nanomaterials have been selected and are investigated, including associated LCA relevant test materials (such as prototype products, dusts):
Nanosustain considers four main aspects of the life-cycle of selected nanomaterials including, (a) selection and design, (b) manufacture, (c) application, and (d) recycling/disposal. Although most studies still focus on possible toxic effects of nanocomponents after exposure for risk assessment, the potential contribution of these materials to all impacts will be 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 encompasses the following specific 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 is structured and organized around 4 technical (vertical) and 2 horizontal Work Packages (WPs), each with distinct tasks, deliverables and milestones:

- **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 (WP6):** Dissemination and exploitation of results.

So far, the following tasks have been realized during the first 18 months:

WP1: Administrative and operational project management and coordination of the scientific work to ensure regular monitoring, update and control of the quality and progress of work and results achieved, organizing regular consortium and progress meetings, reviewing, editing and archiving of produced deliverables and reports, and establishing and maintaining a smooth communication and information system between partners, with the Commission and main stakeholders (see also WP6).

WP2: (1) A comprehensive data and literature survey on testing and characterization of selected nanomaterials to prepare and organize the (2) design and structure of a materials/product and (3) of a literature database, to systematically collect, store, evaluate and validate already existing and continuously generated new data.

WP3: (1) Production of pure test samples and prototype products from selected nanomaterials, as well as various types of life-cycle dusts from sanding of CNTs containing epoxy-plates and TiO2 containing painted boards, to simulate and assess human exposure during handling and reworking, and their toxicology. (2) Studies on the effect of weathering and abrasion on emission of selected ENM from glass sheets coated with nano-ZnO and from TiO2 containing painted boards during sanding, and on testing the suitability of eco-toxicological tests for ENM. (3) Evaluation of newly generated hazard and exposure data to improve existing test strategies and methods for environmental risk assessment.

WP4: (1) Performance of a comprehensive survey of studies and of a questionnaire on manufacture and LCA of selected ENM. (2) Development of two process models for their application/use and end-of-life phase developed. 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.

WP5: (1) Production of nanocellulose-based materials, nano-ZnO coated glass and MWCNT containing epoxy composites and (2) laboratory experiments conducted for their recycling (composting, melting), treatment (incineration) and final disposal (land-filling), in addition to weathering and leaching studies, to explore and develop new technical solutions for the sustainable design, use, reuse/recycling, final treatment and/or disposal of selected ENM.

WP6: (1) A fully functioning and interactive project website and intranet has been established to continuously disseminate the outcome of the project to all stakeholders and to make results available for partners (www.nanosustain.eu). (2) Four dissemination/training events have been organized so far to manage and exploit the new knowledge and outcome produced: 2 dissemination workshops (in Glasgow/UK, May 2011, and in Venice/Italy) to present and discuss first results with relevant stakeholders, and 2 training workshop (on LCA in Bremen/Germany, September 2011, and on advanced characterisation methods in Kaunas, September 2012) to interact with interested experts.

5 Status of the project

5.1 Project management and ST coordination (WP1)

Nanosustain was launched by a kick-off meeting in Sunne, Sweden, on 25-27 May 2010 and 5 regular project meetings have taken place so far, in Ispra/Italy (16-18 November 2010), in Glasgow/UK (10-11 May 2011), Venice-Mestre/Italy (24 November 2011),...
Copenhagen/Denmark (10-11 May 2012) with a joint meeting with NanoValid (9 May 2012), and in Frankfurt/Germany (8-9 November 2012), where partners discussed the progress of work and results obtained during the project, and prepared the next steps to take according to the work plan. The project has now reached its final phase (end of project: 30 April 2013) and is preparing for the final regular meeting in Copenhagen (10-11 April 2013), to conclusively discuss all results and their ongoing dissemination and exploitation. After the 1st Periodic Report prepared and submitted to the EU Commission in December 2011, a Final Project Report will be prepared at the end of June 2013 and subsequently submitted to the EU Commission.

5.2 Data gathering, generation, evaluation and validation (WP2)

In WP2, a central material database was designed and the main structure, parameters and functions established to continuously collect, store and evaluate pc and biological data on selected ENM provided by manufactures involved in the project, but in particular newly generated within the project (in WP3), to be fed into the LCA (WP4) and RA (WP3), or used for exploring new technical solution for reuse/recycling, treatment and final disposal (WP5). Before entering the database, the newly generated data is collected in Material Data Sheets (MDS) that have been prepared for the tested materials and that are online available on the partner intranet, to allow direct data input, use, treatment and validation by all project partners, but also to identify significant relationships between material characteristics and biological effects. From the MDS, the newly generated data is finally taken up by a “project-specific results database” to store and evaluate the pc and (eco) toxicological data (WP3), the human exposure data (WP3), together with the LCIA (WP4) and risk assessment results (WP3). A comprehensive inter-lab comparison campaign on validation of characterization, measurement and testing, in cooperation with an external reference laboratory, is still ongoing and will be finalized in March 2013, to assess the quality and verify the validity of the data and methods used.

To keep all partners up to date with new research developments and to consider new findings for the ongoing work, but also to integrate the project outcome in a wider context, a continuously updated scientific database has been established (see Figure 1) based on a critical literature survey and is available on the project website (for registered users) and equipped with particular search functions. The topics covered by the database are: production methods and uses, occupational, consumer and environmental exposure, environmental fate and transport, eco-toxicity, human toxicity, ADME parameters, test standardisation, end of life treatments. Only publications that prove minimum requirements regarding data quality and metrics of tested materials have been considered. Most studies show that characterization of ENM is a key criterion for the success of any subsequent toxicity testing and critical for identifying any significant relationships between metrics and effect endpoints, but also when comparing the toxicity of different studies or when assessing possible risks. The survey also confirmed that false conclusions may be drawn on the toxicity of ENM with similar compositions but different metrics in the absence of a qualified characterization.

Nanosustain is prepared to share the generated data and expertise with other relevant EU FP7 projects, such as NanoFate, NanoHouse, NanoPolyTox and NEPHH, and with the Database Working Group 4 of the NSC, to develop common protocols and harmonize formats to support the build-up of a central EU wide database on nanomaterials risk assessment.

![Figure 1: Last batch of publications (December 2012) to be processed by category and ENP](image1)

5.3 Hazard characterization and impact assessment (WP3)

One main goal of WP3 is to provide not only pure nanoparticles, but also life-cycle relevant test materials for hazard and exposure characterization. For this reason, the following test materials have been synthesized and treated for characterization and testing: (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 nanoTiO2, and paper prepared with and without nanocellulose (see Table 1). Also five types of life cycle dusts have been generated by sanding of the produced epoxy-plates (CNTs) and painted boards (TiO2), and distributed to the project laboratories for measurement and testing.

![Table 1: Test materials: Pure nanoparticles and sanding dusts from products (with and without nanoparticles)](image2)
A comprehensive characterization round of the obtained pure nanoparticles has been finalized 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. To validate the used methods and the quality of the produced data (see WP2), a number of standards and benchmark materials are being tested during the first months of 2013 by an inter-lab comparison study.

To assess the emission potential and risk for workers during powder handling, data from simulated work activities (sandig) and dustiness testing (miniaturized EN15051 test) have been generated and evaluated. Characterization of the emission source potential and the dustiness testing of pure nanomaterials has been finalized. Work to evaluate the effect of weathering and abraison 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-TiO2, has been performed by sanding before and after exposure to weathering, in close collaboration with the EU FP7 NanoHouse project and Flügger Denmark. Tests have been finalized and data analysis is ongoing.

The toxicological testing of pure ENM and lifecycle material is almost finalized: 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. Histological investigation of the tissue and of the toxicological testing of life-cycle materials is still ongoing.

Another focus in WP3 is to assess the suitability of existing ecotoxicalogical test methods for risk assessment of the selected ENM. As an example, the aquatic toxicity of various micro-fibrillated cellulose (MFC) samples (“nanocellulose”) was evaluated by using the conventional kinetic luminescent bacteria Vibrio fischeri test based on ISO 21338. Studied samples showed no acute toxicity at relatively high concentrations, while a tendency of MFC to form aggregates/agglomerates in the 2% NaCl test medium was observed. Composting of MFC based cellulose products was also assessed by using the same luminescent bacteria test. Also the aquatic toxicity of metal oxide nanoparticles (ZnO, several TiO2) as well as CNT was evaluated. Evaluation of the effect of organic matter on the toxicity of nano-ZnO will be completed soon.

Also in WP3, a critical survey and evaluation of existing strategies and methods for environmental risk assessment has been carried out, to determine where in the product chain nanomaterials are likely to present hazards that are different from bulk chemicals. Basic principles of RA have been summarized and applications in industry given and discussed in a special report. Also international guidelines have been reviewed in relation to potential risks in relation to still existing uncertainties. New results from our survey on nanomaterials risk assessment practices in industry are presented. Based on existing methods, nano-specific risk assessment tools have been identified, compared and critically evaluated to determine their applicability to the materials tested by NanoSustain. The generated data has been compiled in a suitable format for entry into risk assessment models, and gaps identified where data are still insufficient.

5.4 Life Cycle Assessment (LCA) and preliminary Assessment (WP4)

NanoSustain is assessing the impact of selected organic and inorganic ENMs and products along their whole life cycle: (i) nanocellulose used as paper additive, industrial thickener, or rheology modifier, (ii) nano-TiO2 used in paint applications, (iii) nano-ZnO used in glass coatings, and (iv) MWCNT used in epoxy plates and solar cells. Based on a comprehensive literature search and a questionnaire sent to all 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.

As an example, the results of the global warming potential of the case study ‘Life cycle modelling Nano-TiO2 in paint application’ are summarized in Figure 2.

Figure 2: Nano-TiO2 in paint application including Altair hydrochloride process (Important assumption: Alkyd paint with 60% solvent, content rate: TiO2 white paint (conv.) ~30%TiO2; NanoTiO2 paint 10% nano-TiO2, ~20% TiO2)

Also as an example, the predicted environmental concentrations (PEC) for surface waters for nano-TiO2 in paint application are presented in Figure 3.
Figure 3: Predicted environmental concentrations (PEC) for surface waters: a) current scenario based on current (optimistic) production/application estimates of nano-TiO2 in paint coatings; b) PECs of the total amount of nano-TiO2 reaching the aquatic environment recomputed as modelled by Gottschalk et al. 2009, c) maximal exposure scenario reflecting capacity limits of production/manufacturing sites for nano-TiO2 in paint coatings.

For the precautionary design and for improved recyclability of ENM a comprehensive approach is now derived from the presented approaches and is supplemented with environmental impact categories of the Life Cycle Assessment. The concept includes:

- **Precautionary risk aspects**,
- **Resource aspects**, and
- **Environmental impact categories**.

<table>
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<tr>
<th>Categories and aspects</th>
<th>Data quality</th>
<th>Source</th>
</tr>
</thead>
<tbody>
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<td>Precautionary risk aspects</td>
<td>Qualitative</td>
<td>German SRU to precautionary strategies for managing nanomaterials</td>
</tr>
<tr>
<td>Potential exposure of humans</td>
<td>Semi-quantitative</td>
<td>Swiss precautionary matrix for synthetic nanomaterials</td>
</tr>
<tr>
<td>Potential input into the environment</td>
<td>Semi-quantitative</td>
<td>Swiss precautionary matrix for synthetic nanomaterials</td>
</tr>
<tr>
<td>Potential of incident</td>
<td>Semi-quantitative</td>
<td>German ÖI Sustainability check, orientation on Swiss precautionary matrix</td>
</tr>
</tbody>
</table>

**Ressource aspects**

- Criticality: Qualitative, Semi-quantitative, EU concept of criticality
- Recycling capability / tendency to dissipation: Qualitative, In orientation on German ÖI Sustainability check
- Abiotic resource 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 2: Criteria for precautionary design and for improved recyclability of engineered nanomaterials |

In a next final step, the applicability of this approach for the precautionary design and for improved recyclability will be assessed for the selected nanomaterials.

### 5.5 Exploring technical solutions for recycling, treatment and disposal (WP5)

An internal nanocellulose standard was produced and characterized in WP5 for quality control, validation, and reproducibility check for the different planned laboratory studies. Also nanocellulose based products, such as various papers, have been prepared for experiments on dustiness (in WP3), biodegradability and recycling (in WP5).

Composting is recognised as a valuable method for organic waste treatment and can be considered also as an option for recycling of organic nanomaterials containing wastes. According to European compost stability norms (EN 13432), compostable materials should fulfill the criteria of biodegradability, disintegration during composting and compost quality. Biodegradability of the nanocellulose-based products was evaluated by biodegradability tests under composting conditions and in an aquatic environment. According to the obtained results, all tested nanocellulose products can be considered as biodegradable under compost conditions and they also disintegrated in pilot-scale composting experiments. Compost quality requirements include both chemical and biological analyses. Bio-tests have shown to give relevant and valuable information on the possible eco-toxicity of materials under composting process. Eco-toxicity of the test materials during biodegradation was analyzed by the Flash bioluminescence test with Vibrio fischeri.

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.

Multi-walled carbon nanotubes (MWCNT) containing epoxy composites are not suitable for recycling, why incineration 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.

To study the feasibility of land-filling as a final disposal option, the leaching of nanoparticles released from nanomaterials containing waste material has been studied in WP5. 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 been carried out. Experiments and modelling approaches to predict transport in environmental media will be ready by March 2013.

5.6 **Dissemination and exploitation of results (WP6)**

**Website and intranet:**

Up to date news on the NanoSustain project is available via the interactive project website (www.nanosustain.eu), which also hosts current and previous newsletters and, importantly, the project results. Registered users can access additional information such as the output of dissemination events and other presentations. A partner intranet and database allows all partners to view the technical materials and literature database and all project deliverables (draft and final) in a password protected area.

The partners have produced the final structure of the project-specific database and agreed on a common format to allow easy comparison with data from other projects, but in particular to facilitate the collection, validation, analysis and use of all the physical/chemical and biological data continuously generated by the various measurements, assays and experiments.

The project continues to promote the possibilities for exploitation of its data through quarterly newsletters and information sent to the contacts databases, project website, partner websites, NanoSafety Cluster Partners, RTD community and wider community of stakeholders.

**Specific project meetings:**

In May 2012, project partner laboratories came together at the headquarters of the National Research Centre for the Working Environment (NRCWE), Copenhagen, to discuss work progress and plan the implementation of a comprehensive inter-calibration study (round-robin validation) to assess the accuracy and reproducibility of the characterization and measurement methods.

To support further collaboration between project partners, regular online meetings have been organized throughout the project by using modern video-conferencing software.

**Dissemination and exploitation of final results:**

**i) Training**

A training workshop on Life Cycle Analysis of nanomaterials was held at the University of Bremen in September 2011 and presentations are available on the registered user’s area of the project website. Also two dissemination events were held in Glasgow/UK (May 2011) and Venice/Italy (November 2011) to provide a platform for project partners to present the first results of their work, but also to interact with invited stakeholders.

An Autumn School on ‘New Methods for Nanoparticle Characterization’ was held on 17 – 18th September, 2012, hosted by NanoSustain partner Kaunas University of Technology, Lithuania. 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), the event updated current scientific knowledge as well as technical tools for an integrated risk assessment in the light of results produced within the project. The school was aimed at young researchers (PhD students, post-docs, etc.) and provided an interactive learning environment, highlighting best practices and approaches in the project focus areas of physicochemical characterization, (eco) toxicity testing, exposure, risk and lifecycle assessment of ENMs.

**ii) Data sharing and exploitation**

The consortium members have now prepared and begun to implement a strategy for an intense period of dissemination of the project results for the benefit of end-users and key stakeholders via e-communications and interfacing with targeted stakeholders in research, production and industry. One outcome is that NanoSustain will collaborate with the NanoPuzzles project, which is developing new computational methods of estimating the risk of nanoparticles, and can now use empirical data generated by the NanoSustain project to calibrate and validate the new models.

In addition, the consortium is planning a unique exchange with the EU FP7 NanoPolytox (www.nanopolytox.eu) and NanoFate (www.nanofate.eu) projects in a final two-day scientific workshop to be held in Barcelona in May 2013, at which the outcomes of the three projects can be shared and evaluated with a view to wider dissemination and exploitation, and so increase the final impact of each individual project. NanoSustain will use this opportunity to organize and implement together with the other 2 projects a one-day training workshop 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 is the production and distribution of concise case studies and fact sheets which will 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. The disseminated information will also describe the data evaluation and categorization processes according to different material and environmental attributes, such as toxicology, eco-toxicology, degradability, exposure and fate,
and relevance for LCA. This data will help to guide the development of new sustainable products and industrial applications, and thereby help to strengthen the competitiveness of the European nanotechnology industry.

Finally, to further engage with potential end-users, several NanoSustain partners will have oral/poster presentations at various forthcoming international workshops and conferences, and a synthesis of main results will be presented at the biannual NanoEuroForum conference in Dublin 2013 (www.euronanoforum2013.eu/). Also a special book volume on NanoSustain main results will be published in the second half of 2013.

6 Expected Impact

NanoSustain will contribute to improve our current knowledge on hazard, impact and sustainability of nanomaterials and products, in particular in relation to end-of-life stages. The produced new data will help to 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 and their probability of occurring during reuse/recycling and final treatment or disposal will be produced and made available. For the first time new and innovative technical solutions are explored on a lab-scale for the (1) recycling (by composting and melting) of nanomaterials from waste products, 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 these new technical solutions will help to overcome some still unresolved technical barriers towards the environmentally benign and sustainable design, use and development of nanomaterials based technologies. Although not a main target, NanoValid will naturally 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 Cooperation

NanoSustain is represented and actively participating in most Working Groups (WG) of the EU Nanosafety cluster. In addition, the project is closely cooperating with the EU FP7 NanoHouse project and with the Danish Flügger company on the performance of weathering and abrasion tests, with the EU FP7 NEPHH project on LCA-methodology and collection of relevant LCA data, with EMPA/Switzerland on developing an exposure model, with the University of eastern Finland on the incineration of MWCNT containing wastes, and with Health Canada on micro array analyses of tissue from the animal experiments.

8 Directory

Table 1 Directory of people involved in this project.

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NanoSafetyCluster - Compendium 2011

200 Compendium of Projects in the European NanoSafety Cluster

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NanoTransKinetics

Modelling the basis and kinetics of nanoparticle cellular interaction and transport

Contract Agreement: NMP4-2010- 266737
Website: http://www.nanotranskinetics.eu
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* EU-US modelling call – project is paired with the project “Nanoparticle Transport: From Cells to Organisms” funded via the the EPA STAR programme (EPA-G2010-STAR-N1 Fate, Transport, and Transformation).

The NanoTransKinetics project is cooperating with ongoing projects in Duke University and University of North Carolina, as well as with the EU modelling cluster (initially ModNanoTox coordinated by University of Birmingham).

Contents

1 Summary .......................................................... 201
2 Introduction .................................................... 201
3 Background ..................................................... 202
4 Project Description and Organisation .................. 204
5 NanoTransKinetics Year 1 results ......................... 206
6 Expected impacts ............................................. 208
7 References ...................................................... 208
8 Directory .......................................................... 209
9 Copyright ......................................................... 209

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). A second joint meeting was held in Brussels on the 28th and 29th of January 2013.

2 Introduction

Nanomedicine (and Nanodiagnostics) recognize the capacity to treat (and diagnose) most of the remaining intractable disease classes (viral, genetic, cancer). They are based on the central observation that objects of such small size can (uniquely) gain access to, and operate in all parts of the body (including the brain), and within cells. Nanosafety acknowledges that (as with blue asbestos - a nano-rod that is non-toxic in bulk form but the agent for the increasingly common cancer, mesothelioma) there exists potential for new, serious and unpredictable diseases originating from the interaction of such small-scale objects with living organisms. There have been, as yet, limited numbers of clearly identified hazards from early phase nanoparticles, but caution is being shown worldwide in developing strategies to address the issues.

It is becoming increasingly clear that Nanomedicine and Nanosafety will rely on the same fundamentally new scientific enterprise, based on understanding (and in the medium term, predicting) the interactions between nanoscale objects and...
living systems. Indeed, results from experimental projects (such as those previously lead by Partner 1 - FP6 NanoInteract and FP7 NeuroNano) have produced extensive experimental information that now needs to be integrated in early stage computational models. Early experimental data now begins to clarify the basic scientific issues, and it is clear that we are at the dawn of a new interdisciplinary science (bionanointeractions).

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 - this is the primary purpose of the NanoTransKinetics program. Based on this information, toxicological impacts can potentially be deduced, which is the goal of the project. Thus, we frame models by abstracting the essential, relevant principles of particle-protein (and matrix) interactions, and cellular and barrier transport mechanisms for nanoparticles. Based on preliminary studies, we find a limited number of interactions, particles fluxes (and control parameters) between prescribed sites are sufficient to specify the essential features at each level of description. In all cases, and at each level, these hypotheses are under direct experimental observation.

3 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.

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’.

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 ‘bimolecular 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 biomolecules, 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.[6]

![Figure 1. Comparison of uptake of 50nm and 100nm SiO₂ nanoparticles at 100μg/ml in the presence (complete MEM) and absence (serum free media) of serum proteins. Note the very significant particle uptake in the absence of serum, compared to the much lower uptake in the presence of serum. It has been shown that serum reduces the non-specific interactions between that nanoparticles and the cell surface. Other differences in details of the uptake cannot be discussed here. Data from Partner 1.[5].](image)

On the contrary, nanomaterials are too large to 'dissolve' across membranes in a passive manner, and no such processes have (so far) been observed in all (our and other) experimental work across many particles types down to sizes of 5nm. On the contrary, nanoparticle uptake across the biological membrane is rapid, and cellular energy dependent (see Figure 1B where we show effects of cell energy depletion on nanoparticles uptake), driven by active biological processes that are currently being uncovered by various EU (including those of the current Partners) and National programs around EU and US. Sufficient preliminary information now exists[6,7] for us to identify a broad range of active biological processes (receptor mediated and other) responsible for this uptake of nanoparticles. Here it is sufficient to say that particles use a combination of endogenous entry portals (receptors etc) and membrane adhesion[5] (followed by membrane turnover) together producing internalization using the cells own energy.

** 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.

![Figure 2. A. Uptake of green fluorescent dye (molecular) by A549 cells – no effect of energy depletion. B. Effect of cellular energy depletion on uptake of 50nm nanoparticles of similar composition to the dye. C. Confocal image showing the localization of those 50nm nanoparticles in the lysosomes of A549 cells. D. Lack of export of those 50nm nanoparticles from A549 following removal of the particle source (I), compared to rapid release of molecular dye (YG). All data from Partner 1.[6].](image)
4 Project Description and Organisation

There are several striking features of the program that require particular emphasis and attention in the S/T methodology. These issues, and their impact on methodology are:

(i) Relative immaturity of the experimental field, lack of clearly validated data, and lack of uniformity on the understanding of the role and methods by which quantitative and reproducible data are acquired.

(ii) The long time required to generate extensive collections of such data, and the requirement to be more pro-active and constructive in the interim.

(iii) The need for realistic, achievable outcomes that can be checked at every point.

Figure 3. Electron Microscopy images after 10 minutes, 1 hour and 24 hours of exposure to 25 µg/ml 50nm red-fluorescently labelled SiO2 nanoparticles. From these images the localization of the nanoparticles on the surface (some in clathrin and caveolin receptors), early endosomes and finally lysosomes can be observed. Co-localisation of the red-fluorescent SiO2 nanoparticles with green LysoTracker dye is shown in the confocal microscopy image in the top right, confirming the final end point as the lysosomes. Data such as these are now developed to a quantitative level, including quantitative sub cellular localizations. EM and confocal data from Partner 1,[7] sketch of cell re-drawn from Watson et al.[8]

Intimate Collaboration between experiment-modeller-theorist

The current lack of large amounts of data that can be considered reproducible suggests that modellers must rely heavily on the science and understanding, which is now growing rapidly. This understanding of relevant parameters and mechanisms can be gained using a few examples, well studied, and can be widely applied in models. Thus, our approach is based on an implementation of the mechanistic understanding in an interactive manner. Thus, the model when created is tested using new sets of experimental data, and checkpoints applied to ensure success in a modular approach. Success in this kind of approach (albeit in a limited manner in the early days) is immediate. For example, the simplest uptake model has already been checked experimentally, and the most interesting outcome noted that one had to include the effects of cell division to obtain quantitative agreement.[6] Expansion of this concept allows for a proactive impact on the broader experimental community, from the earliest days, and does not require very large amounts of data before this can occur.

Robust organization to filter data inputs to ensure quality

At all points of the program, the acquisition of high quality data is key, and this is reflected in the accompanying chart, as well as the WP descriptions and the management processes. It is helpful that Partner 1 has an extensive experimental as well as modelling program, as this allows the Unit leaders to be in daily contact, interrogating the experimental information, and models. It ensures such details that the correct parameters and characteristics of the particles are recorded for the models. This allows us to template the process into a more formal management group activity where the young leaders from experiment and theory-modelling are required to evaluate data also emerging from other collaborators outside of the present program. This intimate day-to-day link between modeller and experimentalist we consider as foundation for the success of the project.

Progressive and systematic checks at modular level

Whilst we consider the program highly ambitious, we do feel that it will succeed, and that it is an essential building block on the constellation of such projects. The careful preliminary research and preliminary results in each segment enabled by a series of exchanges and visits in the last year as the program was built certainly gave us much assurance. However, the design and modularity of the project, with the capacity of exposure to the critical evaluation of experimentalists at each step and at each level is a key element. Thus, experience of the modellers and theorists in the program suggests that the developments of highly complex models that can only be tested at late stages are risky, and prone to failure. Here, for example, the capacity of a model module to predict the effective interaction between a nanoparticle (complete with corona) and cell membrane can be explicitly tested in a simple experiment. Similarly, the phenomenological model’s capacity to model the steady state concentration (for example, in basolateral endosomes in the BBB model) and link that correctly to the macroscopic flux across the BBB barrier can be explicitly checked with live cell imaging, where we have already established the reproducibility of such measurements. Thus, each modular component can be exposed to scientific checks, as well as the usual software validity checks.

The final Integration and Co-ordination tasks (within WP5) ensure that the modules remain in overall conceptual and operational alignment with each other within the program, to allow for a later integration with the future objective of modelling nanoparticle in vivo biodistribution. Crucially, this work package also allows for the co-ordination of communication with a variety of groups in EU and US, and Japan with similar objectives. Key collaborations already exist between the program and US partners. However, key collaborations also
exist with other major US and Japanese centres, and these have been aligned to the program. Still, we recognise the need to adopt a more flexible approach that takes account of realities on the ground after review of these programs, aligning with those programs that are funded, and newly emerging ones both in modelling, and in collection of experimental data.

We consider that these models, and this methodology, will point the way to the key science, and its relevance for society. 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 nanoparticles leading to different impacts, and above all, clearly identify the causal link between them. This link is the key to safety by design.

The interaction of the workpackages and the flow of information between them and the external experimental projects are shown in the Pert Chart in Figure 4, below.

NanoTransKinetics addresses the following objectives:

- To establish techniques for modelling relationships between nanoparticle properties and toxicity (including interactions of nanoparticles with biological systems);

NanoTransKinetics focuses on understanding the mechanisms of nanoparticle uptake into, and sub-cellular transport within cells and through biological barriers with the objective of enabling much more rapid progress towards a screening approach, where predictions of nanoparticle bioaccumulation could be made on the basis of limited in vitro screening data. NanoTransKinetics is the first integrated effort to develop phenomenological models based on high quality experimental data of nanoparticles interactions with cells and biological barriers. It aims to characterize the hazard posed by nanoparticles in relation to their ability to cross biological barriers, based on nanoparticle concentration fluxes (rather than the traditional ADME approaches which are based on equilibrium properties, which are not applicable to nanoparticles as they interact with cells in a biological manner, and are actively transported within cells). A four tiered approach (interaction with biological fluids, interaction with cellular membranes, interaction with cells in vitro, and interaction with biological barriers, such as the blood-brain barrier based on in vitro and in vivo data), as shown graphically in the Pert Chart in Figure 4, will ensure sufficient understanding of the role of nanoparticle-protein interactions in mediating nanoparticle-membrane and nanoparticle-protein-cell interactions, whilst allowing sufficient flexibility to be built into the models to allow modelling of data from a wide range of sources, including high throughput data such as high content analysis, thereby also providing a useful route for these data to be integrated into predictive approaches.

- Identification of physicochemical properties chosen for establishing groups of structurally similar particles, the characterisation and classification techniques, the test methods, and the relation of structural descriptors to toxicological targets;

One hypothesis of our approach is that nanoparticles in contact with biological systems are immediately coated by a layer of biomolecules which confers to them a “biological identity” which determines how the particles are seen by the cell, and how they interact with the cell. However, a deeper view of this that we have sought to clarify here is that the nanoparticle-environmental interaction cannot be ignored. Partner 2 and Partner 1 were both engaged in a previous EU program (lead by Partner 2) on gene transfer using liposomal and other carriers that though overall successful was striking in illustrating how weak the connection and efficiency between cell level and in vivo predictions was. Considerable investigation revealed that a major element of that was that cell culture takes poor account of the nanoparticle interactions with proteins, extracellular matrix and other biological environmental aspects. Here these elements are built into the program. Learning to predict the biological identities of nanoparticles and to correlate this with uptake, transport and clearance is the only way that we can truly determine a priori, the fate and behaviour of nanoparticles, and their safety implications for human health and the environment. Thus, the key to establishing categories of particles is via their biological identity, or what they actually present to cells. The endpoints that we have chosen to focus on in this programme are thus interactions with biofluids (e.g. plasma / cell culture medium), interactions with biological membranes (involved in uptake processes), interaction with cells (specifically transport kinetics and sub-cellular concentrations) and interaction with biological barriers (to begin the connection to in vivo predictions). Connecting the biological identity of nanoparticles to specific accumulation in certain organelles, and consequently to specific impacts such as apoptosis, enables us to categorize nanoparticles and to begin the process of predicting biological impacts based on biological identity. The capacity to quantitatively track the particles over long periods of time allows us to determine their biological fate, opening the way for significant advances in our understanding of the transport pathways used by nanoparticles to access the brain, using advanced quantitative dosimetrics, selected and controlled exposure scenarios, long-lifetime radiolabelled nanoparticles, and biophysical approaches such as fluorescently...
labelling certain proteins involved in the transport pathways and determining co-localization of the proteins and nanoparticles.

- deliver the basis for an inventory of nanoparticles based on potential for exposure, categorising nanoparticles on the basis of physicochemical, structural and toxicological properties:

As above, the capacity to categorise nanoparticles based on their biological identity will offer key advances in our predictive skills, and enable us to connect high throughput screening data to a screening predictive phenomenological models

5 NanoTransKinetics Year 1 results

NanoTransKinetics has made excellent progress in its first year, in part facilitated by the close collaborations between the three European partners, and also in light of the fact that a significant body of high quality data was immediately available to the consortium from the FP6 project Nanointeract (http://nanointeract.nanosafetycluster.eu/) and the FP7 project NeuroNano (http://www.ucd.ie/cbni/projects/european-projects/neuronano/) (both coordinated by the NanoTransKinetics coordinator) such that the modelling efforts were able to commence immediately for modelling of nanoparticle-protein interactions and nanoparticle uptake and localisation in cells.

WP: INPUT DATA QUALITY CONTROL

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.

As the modelling approaches described are phenomenological initially, they rely directly on the experimental data, in order to reproduce the phenomena, such as protein corona formation, nanoparticles uptake and sub-cellular transport and localisation) and we see a key outcome of the project as being a set of modelling tools that can be linked directly to high content analysis assays, such as lysosomal load, in order to correlate impacts with sub-cellular localisation and bioaccumulation potential, for example. The NanoTransKinetics partners believe that the approach outlined here is the only realistic possibility for understanding and predicting the implications of nanoparticles for living systems, as it is based on understanding the mechanisms of interaction of nanoparticles with cells.

During the first year, a clear focus has been placed on establishing the processes for data sourcing, and evaluation of incoming data quality, to ensure that experimental basis for the phenomenological models is of the highest possible quality and standards (WP1). To this end, an evaluation of the range, scope and completeness of in-house data from FP6 and FP7 projects which the project partners participated in / coordinated (including FP6 Nanointeract and FP7 NeuroNano) was carried out. Additionally, an inventory of other FP and national projects which could potentially have datasets of interest was developed, and contacts are now being made with these projects to source additional data. Similar efforts have been made (in collaboration with the NanoSafety Cluster and the EU-US Community of Research (CoR) on databases) to identify databases that may contain data of interest to NanoTransKinetics and to begin the process of ensuring that data can be shared between the various databases.

An initial analysis and identification of potential sources of suitable data sets for modelling nanoparticle interactions with proteins, cellular membranes, cells and biological barriers was performed in the first months of the project. The primary source of information on data being generated within other EU FP7-funded projects was the NanoSafety Cluster compendium of projects, which is a detailed description of each project, provided by its coordinator, and updated annually. On this basis, 9 EU-funded and 3 national projects were identified as potentially having useful data sets.

As part of this quality assurance process, a checklist for assessing the completeness of data sets being brought into the NanoTransKinetics projects has been developed, and is currently being drafted for publication to share with the wider scientific community. Using this checklist will enable design of experiments to generate data of sufficient quality to be used in the development and initial testing of the models, which is now being translated into a best practice article describing the factors to consider in the design and implementation of in vitro nanoparticle uptake or barrier crossing studies.

WP2: NANOPIRAT - BIOFLUID INTERACTIONS

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 first stages of this work has been published in ACS Nano[9] and the follow-on studies will be published in due course.
The University of Barcelona (UB) partner is using molecular dynamics 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-grained 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.[10-12]

Figure 5: a) FCS measurements of Tf binding to polystyrene NPs in buffer. Experiments performed at a feed concentration of Tf (full square 2.5µg/ml and circles 20µg/ml) by adding NPs. Dashed lines are Langmuir adsorption curves. b) A normalized representation (fraction bound vs. the ratio of protein to NPs) of the same data as presented in Figure 3a shows a universal behaviour. The vertical dashed line indicates the ratio of full surface coverage. The grey line represents the theoretical value of a single strongly bound monolayer.[9]

WP3: NANOPARTICLE-CELL INTERACTIONS

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 nanoparticle load among the daughter cells.[13] An analytical model to describe this was developed and validated against experimental data,[14] as shown in Figure 6.

For nanoparticles 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. In collaboration with experimental efforts within the UCD group, UCD has studied the phenomenology of nanoparticle adhesion to the cell membrane.[15] Experimentally the measurements were performed by removal of the cell energy, so that nanoparticles do not enter cells, and subsequent exposure of the cells to nanoparticles for given amounts of time, thereby providing a measure of the number of nanoparticles that adhere to cells as a function of time. A main conclusion is that adsorption to the cell membrane is strongly correlated with a high uptake rate – i.e. the more nanoparticles that stick, the more nanoparticles are taken up. The adsorption to cells and the subsequent uptake was modelled phenomenologically with the simple rate equation system

\[
\frac{dN_m}{dt} = k_u N_p (N_{m, max} - N_m) - k_d N_m - k_{in} N_m
\]

\[
\frac{dN_i}{dt} = k_{in} N_m
\]

yielding a good description at this level. A most basic conclusion is the vastly stronger adhesion of nanoparticles without a corona (in the absence of serum) compared to nanoparticles with a corona (in the presence of serum). More subtle is the connection between the adhesion and uptake rate constants with properties of the nanoparticle corona; our further work aims to elucidate this.

To date the models developed in WP3 have been applied to fluorescently-labelled polystyrene nanoparticles of various sizes and surface charges. A next step is to demonstrate their applicability to other particle types (e.g. fluorescently-labelled silica) and to unlabelled particles, for example on the basis of Transmission Electron Microscopy imaging of nanoparticle uptake and localisation (although this is less quantitative than confocal imaging) or via other labelling methods developed in UCD (mainly within the FP7 NeuroNano project).

Modelling of effects of nanoparticles on cells, such as on the lysosome-lysosome, lysosome-nucleus and other inter-organelle distances is also underway. Correlation of protein coronas with uptake pathways, uptake kinetics and nanoparticle localisation and impacts is the ultimate goal of this work.

Figure 6. The effect of cell cycle progression on nanoparticle uptake.[14] a, Schematic showing a cell progressing through the cell cycle phases G1, S and G2 and dividing into two new daughter cells during the M phase. Throughout the cell cycle the cell is taking up nanoparticles, potentially with different rates depending upon phase. b, Validation of the model for cell cycle progression to experimental data. The data points show the fraction of cells that divide as a function of time, whereas the solid line shows the parameter-free prediction from the model. The excellent agreement constitutes a strong basis for further modelling. c, Normalised nanoparticle uptake by cells in the different phases (G1, S and G2/M) as a function of time according to the model. In this case cells have been assumed to take up nanoparticles with the same rate regardless of phase; similar curves can be produced for arbitrary conditions, depending upon the nanoparticle and cell system.

Compendium of Projects in the European NanoSafety Cluster
WP4: NANOPARTICLE-BARRIER INTERACTIONS

This workpackage only started in month 12 of the project, and thus no significant modelling effort is reported as yet. However, within the FP7 project NeuroNano, significant data regarding the uptake and localisation of nanoparticles by an in vitro endothelial barrier model was obtained, as shown in Figure 7.

Similar approaches as described for the nanoparticle-cell interactions will be applied to model nanoparticle-barrier interactions, and again whether cell division plays an important role will be elucidated for the first time. Other important outcomes from NeuroNano that will be investigated further include whether the significant accumulation of nanoparticles in lysosomes is a symptom of nanoparticle-impacts on the transcytosis pathways, or suggestive that the blood-brain barrier (and cellular barriers in general) may be a target for nanoparticle accumulation.

WP5: INTEGRATION OF DATA MODULES

While the three modelling modules being developed in WPs 2-4 (nanoparticle-protein; nanoparticle-cell and nanoparticle-barrier) will all be significant research advances in themselves, the truly predictive output will arise from the integration of the modules, such that having experimental data for one module which can be fed into the models will enable prediction of the other end-points. We envision this as being able to start from any end-point and be able to predict any (all) other end-point(s) addressed by the model. The modular nature of the models will also enable new elements to be added as and if new toxicological end-points emerge for nanomaterials (note that at present very few exist, with apoptosis being one of the few confirmed as being induced by some nanoparticles).

This task is at an early stage of development (since the WP commenced only at Month 12). The UB partner has started developing a suit of codes that run on Graphic Processing Units (GPUs), which will be available to the wider community including regulators. These clusters are considered the future of simulations, because they can perform calculations hundreds of times faster than traditionally handled by Central Processing Units (CPUs).

6 Expected impacts

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.

Publications and outputs from Year 1

NanoTransKinetics activities have produced a number of publications (7 publications, 3 in press, 2 submitted by month 12), along with being featured on the Cover of the December 2012 issue of Nature Nanotechnology.[17] Additionally, NanoTransKinetics team members have presented the project and its results at over 15 international conferences and workshops and as visitors to over 15 institutes and departments.

7 References


8 Directory

Table 1 Directory of people involved in the NanoTransKinetics project as beneficiaries.

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<tr>
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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

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**USEPA (no full member of the NanoValid consortium but associated by a LOI to the project)**
Contents

1 Summary .................................................................................................................. 212
2 Scientific / industry needs and problems addressed .............................................. 212
3 Scope and objectives ................................................................................................. 214
4 Technical approach and work description ............................................................. 215
5 Status of the project .................................................................................................. 215
   5.1 Project management (WP1) and ST coordination (WP8) .................................. 215
   5.2 Materials fabrication and methods selection (WP2) ........................................ 215
   5.3 Method validation (WP3) .................................................................................. 216
   5.4 Applicability of validated methods to RA and LCA (WP4) ............................ 216
   5.5 Developing reference methods and materials (WP5) .................................... 216
   5.6 Case studies (WP6) .......................................................................................... 217
   5.7 Dissemination, training and exploitation (WP7) ............................................. 218
6 Expected impact ....................................................................................................... 218
7 Dissemination and exploitation strategy ................................................................. 218
8 Directory .................................................................................................................. 219
9 Copyright .................................................................................................................. 223

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 regulatory requirements and for safeguarding production, application and disposal of nanomaterials.

NanoValid has mobilized the critical mass of international scientific knowledge and technical expertise required to address these questions. Current analytical and toxicity test methods and models are put to the test and subjected to rigorous intercalibration and validation. Where necessary, methods and materials will be modified, adapted and validated, and new reliable reference methods developed, in cooperation with international standardization bodies and the concerned industries, to support pre and co-normative activities and to make existing risk (RA) and life cycle (LCA) assessment schemes more reliable for ENPs.

The feasibility of validated measurement, characterization and test methods will be assessed by selected case studies to help to improve performance of existing exposure monitoring systems as well as risk management and reduction strategies.

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.

In particular, new progress will be made 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: To produce well defined ENPs for toxicology testing, NanoValid will optimize and extend current synthesis protocols for selected nanomaterials (see lists below) and include the generation of 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 toxicity 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 will develop and use highly sensitive EN labeling and tracing methods and will design and use a controlled atmosphere dispersion chamber that allows a more precise and reliable monitoring of the behavior of selected nanoparticles.

Initial test materials will include those listed below, although final prioritization will be made in close coordination with relevant standardization bodies and programs and with projects included in the Nanosafety Cluster (in particular with the EU FP7 MARINA, QNANO and NanoDevice project):

**Priority 1 test materials**: metal oxides (SiO₂, TiO₂, ZnO, CuO), metals (Ag, Au and Pd), CNTs (SWCNTs and MWCNTs) and fullerenes.

**Priority 2 test materials**: quantum dots (CdSe, CdS, CeO₂), 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 will establish and implement the following specific tools to address 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 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 will use the most efficient cell-based assays as a basis for the first biological test 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 will be 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.

A collection device for 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 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 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. NanoValid will in particular shed light 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 will use this method to examine the compatibility of various exposure media with in vitro models tested, 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 will further develop and validate a specific 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 will develop 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 will also assess 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 will develop 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 materials, 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 currently selected and tested, and new nanomaterials will be synthesized, characterized and stabilized for final method validation.

Already existing industrial or newly designed nanomaterials (ENMs) will be submitted to a comprehensive inter-laboratory validation campaign that includes the 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 will be 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 validated pc methods derived from the extensive intercalibration and inter-comparison of selected methods and materials will be used to design well-defined reference materials, which in turn will be employed to validate, and where necessary adapt, modify and further develop current biological approaches (in vitro, in vivo and in silico) for assessing the toxicity of ENMs and associated 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 comprehensive and critical review 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 will be developed and validated by using relevant and representative industrial and/or 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.marina-fp7.eu), the other large-scale EU FP7 nanosafety project. Both NanoValid and MARINA will coordinate and synchronize their R&D activities, to effectively use synergies, share data and test materials, and to organize common dissemination events.

In the following, a short overview is given on the work done and results achieved so far during the first project year (M12).

5.1 Project management (WP1) and ST coordination (WP8)

Beside the kick-off, NanoValid had 2 following regular meetings, 1 in Copenhagen (8-9 May 2012) and another one in Berlin (29-30 November 2012), and the next regular meeting will be in Ljubljana, 5-7 June 2013. Besides, NanoValid had several Webex-based online progress meetings and 3 synchronization online meetings with MARINA on material selection, toxicity testing and exposure assessment. NanoValid partner BAUA organized on 27-28 November 2012 an open workshop on safe handling of nanomaterials in Berlin. In M18, NanoValid will reach the end of the 1st reporting period. For this, the 1st interim Report will be prepared and the 1st technical review implemented together with the Commission.

Right away from the start of the project, quarterly progress reports are prepared by all project partners and evaluated by work package leaders (WPL) and the coordinator, to continuously update the progress of work according to the road map and work plan.

5.2 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 CCBM) and 1 prototype of Au (dispersed in H2O and in-house fabricated by INMETRO). 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 Colorobbia). In the 2nd half year of the project, another 2nd set of test materials was prepared and shipped to partners from WP2, including prototypes of Au (from INMETRO), TiO2 (from CCBM) CNTs (fabricated by MARINA partner Nanocyl).

Main pc properties of the 1st set of nanomaterials have been fully characterized and a preliminary approach for their biological screening applied and evaluated, including bacteria, protozoan (Tetrahymena thermophila), crustacean (Daphnia magna), isopod (Porcellio scaber), plant (Allium cepa), yeast and mammalian cells. PC characterization and biological screening of the 2nd set of test materials is still ongoing. Both preparation and distribution of the first 2 sets of test materials have been duly documented in various deliverable update and milestones reports, including a description of SOPs established for the synthesis of tested materials, their pc characterization and biological screening. Table 1 gives an overview of the methods used in WP2 and of partners involved in the pc characterization of the 2nd set of test materials:
At present (M15), synthesis protocols for TiO2 and for the upscale synthesis of a 3rd prototype of SiO2 by BAM are further optimized and the synthesis and delivery of a 3rd set of test materials are in preparation.

Table 1: PC characterization of the 2nd set of test materials (Au, TiO2, CNTs)

<table>
<thead>
<tr>
<th>Technique</th>
<th>Property</th>
<th>Partner involved</th>
</tr>
</thead>
<tbody>
<tr>
<td>XRD</td>
<td>Crystalline phase</td>
<td>IMT, NLab</td>
</tr>
<tr>
<td>SEM</td>
<td>Morphology, Particle size (dry)</td>
<td>NLAB, CRF</td>
</tr>
<tr>
<td>TEM</td>
<td>Crystalline phase, Symmetry of porous (porous SiO2), Composition</td>
<td>NHM</td>
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<tr>
<td>DLS</td>
<td>Particle size (dry), Agglomeration/Aggregation</td>
<td>FBG, IMT</td>
</tr>
<tr>
<td>Z-potential</td>
<td>Electrical charge</td>
<td>FBG, IMT</td>
</tr>
<tr>
<td>BET</td>
<td>Surface area, porous size</td>
<td>NLAB, FBG</td>
</tr>
<tr>
<td>ICP</td>
<td>Composition</td>
<td>VN</td>
</tr>
</tbody>
</table>

5.3 Method validation (WP3)

All WP3 partners have received from WP2 two prototypes of particles (i.e. silver and silica) for the various tests planned. The approximate total quantity of particles delivered was 180g of silver and 170g of silica. 2 milestones reports have been completed, i.e. MS8 and MS15 concerning prioritization and selection of model nanoparticles and harmonization of test conditions. Also a list of methods selected for WP3 has been compiled and circulated to all partners participating in the planned round robin studies (RR1 on pc characterization and RR2 on dispersion) aimed towards method development and validation.

The 1st set particles (i.e. silver and silica) has been fully characterised using a RR type exercise to evaluate a range of key pc properties of the materials tested (viz. size, size distribution, shape, surface charge, surface chemistry, purity, surface area, hydrodynamic size etc.). Also SOPs have been created regarding cell culture conditions, the growth of A549 cells, and potential myco-plasma contamination, to finally evaluate possible toxic effects of selected NPs by the MTS assay in 2 RR studies. In addition, equipments 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, NPS500, FMPS, ELPI and AMS.

Established SOPs will be a main outcome of the first RR studies and preliminary work has started to ensure validation of methods, e.g. to monitor human workplace exposure.

5.4 Applicability of validated methods to RA and LCA (WP4)

A first data evaluation on the hazard of nanomaterial for human and environmental health was carried out with specific emphasis on eco-toxic effects of CNTs, in this context a Review paper was prepared on Bioaccumulation and eco-toxicity of Carbon Nanotubes (Petrà Jackson, Nicklas Raun Jacobsen, Anders Baun, Dana Kühnel, Keld Alstrup Jensen, Ulla Vogel, Håkan Wallin, 2012, submitted to: Critical Reviews in Environmental Science and Technology). Also, current risk assessment case studies and strategies specifically developed for nanomaterials were critically evaluated. For the planned life cycle analysis and life cycle impact assessment, data collection sheets have been developed 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 set of test materials from WP2 and WP3, to develop the framework for a new risk assessment strategy for both human and environmental health. Also the life cycle inventory analysis has started with an extensive data collection process to get all relevant data available or continuously generated by NanoValid partners.

Some of the problems still faced today are due to the fact that

- Many of the physical and chemical properties of nanomaterials are unavailable or unknown
- Few and inconclusive data on health and environmental impacts exist
- Risk assessment seldom considered due to present low volume of production, diversity of materials and uncertainty about exposure
- Exposure levels cannot be established with precision

Next steps to be taken within WP4 include:

- Complete description of materials and applications
- Development of profile lifecycles
- Generation of the necessary pc characterization data
- Assessing health & environmental safety aspects
- Perform risk assessments for selected materials.

5.5 Developing reference methods and materials (WP5)

Methods identified under WP2 and validated under WP3 will be evaluated for their potential to be developed as reference methods for a special task in WP5. Also, test materials selected and/or newly fabricated in WP2 and validated in WP3 will be used to develop appropriate CRMs in this particular WP. 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 (in-house produced by BAM) is currently developed, which shows a high potential to become a certified reference materials (CRM). Also a nano-Ag CRM (not...
developed within NanoValid) will be soon made available by BAM to be used as an internal benchmark standard.

Together with WPL2 and WPL3, WPL5 BAM has developing an internal set of standard criteria for the specification of test samples that can be used for toxicity testing.

A literature study on existing methods for the dispersion of nanomaterials in water as well as in cell culture media has been prepared and first results of validation measurements that use different dispersing methods for standard nano-TiO2 and nano-SiO2 have been obtained. The preparation of protocols and the selection of test samples for inter-laboratory comparison (RR) on dispersion measurements are still ongoing. Measurements on suspended NM within WP2 + WP3 are a prerequisite for the round-robin tests in WP5.

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 participating laboratories, to handle them in toxicological and environmental test systems and particle size measurements in appropriate concentrations will be done by dynamic light scattering and centrifugal methods and qualified by zeta potential determination (microelectrophoresis).

To establish reference methods for NP labeling, first labeled ENPs have been synthesized, e.g. SiO2/fluorescent labeled NPs by reverse micro-emulsion method, and TiO2/lanthanides labeled NPs by sol-gel method and microwave heating, and are currently characterized. Synthesis methods will be further modified to reduce particles size but maintaining the capacity of labeled ENPs detection.

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.

To establish reference methods to study uptake and distribution of nanomaterials in the body, the nanotoxicity of the 2 SiO2 prototypes and the Ag delivered by WP2, as well as of carbon nanowhisker delivered by MARINA were tested 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. Within the next months, biocompatibility, membrane penetration and distribution of SiO2, TiO2, C60, and liposome NPs will be evaluated.

In addition, the performance of various eco-toxicological assays (aquatic and terrestrial) has been tested 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.

As next steps, protocol templates specific for (1) particle synthesis/production, (2) particle characterization, and (3) toxicity testing will be compiled and some SOPs provided by the EU FP7 Nanommune Handbook will be adopted. SOPs established for the tests with crustaceans and bacteria will be further improved. Also safety aspects will be addressed by the used SOP, e.g. a cell line identity check will be included.

To transfer relevant results to method and materials standardization, NanoValid has signed a project liaison with CEN and is presently discussing direct participation in various CEN and ISO/TCs. A recent internal survey identified those TCs which are most interesting and where NanoValid could contribute with standardization proposals, 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.

5.6 Case studies (WP6)

Guidelines for safe handling and monitoring of ENPs, and a Draft guidance document based on the code of practices have been developed and intensively discussed internally and with external experts during an open workshop (BAUA Berlin, 27-28 November 2012), to ensure safe and standardized handling of ENP in all partner labs, and beyond. The proceedings of this workshop have been finalized and are available on the project website. Also a standardized procedure for field studies to assess the feasibility of the developed guidelines is expected in M18.

Concerning the use of the developed validated methods for accidental risk management, a report on the selection of accident scenarios is in preparation and will be ready in M18. Two different situations will be considered: 1) Industrial accident leading to an explosion or massive release of a nanoparticle-containing cloud within the processing plant. 2) Transport accident leading to a partial or entire spill of NPs on a public waterway (river or lake). Work is presently focusing on finding suitable industrial partners, finalizing accident scenarios, and on contracting a subcontractor for release modeling.

The selection of nanomaterials to be used and tested in the automotive case study has been changed due to new developments, and work will now concentrate on nanomodified adhesives activatable by external triggers (instead of functionalized polymers), and on nanomodified coatings and nanocontainers for the improvement of corrosion resistance (instead of electrodes for battery/supercapacitor systems). By this, new research priorities and new material preferences within the automotive industry will be duly taken into account.

Also bystander substances have been defined for controlled contamination of ENPs, to investigate the robustness of developed and validated biological test methods applied to non-clean samples.

A specific WP6 meeting is planned on 6-7 February 2013 at the University of Salzburg (PLUS = WPL6) to coordinate and back up the work to be done within the various case studies.
5.7 Dissemination, training and exploitation (WP7)

A project website and discussion forum has been created together with a web-supported project database and partner intranet; 3 Newsletters have been issued so far (see more information on dissemination below). A 1-week training workshop is planned in September 2013 together with other projects (MARINA, QNano) for postgraduate and PhD students and young researchers on advanced methods and materials used within the project.

6 Expected impact

The project aims to compare and validate current test methods, and if necessary modify and adapt these methods, and/or develop new methods for reliable measurement and testing to improve exposure and risk assessment as well as life cycle analysis of nanoscale materials, with the ultimate goal of establishing reference methods and materials. To achieve these objectives, the project will implement a comprehensive inter-laboratory, inter-comparison and intercalibration campaign between all participating laboratories and a set of case studies to assess the feasibility of the established methods. It is a first step in the generation of reliable quantitative data on the toxicology and ecotoxicology of nanomaterials and the development of accurate methods required to generate the data. The validated methods and new knowledge developed will trigger a step change in the early scientific assessment of potential health, safety and environmental risks associated with nanotechnology-based materials and associated products. It will help to meet existing regulatory requirements and/or develop new legal requirements for safe, responsible and sustainable development.

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 assessment and the design of sustainable solutions for safe production, use and disposal of ENMs. NanoValid will generate a comprehensive knowledge and database on the intrinsic properties of particular ENMs and associated ENPs and will contribute to a better mechanistic understanding of their behaviour in various test media, physiological solutions and environmental matrices. The generation of scientifically sound and well-defined reference tools will support standardization efforts to characterize ENPs and their potential effects and hence improve current test schemes for risk management, reduction and monitoring. Providing reliable methods for RA and LCA of ENPs, including new in vitro cell panels and other models to assess their bioaccumulation, persistence and bioavailability will help to identify problematic materials early on, which in turn will stimulate the development of safe production processes, novel properties and innovative sustainable products (“green nanotechnology”).

The proposed toxicological work will address critical issues, such as the validation of current standardized in vitro tests, the follow up of biodistribution, persistence and bioaccumulation of NPs, tracing of NP excretion or immune effects as well as possible genotoxicity and effects on sensory organs or neural tissue. Results from NP testing will contribute to new conceptual and experimental standards for toxicity testing by in vitro test systems, which in turn helps to reduce animal testing.

New knowledge on the toxicity of ENMs to particle-ingesting organisms and to those not internalizing NPs, and on mechanisms that control particle solubility and bioavailability, 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 under various laboratory and field conditions by various measurement and testing methods 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 will develop standardized procedures for labelling and dispersing 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 suspensions.

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

The NanoValid work programme aims to develop a series of new collecting, characterizing, and testing methodologies. A number of these have clear commercialization opportunities. Through the participation of industrial partners and partners that have an established background in the commercialization of new devices, it is expected that project results will lead to:

- standardized test materials that can be used by different industries to validate the physicochemical properties of ENMs they manufacture or purchase;
- novel ENP samplers, e.g. for hot gases;
- novel real-time collection and assessment devices which collect airborne ENPs and assay them on an integrated biomodule, thus addressing issues of bioactivity and transport, and ensuing loss or change of reactivity and physical properties;
- a multi-compartment cell barrier model to mimic physiological systems and provide a more realistic evaluation of NP 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.

NanoValid has established a dedicated project website (www.nanovlaid.eu) and database, where users can find the latest news and developments as well as additional information such as the output of dissemination events and other presentations. NanoValid dissemination activities directly target stakeholders through regular newsletters and press-releases, which are also hosted on the website. As the
project progresses, registered users can access project results and engage through feedback and a discussion forum. A partner intranet and database enable all partners to view the technical materials and literature database and all project deliverables (draft and final) in a password protected area.

The project continues to promote the possibilities for exploitation of its data through partner websites, NanoSafety Cluster Partners, RTD community and wider community of stakeholders. It closely liaises with counterparts in complementary projects and actively engages with individuals through a series of physical and online events. To ensure all relevant stakeholders are reached, the consortium leverage its 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 commercialization potential of diagnostic tools developed within the project, and propose new standards to ISO/CEN and OECD committees using the expertise within the consortium. To achieve this, the consortium will utilise established information channels including (but not limited to): the ION website (over 65,000 nanotechnologists in its database, receives over 100,000 visits and 1 million hits each month), the EU FP7 observatoryNANO website (providing analysis of nanotechnology developments and impacts from a European perspective), NanoForum (gateway to European nanotechnology with over 20,000 users registered), and existing news services and aggregators, such as Cordis and Alpha Galileo.

8 Directory

Table 1 Directory of people involved in this project.

<table>
<thead>
<tr>
<th>First Name</th>
<th>Last Name</th>
<th>Affiliation</th>
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Nan Safety Cluster - Compendium 2011

220 Compendium of Projects in the European NanoSafety Cluster

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QualityNano
A pan-European infrastructure for quality in nanomaterials safety testing

Contract Agreement: SP4-Capacities-2010-262163  Website: http://www.qnano-ri.eu
Coordinator: Kenneth Dawson, Centre for BioNano Interactions, University College Dublin, Belfield, Dublin 4, Ireland

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* Grant agreement amended in 2012.

Note: From 1st February 2013, QNano is being 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 ................................................................. 226
2 Background .......................................................... 226
3 Project Description and Organisation ......................... 227
4 Key Challenges being addressed by QualityNano ........... 228
5 QualityNano activities ............................................. 230
6 Expected final results and their potential impacts and use ... 234
7 References ............................................................ 235
8 Directory ............................................................... 236
9 Copyright ............................................................... 238

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.

2 Background

Nanoscience constitutes a new scientific frontier in which we can, for the first time, engineer materials on the length scale of some millions 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.

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. Physicochemical 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) physicochemical 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 - Working Groups to drive future development and sustainability of the infrastructure.

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 8m² 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 situs 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 15 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 aims of the QualityNano annual Integrating conference are:

- To serve as the European showcase for research excellence in nanosafety assessment, including new scientific directions and key advances, to inspire young researchers;
- To build and sustain research linkages among the European and International communities working on the topics of nanosafety assessment and nanomedicine;
To reflect the hand-over of activities from the Coordinator Support Action NanolmpactNet to QualityNano, a joint conference was organised by the two projects from 27th February - 2nd March 2012, which was held at the O’Reilly Hall in University College Dublin, Ireland. Reflecting the three pillars of activity of QNano (JRA, NA and TA) the theme of this 1st QualityNano conference was “From theory to practice - development, training and enabling nanosafety and health research”.

The conference was attended by over 250 delegates from all over Europe, America, Africa and Asia, and had an excellent representation from industry including the insurance industry (10%), governments & the European Commission (11%) and NGOs and other stakeholder groups (12%) as well as from academic (67%).

Representatives from 18 EU countries plus Norway and Switzerland demonstrate the importance of the conference as a European meeting place, and indeed 90% of the delegates were European.

All oral presentations were recorded and are available for viewing via the QualityNano Knowledge Hub, e.g. conference day 3’s presentations can be viewed at: http://www.qnano-ri.eu/knowledge-hub/the-qnano-knowledge-hub/repository-of-conference-materials/nanolmpactnet-qnano-2012/video-recordings/day.3.html. We are currently working to make this element of the site easier to navigate and more user-friendly, although with the size of the files this is proving somewhat challenging.

A key observation from the 1st conference was that the participation of the newer member states was quite low, with participants only from Slovenia, Slovakia and Poland. Thus, targeting participation of researchers from new member states is a key goal for the 2nd QualityNano conference, and indeed the location was selected in order to facilitate this: the 2nd QualityNano conference is taking place at the IMG Conference Centre on Prague, Czech Republic from 27th February - 1st March 2013. The theme this year will be “Quality in nano safety assessment – driving best practice and innovation”, reflecting the increasing awareness of the need for high quality data and the Innovation 2020 agenda. The conference website is: www.qnano-ri.eu/conference.

An important element of the 2013 QualityNano conference will be the showcase of the efforts and outputs of the first round of QualityNano Transnational Access Users, via the Transnational Access session, which has a central spot on day 2 of the conference, to ensure maximal participation. 5 QualityNano TA Users will give short presentations on their QualityNano-funded work at JRC, UCD, KIT, UU and FUNDP. Plans are also in place to conduct short interviews with the TA Users to add to the QualityNano Transnational Access website as further promotion and User endormements.

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 will coordinate all aspects of training and good practice (guidance, hands-on training, industry training etc.). As part of the Knowledge Hub, QualityNano is currently setting up a system that can be accessed and utilised by all and current and future Users1. The first step in this task is gathering of existing information regarding training offers into one single location. Already existing courses have been invited to add their details to a content management site to host the materials, and later indexing and search features will be added to increase the User experience.

As part of this, identification of knowledge needs is also underway in conjunction with the NanoTOES Initial Training Network and the NanoEIS Coordination support Action on nanoeducation.

A key feature of the Knowledge Hub will be 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.

Initial approaches at populating the Knowledge Hub have included video-recording the QualityNano conference and Training School lectures and most speakers gave approval for using these recordings for the purpose of the QualityNano knowledge-hub (http://www.qnano-ri.eu/knowledge-hub/the-qnano-knowledge-hub.html).


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;

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.
• 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.

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-NH2), 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.

Data from DLS and TEM were reasonably good, although more scattered about the mean size that would be considered acceptable especially for the IRMM certified size particles, as shown in Figure 1. This significant variability in the baseline data confirms the need for QualityNano and its processes to improve data quality and data acquisition processes. More significant differences were observed in the NanoSight and disc centrifuge data, and indeed some technical problems were also encountered by partners using the disc centrifuge, especially for the PS-NH2 nanoparticles which appeared to interact with the sucrose gradient, and therefore, a common protocol has been developed and alternative test nanomaterials identified. UCD proposed that two new samples are selected that have larger particle size. All partners will re-run TEM, DLS and NTA on the new samples.

For all four methods protocols have now been circulated to all partners and the study is being repeated to assess the implementability of the protocols. The DLS protocol is the one utilised in the IANH round robin and published in J. Nanoparticle Res.,2 the TEM protocol was developed in UCD, NanoSight led the development of the protocol for NanoSight Nanoparticle Tracking Analysis and the Differential Centrifugal Sedimentation (DCS, also named AUC Analytical Ultra-Centrifugation, term used in TA) protocol was developed collectively by UCD, FUNDP and UNIVLEEDS. Based on the outcome of the study with the test nanomaterials and the protocols, further refinement may be required to remove any last uncertainties (step 3 above) and a re-run of the RR will be performed.

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 1st and 2nd Calls for QualityNano TA are now underway, with several of the partners having fully

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completed all of the TA visits. The 3rd call for TA opened on the 1st November, with a closing date of the 15th March 2013 – this date has been shifted to after the 2nd QualityNano Conference to allow new users who hear about QualityNano TA at the 2013 Conference time to apply after the conference.

Transnational Access is provided according to four thematic categories: nanomaterial synthesis, labelling and processing, characterisation in situ, exposure assessment. As shown in Figure 3 the 15 transnational access facilities 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. The process is shown schematically in Figure 3. 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.

Figure 1: Compilation of size data obtained from the Quality-Nanobaseline RR of nanoparticle size determination by DLS or TEM using IRMM certified reference material ERM FD100. The Certified diameter by DLS is 19.0 nm with uncertainty 0.6 nm, while the certified diameter by TEM: 19.4 nm with uncertainty 1.3 nm.

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 2: 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.
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.

7 References

3. The EU 6th Research Framework Programme (2002-2006) devoted over €1.3 billion to nanotechnology and new materials, and has allocated € 3.5 billion to the NMP theme for the period 2009-2013 (FP7).
## Directory

Table 1 Directory of people involved in the QualityNano project as beneficiaries.

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</tbody>
</table>

* Note there are also additional partners who are non-funded who are not included here.
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**REACHnano**

Development of a web based REACH Toolkit to support the chemical safety assessment of nanomaterials

Coordinator: Carlos Fito, Packaging, Transport and Logistics Research Center , Valencia, Spain

<table>
<thead>
<tr>
<th>No.</th>
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<td>2</td>
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<td>3</td>
<td>Nanotechnology Industries Association</td>
<td>NIA</td>
<td>Belgium</td>
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<td>4</td>
<td>Instituto Valenciano de Seguridad y Salud en el Trabajo</td>
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Contents

REACHnano ..................................................................................... 239
1 Summary ................................................................................... 239 5 Advances over the state of the art ......................................... 242
2 Background .............................................................................. 240 6 Expected Results ................................................................. 243
3 Concept and Objectives ........................................................... 240 7 Progress to date ................................................................. 243
3.1 Project Concept ................................................................... 240 8 Conclusion To-Date & Plan for the Future ......................... 243
3.2 Project Objectives ............................................................... 241 9 Directory ............................................................................ 244
4 Overall view of the Workplan ................................................... 241 10 Copyright .......................................................................... 244

1 Summary

Within the context of REACH regulation, the main issue pertaining to the use of chemicals in whatever size, shape or physical state, is to ensure their safety to the human health and the environment, therefore, since its entry into force on 1 June 2007, **REACH plays a central role to ensure the protection of environment and health from risk posed by chemicals** and to promote sustainable development.

In this context, all available information on the substance has to be gathered and considered for the registration, however in certain cases already exists a lack of information or even standardized risk assessment methods, which make difficult the direct application of REACH regulation. This is the case of nanomaterials, which properties are often exceedingly different to those demonstrated by the bulk forms.

In order to address these major concerns and considering the priority areas of LIFE +, the main objective of the project is to provide innovative instruments to improve the implementation of REACH when manufacturing or handling materials or substances at the nanometer scale through the development of a web based Help Desk tool to support the risk assessment and promote the safety use of nanomaterials along their life cycle, providing the industry and stakeholders with easy to use tools to support the implementation of REACH regulation.

The toolkit to be developed within REACHnano project will take into account the needs and specifications of end-users and stakeholders, including advanced functionalities that will support industry and authorities to fulfill their main task under REACH, with special concern to those provisions aimed at ensuring high levels of human health and environmental protection such as the generation of reliable information in terms of REACH information requirements , the assessment of risk for the specific uses of the substances (i.e. exposure scenarios) and the characterization of effective risk managements measures (RMM). Moreover, this interactive web application will provide an innovative tool to share and exchange information between the scientific community and politicians, enhancing science-policy integration in support of REACH legislation.

Project Duration: 1 October 2012 – 30 November 2015
Project Funding: 915,861 EUR
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. The uncertainties are extensive since the properties exhibited by substances and materials at the nanometer scale are often completely different from those demonstrated by bulk forms, affecting their physicochemical and biological behaviour, which results, in more toxic properties. Research on human and environmental toxicity (i.e. ecotoxicity) of this group of materials and substances has recently started, and draws upon existing knowledge in toxicology, ecotoxicology and environmental sciences in an attempt to predict potential future problems related to spreading of nanomaterials in the environment, notwithstanding, studies of existing nanometer-sized particles of many materials have shown adverse health effects in humans exposed, and animal studies have shown that ultrafine particles are more inflammogenic and tumorigenic than an equal mass of larger particles of similar composition.

Taking into account the current situation, the rising production and use of materials and substances at the nanometer scale is generating both environmental and human health impacts, which are increasing the likelihood of human diseases and environmental pollution, with a special concern for water, soil and atmosphere, key compartments where organisms are likely to be exposed in different ways.

The REACH regulation is the main legal instrument to protect the environment and health from risk posed by chemicals, as well as to ensure the safety use of chemicals in the European market. Since REACH deals with substances, in whatever size, shape or physical state, substances at the nanoscale are also covered by REACH and its provisions apply. This implies that also the safety of nanomaterials to human health and the environment should be ensured under REACH, covering their whole life cycle.

On the basis of the current situation, activities supporting the implementation of REACH will improve the protection of environment. In addition, the enhancement of knowledge regarding information on physicochemical, toxico logical and ecotoxicological properties of materials and substances at the nanometer scale, as well as exposure, use and risk management measures, will provide new data to support the risk assessment of nanomaterials.

3 Concept and Objectives

3.1 Project Concept

The REACHnano project deals with the enhancement of knowledge base on risk assessment of nanomaterials through the identification and evaluation of available information under REACH requirements, as well as with the development of innovative alternatives to support the implementation of chemicals legislation, in particular REACH regulation.

The overall aim of REACHnano 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 information sources and the characterization of the information requirements to perform the chemical safety assessment (CSA).

b- Characterization of current lack of data to prepare the REACH registration dossier by means of authorized reporting tools (IUCLID 5/Chesar)

c- Characterization of current lack of data to prepare the REACH registration dossier by means of authorized reporting tools (IUCLID 5/Chesar)

d- Design and Development of the web based REACH Toolkit

e- Promotion of REACH fulfillment by implementing REACHnano Toolkit
3.2 Project Objectives

The main objective of the project is to provide innovative instruments to improve the implementation of REACH when manufacturing or handling materials or substances at the nanometer scale through the development of a web-based Help Desk tool to support the risk assessment and promote the safety use of nanomaterials along their life cycle, providing the industry and stakeholders with easy-to-use tools to support the implementation of REACH regulation. This interactive web application will provide an innovative tool to share and exchange information between the scientific community and politicians, enhancing science-policy integration in support of REACH legislation.

In detail, our key aims are:

• To develop a functional and user-friendly web-based toolkit to support the implementation of REACH
• To disseminate the project results for a large community of SMEs and potential stakeholders
• To enhance the knowledge base on nanomaterials related risk and risk assessment by means of the collection, evaluation of adequacy and selection of the available information on physicochemical, toxicological and ecotoxicological properties, exposure, use and risk management measures to be provided upon registration
• To demonstrate the effectiveness of REACH regulation to protect the environment and health from risk posed by chemicals, and especially by substances at nanoscale.
• To support the monitoring of REACH compliment and its impact on risk mitigation and prevention of pollution posed by hazardous chemicals.

In summary, the REACHnano project deals with the set-up of an innovative web application to improve the protection of environment and health from risk posed by materials and substances at the nanometer scale through the implementation of REACH provisions, and bridge the gaps of knowledge on nanomaterials properties, hazard and exposure.

4 Overall view of the Workplan

The REACHnano 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 REACHnano

<table>
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<tr>
<th>WP nº</th>
<th>WP Title</th>
<th>Action Leader</th>
</tr>
</thead>
<tbody>
<tr>
<td>Preparatory Actions</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A.1</td>
<td>Selection of representative nanomaterials</td>
<td>NIA</td>
</tr>
<tr>
<td>A.2</td>
<td>Identification of information requirements to complete the CSA of nanomaterials</td>
<td>ITENE</td>
</tr>
<tr>
<td>A.3</td>
<td>Identification of information sources</td>
<td>LEITAT</td>
</tr>
<tr>
<td>A.4</td>
<td>Identification of REACHNano Toolkit functionalities</td>
<td>ITENE</td>
</tr>
<tr>
<td>Implementation Actions</td>
<td></td>
<td></td>
</tr>
<tr>
<td>B.1</td>
<td>Compilation, analysis and evaluation of data to be provided upon registration of nanomaterials</td>
<td>ITENE</td>
</tr>
<tr>
<td>B.2</td>
<td>Compilation and critical evaluation of possible approaches to assess the risk posed by nanomaterials</td>
<td>LEITAT</td>
</tr>
<tr>
<td>B.3</td>
<td>Characterization of current lack of data to prepare the REACH registration dossier by means of authorized reporting tools</td>
<td>ITENE</td>
</tr>
<tr>
<td>B.4</td>
<td>Design and Development of the web based REACH Toolkit</td>
<td>ITENE</td>
</tr>
<tr>
<td>B.5</td>
<td>Development of complementary tools and plugins of the REACHNano Toolkit</td>
<td>ITENE</td>
</tr>
<tr>
<td>B.6</td>
<td>Validation by application end users</td>
<td>ITENE</td>
</tr>
<tr>
<td>B.7</td>
<td>Development of training support materials and training sessions</td>
<td>INVASSAT</td>
</tr>
<tr>
<td>Monitoring Action</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C.1</td>
<td>Definition of the starting situation regarding REACH regulation fulfillment and environmental problems targeted by the project</td>
<td>NIA</td>
</tr>
<tr>
<td>C.2</td>
<td>Strengthening of the knowledge base on nanomaterials properties, and environmental impacts and risk assessment</td>
<td>ITENE</td>
</tr>
<tr>
<td>C.3</td>
<td>Promotion of REACH fulfillment by implementing REACHnano Toolkit</td>
<td>LEITAT</td>
</tr>
<tr>
<td>C.4</td>
<td>Integrative assessment of risk characterization ratios when implementing risk management measures</td>
<td>ITENE</td>
</tr>
</tbody>
</table>

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

These actions will be focussed on the generation of practical information to be used during the implementation phase, including the selection of representative nanoscale materials, the identification of information sources and the characterization of the information requirements to perform the chemical safety assessment (CSA)

2. Implementation Actions

The implementation action will be focussed on the development of the web application REACHnano Toolkit and its further validation. To this end, a complete compilation and evaluation of available data will be conducted to select reliable information to be used in the risk assessment process. Secondly, the current approaches and data requirements of authorized reporting tools will be analysed in depth to provide a strong basis to the further development of the web toolkit. In addition, demonstration and training activities have been considered.
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 REACHnano project.

The scheduled actions and their interdependence are shown schematically below:

5 Advances over the state of the art

Research on environmental impact has merely started, and will face major methodological obstacles regarding detection, characterization and tracing. The first is due to the small size and the complexity of the environments, and the latter to the multitude of NMs that exist and their derivatives.

In relation to the ecotoxicological properties, data on biological effects show that NMs can be toxic to bacteria, algae, invertebrates and fish species, as well as mammals. However, much of the data are limited to species used in regulatory testing and freshwater organism, and quantitative data on ecotoxicological effects are still scarce even at the single organism level. Similarly, data on potential harmful properties on ecosystems are just emerging. In this sense, ecotoxicological studies have not been performed to the level of detail that would enable a high degree of protection, nevertheless, at present, several projects are being carried out within this field (e.g. ENNSATOX, MARINA, NanoFATE, NanoReTox, NERFH, NanoPolytox or Qnano). In addition, since 2008, the International Consortium for the Environmental Implication of Nanotechnology (iCEINT) is working to assess the potential impacts of NMs on environmental health.

In relation to the progress beyond the current state of the art, REACHnano project will work on the identification of reliable information to be used to assess the environmental impact of nanomaterials on the basis of REACH requirements.

In relation to the innovative approach, both toxicological and ecotoxicological parameters will be placed in a nanomaterials inventory that will be developed as a complementary tool of the REACHnano help desk.

Besides the above, the inventory will contain reliable information on physicochemical properties, conditions of use and exposure data that will support the development of exposure scenario and the risk characterization process, enhancing the knowledge base on risk assessment of NMs.

As a key action within the project, the data collected and validated during the previous tasks and actions will be transferred to several IUCLID files (i.e. TiO2 Nanoform.i5z), making the information available to their direct use by registrants, end users and other stakeholders.

On the other hand, the solution proposed, the REACHnano helpdesk will be a free of charge tool, including interactive templates to support the risk assessment of nanomaterials in their specific conditions of use.

In addition, our proposal will be designed to enable a continuous actualization, considering also the publication of on line information, latest news on REACH revisions and new findings regarding information on health and environmental impacts.

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 nanomaterials, in particular REACH regulation. 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 reliable data to establish new legal requirements to the use of nanomaterials.

The main characteristics and software architecture of the REACHnano Tool can be split as follow:

6 Expected Results

It’s expected to produce the following results:

- Development of a web based toolkit for decision making support on risk assessment and REACH compliment (REACHnano Toolkit), when manufacturing or handling substances at nanoscale,
- Development of a set of 3 complementary tools to support the risk assessment process, information exchange and the information search process.
- A structured compendium of free Webinars and workshops to support the training of end users and stakeholders in the use of the REACHnano help desk to promote the implementation of REACH
- A set of informative material to disseminate the project actions at a Regional, National and European level.
- A structured compendium of reliable information to be included into the chemical safety assessment report-CSR.
- A complete selection of standard testing models to be used in the risk characterization process
- A complete description of the current exposure scenarios (ES) across the nanomaterials life cycle, including a complete description of the existing operating conditions (OCs), efficient risk management measures (RMMs) and estimated exposure levels (ELs).

7 Progress to date

The project started officially on October 1st 2012 and had its kick-off meeting at the coordinator facilities on October 16.

At the moment, much of the work is concentrated on the selection of relevant nanomaterial under REACH, considering the tonnage band, sectors of use and hazardous properties as main criteria.

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 LEITAT, both involved in the characterization of the risks on the basis of the requirements laid down on REACH regulation.

Finally, the responsible partner for dissemination activities (NIA) is preparing the first brochure of the project, which will be presented jointly with the project web site.

8 Conclusion To-Date & Plan for the Future

The project is an early stage at the moment, but the Partners are working in the definition a set of representative manufactured nanomaterials in the context of REACH, taking into account the scope and exemptions of REACH provisions.

During the first period of 2013, we will identify the information needed for safety assessment and risk management of nanomaterials beyond current REACH information requirements listed in annexes VI to X, as well as any other information needed to prepare a complete dossier and fulfill other obligations such as authorisation procedures or data sharing.

A list of the main milestones to be completed during 2013 is presented below:

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<td>Identification of reliable information resources</td>
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</tr>
<tr>
<td>Selection and definition of reliable data on NMs properties</td>
<td>B.1</td>
</tr>
<tr>
<td>Definition of relevant testing methods under REACH</td>
<td>B.1</td>
</tr>
<tr>
<td>Database on Exposure Scenarios over NMs life cycle</td>
<td>B.1</td>
</tr>
</tbody>
</table>

All the activities described above will be performed with the support of the consortium partners, and relevant stakeholders and industries representatives.

The dissemination related activities will continue with the scheduled task, especially with the preparation of the project web site, which will be the key tool to disseminate the results of the project.
9 Directory

Table 2. Directory of people involved in this project.

<table>
<thead>
<tr>
<th>First Name</th>
<th>Last Name</th>
<th>Affiliation</th>
<th>Address</th>
<th>e-mail</th>
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<td>Eva</td>
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<td>Juan</td>
<td>Uriol</td>
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<td>Institut Valencià De Seguretat I Salut En El Treball. C/ Valencia, 32 46100 Burjassot</td>
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SANOWORK
Safe Nano Worker Exposure Scenarios

Contract Agreement: Under Negotiation  Website: http://www.sanowork.eu
Coordinator: Anna Luisa Costa, ISTEC-CNR, Faenza, Italy

<table>
<thead>
<tr>
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<td>ISTEC</td>
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<td>Institute of Occupational Medicine</td>
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<td>Colorobbia Italia SPA</td>
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<td>7</td>
<td>Bayer Technology Services GmbH</td>
<td>BAYER</td>
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<td>8</td>
<td>Institut National de l'Environnement Industriel et des Risques INERIS</td>
<td>INERIS</td>
<td>France</td>
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<tr>
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<td>UL</td>
<td>Ireland</td>
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<tr>
<td>10</td>
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<td>UNIPR</td>
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<td>11</td>
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<tr>
<td>12</td>
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</tr>
<tr>
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<td>INAIL</td>
<td>Italy</td>
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</tbody>
</table>

Contents

1  Summary ................................................................................... 245
2  Background .............................................................................. 246
3  What is Sanowork .................................................................... 246
   3.1 Summary of SANOWORK’s key strengths ........................... 246
4  Organisation of SANOWORK ................................................... 247
5  Sanowork progress to date..................................................... 249
6  Directory .................................................................................... 249
7  Copyright ................................................................................... 252

Project Duration: 1 March 2012 – 28 Feb 2015  Project Funding: 3.5 Mio. EUR

1  Summary

The main goal of Sanowork project is to identify a safe occupational exposure scenario by exposure assessment in real conditions and at all stages of nanomaterials (NM) production, use and disposal. In order to address this and more specifically the issues introduced by NMP.2011.1.3-2 call, we intend to: 1. contain hazard and worker exposure potential by developing exposure mitigation strategy based on “Prevention through Design” approach. 2. implement a rigorous exposure assessment in the workplace in order to evaluate the effectiveness of existing and proposed exposure reduction strategies. 3. 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 Sanowork proposed risk remediation strategy will be applied to nanomaterial properties. The following “representative” pool of NM and nanoproducts have been selected: ZrO2 (chemical or ceramic raw material); TiO2 and Ag (ceramic or textile photocatalytic/antibacterial surfaces); CNTs (polymeric nanocomposites); organic/inorganic nanofibers (nanostructured membranes for water depuration system). 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, will allow to evaluate the effectiveness of existing and proposed exposure reduction strategies. The cooperation with industrial key partners such as Plasmachem, Elmarco, GEA Niro, Colorobbia, Bayer will guarantee an accurate exposure assessment in the workplace.
2 Background

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.

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 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 Strategies with a balanced approach between design for manufacturing and design for safety.
KEY BENEFITS

- Cost-effective tools for safer manufacturing processes.
- Primary prevention of potential risks that can occur during worker manufacturing or customer use.

4 Organisation of SANOWORK

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.

![Sanowork WPs description (Pert chart).](image)

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).

Table 1 Workpackages (WP) of SANOWORK

<table>
<thead>
<tr>
<th>WP Title</th>
<th>WP</th>
<th>Topic</th>
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<tbody>
<tr>
<td>1 Administrative Management</td>
<td>WP1</td>
<td>This workpackage is aimed to: coordinate and manage the administrative, financial and legal-contractual activities of the project and ensure an accurate and on-time communication flow with the European Commission (EC) and the Project Consortium in order to track the project progress and meet its administrative objectives.</td>
</tr>
<tr>
<td>2 Risk Analysis</td>
<td>WP2</td>
<td>The main objective of this work package is to assess the risks caused by the NMs studied in SANOWORK in all the stages of their life cycle. The risk assessment characterization will be done on the different NMs before and after applying the risk management strategies proposed by SANOWORK. The data obtained from the risk assessment will be used to inform the industrial partners about the strategies to implement in their processes to create a safer workplace environment.</td>
</tr>
</tbody>
</table>
In this WP, we will develop databases for NMs along their life cycle which will be versatile to include new data from other sources. These databases will include description of materials and processes, their applications, their physico-chemical properties and their toxicological profile. These data will be generated in the project as well as included from literature sources and will be used in the risk characterization. The collected information and risk characterization transfer to insurance companies will be one of the relevant objectives of this WP2.

Strictly connected with the sustainability assessment of the proposed strategies is the Cost/Benefit evaluation of the proposed strategies, that will be based on cost analysis and evaluation of process efficiency and NMs functional properties BEFORE and AFTER the introduction of Risk Remediation Strategies.

The aim of this work package is to assess risks associated with chronic and accidental worker exposure scenarios applied to Processing Lines 1-6 at pilot-scale levels. Therefore the following specific objectives are proposed:
- Devise a qualitative worker exposure assessment method based on design or control approaches;
- Develop a control banding risk assessment methodology to evaluate risks at the pilot industrial scale level;
- Develop a quantitative worker exposure methodology by performing on-site exposure measurements, to provide inputs to the control banding (CB) risk assessment methodology.

The main objective of this WP is the development of the following Risk Remediation Strategies and their integration within the target processing lines:
- Strategy I: Coating by organic or inorganic based additives.
- Strategy II: Control of colloidal forces and disintegration of NPs.
- Strategy III: Integration of spray-drying process.
- Strategy IV: Immobilization by organic film coating deposition.
- Strategy V: Wet milling.

Target Processing Lines:
- Processing Line 1: ZrO2 NPs as additives in the chemical industry.
- Processing Line 2: ZrO2 NPs as additives in ceramic materials.
- Processing Line 3: Polyamide Nanofibers as membrane components for filter industry.
- Processing Line 5: TiO2 NPs as photocatalytic additives and Ag NPs as antibacterial additives in the ceramic industry.
- Processing Line 6: MWCNTs as additives in plastic industry.

Strictly connected with the above described objective is the assessment of process efficiency as well as of NMs functional properties. This objective aims to provide useful information for COST/BENEFIT evaluation performed by WP6.

In order to assess the performance and the validity of proposed Risk Remediation Strategies (WP4) the WP5 will:
1) Identify the hazardous properties of NMs, by reviewing the available literature data and relying on the thorough physico-chemical characterization performed within WP4;
2) Assess the impact of pristine and surface modified NMs by using representative of exposure pathways in vitro models: a) primary human cells; b) established mammalian including human cell lines;
3) Determine how the cellular bio-reactivity of NMs relates to their physicochemical properties and give indications for the development of a safe and sustainable manufacturing of nanoproducts.

The following specific objectives will be pursued:
1) Review toxicological data on selected NMs and identify knowledge gaps in hazard which are expected for such newly synthesized NMs (e.g. nanofibers, new functionalized nanostructures);
2) Assess cellular responses to the pristine and surface modified NMs delivered by WP4 by developing a testing strategy based on in vitro tests, using human cell models representative of the pulmonary and cardio-vascular systems;
3) Implement an hazard characterization strategy in close collaborations with SMEs, by assess cellular responses of nanoproducts collected by WP3, during exposure assessment of processing lines implemented by WP6.

The general objective of this WP is the implementation of target processing lines, BEFORE and AFTER the introduction of the Risk Remediation Strategies proposed in WP4.
The activities of WP7 aim to disseminate information and results of the project within the partners and outside the Consortium to all relevant stakeholders: national, international and EU regulatory bodies, NGO and representatives of the Nanotechnology industry, as well as proposing exploitation transfer plans and managing the Intellectual Property Rights. Moreover, it aims to collaborate with existing EC funded activities such as NanoSafety Cluster.

The general aim of WP8 is to coordinate the scientific and technical management of the project and maintain the project work plan and complete it within the agreed time schedule. Scientific coordination:
- Scientific review of the work performed by the partners including scientific deliverables and milestones.
- Monitor annual project meetings and regular interim, mid-term and final reporting, as well as the scientific quality.
- The supervision of project global critical path.

5 Sanowork progress to date

The project reaches the 12 months term at 1st of March 2013. The work performed by each WP and the main activities performed until now are summarized below.

WP2 The progress of WP2 in the first reporting period is according to the original workplan. The most notable results so far are:
- The characteristics of the different process lines have been described and hotspots for exposure identified (D2.1, month 6)
- Templates to collect physicochemical and toxicological data within the project have been developed.
- Physicochemical characterization is available for pristine and modified samples for some of the processing lines.
- An approach to perform risk assessment within the project has been developed. To facilitate this process, reference benchmark materials have been added to the list of materials to be evaluated in WP5
- Information gaps that hinder communication and understanding among different stakeholders (scientists, industry, and insurance companies) have been identified.

Deliverables/Milestone
- D2.1: General description of the NMs and their life cycle stages (Month 3)

WP3 The progress of WP3 in the first reporting period is according to the original work plan. The most notable results are reported in the following.

- Identification of exposure scenarios in the process lines during cases of normal functioning and abnormal conditions (ex: spills or loss of containment).
- Offline lab scale tests
- Characterization of the dustiness on two ZrO₂ nanopowder samples
- Risk analysis using control banding techniques
- CB methods that would be used for process lines have been identified.
- Collection of data necessary for the Control banding (exposure scenarios, physico-chemical characteristics, plan of the process lines, photos).

Deliverables/Milestone
- MS1 “Worker Exposure to Nanomaterials: Handbook on the Strategy and Methods for Offline Characterizations and On-site Measurements” (Month 6)

WP4 The progress of WP4 in the first reporting period is according to the original workplan. The most notable results so far are:
- Once collected all the information related to materials and processes (D 2.1, Month 6), the critical steps and scenarios, to be monitored before and after the introduction of risk remediation strategies (Table 2), have been identified and strategies accordingly planned.
- It has been adopted a product code to identify pristine and modified products and created an updatable data base where code, history and main characteristics of products are reported and updated.
- Pristine and modified samples were produced, sampled and sent to the partners
- Physicochemical characterization addressed to optimize adopted strategies and support hazard specific characterization was performed.
Criteria for the Selection of Risk Remediation Strategies based on NMs risk off-line and performances analysis (MS4 month 15) were identified.

WP5 The progress of all Research Units involved in WP5 (UNIPR, UNIPI and IOM) in the first reporting period complies with the original workplan.

The main results are summarized below:

- An iterative testing strategy was proposed and agreed during the Kick-off Meeting and refined during the 6-Months Meeting. This strategy is aimed to optimize the experimental efforts through: a) an assessment of effects on viability of pristine materials; b) in case of significant effects on viability, an assessment of possible mitigation effects of modified materials; c) in case of absence of significant effects on cell viability, the characterization of other dose-dependent effects, so as to identify appropriate parameters to evaluate remediation efficacy. This strategy will be repeated until evidence of effective remediation will be identified.

- 1) Cell models to be used for in vitro experiments, 2) a panel of tests for cytotoxicity, genotoxicity and carcinogenicity studies with relative protocols, 3) a proper and harmonized dose-metrics for dose-responses studies, and 4) a common dispersion protocol of nanomaterials (NMs) for in vitro experiments, were defined. These elements have been recounted in an Handbook of Toxicological characterization, provided as the first deliverable of this WP in the Project site (MS2).

- 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).

Deliverables/Milestone
- MS2 “Definition of a panel of toxicological tests to assess hazardous properties” (Month 6)

WP7 The progress of WP7 in the first reporting period is according to the original workplan. The most notable results so far are:

- It was created and implemented the Sanowork web site (D7.1, Month 6)
- It was taken the first actions in order to organize the 1st Workshop of projects funded under the same topic: SANOWORK, NANOMICEX and SCAFFOLD. In charge for organization: LEITAT, most suitable data: first week of June)
- It was established on the website a platform with updates of interesting dissemination activities including conferences, workshops, events.
- It was planned the participation of LEITAT to the exploitation seminar organized by EU.
- It was presented a general overview of the project at the following meeting:
  WORKSHOP Safety Issues and Regulatory Challenges of Nanomaterials, San Sebastián, Spain- 3, 4 May 2012
  WORKSHOP Safe implementation of nanotechnologies: Common challenges, MINATEC Grenoble, France, 29, 30, 31 May 2012
  Nanosafe 2012 – 3rd international conference on safe production and use of nanomaterials, Grenoble (FRANCE) – 13, 14, 15 November 2012
  6th European Conference of “N.I.C – Nanotechnology in Chemical Industry” organized by FEDERCHIMICA, Milano (Italy) – 12 December 2012
  International Innovation, EuroFocus – August 2012, publication let available to the partners during the 12th Months Meeting

Deliverables/Milestone
- D7.1 “Project Website” (Month 6)

WP8 The scientific coordination of the project is documented by minutes of:

- Kick-off Meeting, Faenza, Italy 19, 20 March, 2012 (D8.1, month 1)
- 6th Month Meeting, Pisa, Italy 16, 17 Ottobre 2012
- Conference Calls Intra WPs to finalize next actions

Deliverables/Milestone
- D8.1 “Kick-off meeting minute” (Month 6)
### 6 Directory

**Table 2 Directory of people involved in this project.**

<table>
<thead>
<tr>
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<th>Affiliation</th>
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</table>
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Scaffold

Innovative strategies, methods and tools for occupational risks management of manufactured nanomaterials (MNMs) in the construction industry

Contract Agreement: 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 ....................................................................................253
2 Background .............................................................................. 254
3 What is SCAFFOLD .....................................................................255
4 Objectives ..................................................................................255
5 The SCAFFOLD approach and organisation .............................255
6 Progress beyond the state-of-the-art........................................257
7 Impact............................................................................................. 258
8 Citations .......................................................................................... 259
9 Directory .......................................................................................... 259
10 Copyright .......................................................................................... 260

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 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. 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 What is SCAFFOLD

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 3):

- 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-OSEHA 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. SCAFFOLD will focus research in construction. Thus, industrial implementation of the new paradigm in such a very OHS complex sector will allow generalizations face other industrial sectors without too much additional efforts in research. In addition, SCAFFOLD will integrate the NANOHOUSE approach in order to cover the global life cycle of the construction industry.

SCAFFOLD RMM will be constructed on the basis of consistent and effective OHS / Risk models (element 3), applicable to any organisation from all types of business sectors, activities and sizes of which implementation in industry, and specifically in construction, is quickly growing. According to that, SCAFFOLD RMM will be supported by the continuous improvement concept. Thus RMM will be able to incorporate any relevant future input produced by ongoing MNMs 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 Nanocluster 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.

5 The SCAFFOLD approach and organisation

SCAFFOLD 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 solutions.
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. For its development, the SCAFFOLD Consortium analyzed the potential applications of MNMs in the construction sector in the areas of 1) Cement, 2) Pavements and 3) Advanced Materials. Fifteen MNM applications, MNMs involved in each case, as well as potential exposure scenarios along the life cycle were initially identified. The occupational safety research priorities were determined within this preliminary set of applications by using a 3x3 matrix with two decision criteria: Level of risk and Innovation rate. The results of this preliminary analysis were encapsulated in the SCAFFOLD initial roadmap, where the following priorities were selected for the project (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 product 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. The demonstration of SCAFFOLD results will aim to:

1. Test the SCAFFOLD RMM into industrial construction companies in real-life situations to demonstrate their validity and use for effective management of MNMs occupational exposure along Life Cycle in the European Construction Sector.
2. Focus research activities on some specific and priority industrial applications, scenarios and MNMs of the European Construction industry.
3. Focus the project research tasks in the IUC (industrial demonstration) from the very beginning of the project.
4. Develop demonstration activities (IUC) across Europe considering different safe-cultures and awareness levels as well us company scales (large and SMEs).

With the aim of achieving the S&T Objectives set in the project, the Consortium will tackle multidisciplinary research activities that have been grouped into eight Workpackages (WPs): WP1-5 as RTD activities, WP6 as Demonstration activities, WP7 as Other activities (Dissemination and exploitation) and WP8 as Management. The Figure 3 shows the project approach and strategy according to the organization of WPs.
Table 1 Workpackages (WP) of Scaffold

<table>
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<tr>
<th>WP</th>
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<th>Topic</th>
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<tr>
<td>1</td>
<td>Profiling the European construction industry that face MNMs occupational exposure</td>
<td>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 &amp; Toolkit (WP5).</td>
</tr>
<tr>
<td>2</td>
<td>MNMs Risk Prevention</td>
<td>WP2 will aim to develop innovative strategies and methods for Risk Prevention of the occupational exposure to MNMs by safe product design.</td>
</tr>
<tr>
<td>3</td>
<td>MNMs Risk Assessment</td>
<td>WP3 will aim to develop innovative strategies and methods for Risk Assessment of the occupational exposure to MNMs in scenarios of the construction sector.</td>
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<tr>
<td>4</td>
<td>MNMs Risk Protection and control</td>
<td>WP4 will aim to design and develop innovative strategies and methods for Risk Protection and control against the occupational exposure to MNMs in construction.</td>
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<td>5</td>
<td>MNMs Risk Management: Integration of solutions</td>
<td>WP5 will aim to integrate relevant available results from WP1 and innovative results produced in WP2 to WP4 to construct a Risk Management Model (RMM) - ISO 18001 &amp; ISO 31000 based - and a set of innovative tools (Toolkit - Software) to effectively manage the occupational exposure to MNMs in construction.</td>
</tr>
<tr>
<td>6</td>
<td>Testing and validating the RMM &amp; Toolkit in construction industry</td>
<td>WP6 will aim to test and validate the RMM &amp; Toolkit in real conditions - Five Industrial Use Cases (IUC).</td>
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<tr>
<td>7</td>
<td>Dissemination and exploitation</td>
<td>WP7 will deal with raising public awareness, disseminating project results and defining &amp; managing dissemination and exploitation plans.</td>
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<tr>
<td>8</td>
<td>Project management</td>
<td>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.</td>
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</table>

6 Progress beyond the state-of-the-art

The SCAFFOLD Consortium has identified the main technologies that are involved in the S&T Objectives and grouped them in four areas related to the occupational exposure to MNMs: Risk prevention by MNMs design, Risk assessment, Risk protection & control and Risk management. In this respect, the Consortium has conducted a thorough analysis of the state-of-the-art in those four technology areas, identifying the technological challenges associated with the project. The aspects of SCAFFOLD which go beyond the state-of-the-art will be:

1. Risk Prevention by design of MNMs: New intrinsically safe MNMs formulations; new specific fire retardant nanocomposite formulations with minimum risk to heath & safety and new specific strategies for safe nano-filled concrete, bituminous pavements, coatings and insulation.

2. Risk Assessment: New methods - screening/advanced - to measure worker exposure to MNMs and to measure exposure to nanoparticles and toxic fumes in case of accidental situations (selected construction products); an exposure measurement database: inhalation & dermal, at lab scale, pilot and real scale (selected scenarios) and accidental situations (fire); numerical models to obtain exposure data at the breathing zone, deposition in the Human Respiratory Tract and dermal deposition and Proposals on OELs for selected MNMs. Lab scale tests are currently on going addressed to measure and to model particles release from simulated exposure scenarios (Figure 4).

Figure 4. Lab scale tests - Particle characterization in simulated fire scenarios.
3. **Risk Protection and Control**: New method for evaluation of the efficiency of collective protection in complex form, in real conditions of used ventilation systems; new methods for characterization of PPE efficiency (proposed to ISO standardization procedure); a database collecting the determination of effectiveness of main collective protection alternatives operated in different conditions; a novel methodology for the assessment and selection of PPEs against MNMs in the construction sector; new methods and devices for risk protection; Control Banding Approach adapted, tested and customized to the construction sector; new guidance and exposure register model for monitoring the health of workers exposed to MNMs.

4. **Risk Management**: A consistent and cost-effective model for risk management during the complete life cycle (RMM), tested and validated in real industrial conditions (Industrial Use Cases), OHSAS 18001 and ISO 31000 based and fully compatible with ISO 9001 and ISO 14001; a set of innovative tools (Toolkit) to support implementation, including customized applications for SMEs.

### 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.

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, contributing to reaching the overall objective of the Community Strategy aimed at reducing the total incidence rate of accidents at work per 100 000 workers in the EU 27 by 25%. This preventive approach will produce parallel benefits by reducing non-safety bills both in construction companies and in the whole European industry.

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.

This picture means that the implementation of the SCAFFOLD framework in SMEs will have a very relevant impact on the European construction industry. To take this significant issue into account, SCAFFOLD will produce a specific approach for SMEs by customizing both the RMM and Toolkit. Subcontracting, identified as an emerging risk in the sector, will be a significant issue with which to be dealt. It will allow the generation of a practical, easy-to-use and cost effective framework to properly manage the occupational risks associated to MNMs.

A Spanish study shows that a relatively large and increasing percentage of SME subcontractors (23%) recognize the obligation to fulfill 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 RMM will provide SMEs with a competitive advantage, impacting positively on the sector. Finally, an additional benefit for SMEs integrated in the SCAFFOLD Consortium will be the participation in research programmes.

#### Market

Freedonia has recently forecasted that the annual US demand for nanocomposites will more than double by 2011, reaching 130,000 tonnes. By 2025 it is expected that nanocomposites will be a 6.5 billion € market, with volumes approaching 2.2 million tonnes. The authors note that 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 includes nanotechnology as an important topic to be developed in the context of the identification of new and emerging risks. In addition to the previously mentioned Strategy, other specific objectives tangibly impacted by SCAFFOLD will be: 1) the development of new methods to identify and evaluate new potential risks and 2) to support SMEs and the high-risk sectors - like construction - in the implementation of the legislation in force and its adaptation to changes in the workplace.

On the other hand, SCAFFOLD will positively contribute to 1) getting some of the objectives defined by the Action Plan for Construction of the Lead Market Initiative (LMI), 2) supporting nanosafety issues of the European policy on nanotechnology, currently in revision, 3) supporting the Innovation Strategy for Europe by introducing innovation in the core of the construction sector, contributing to enhance Europe’s global economic competitiveness in a global market.

SCAFFOLD will contribute positively to European Regulations by providing new information to elaborate better regulations about issues related to safety of MNMs and support compliance with current legislation requirements. The two main topics to be addressed will be: a) Health and Safety at work in the sector and the future strategy for managing the occupational exposure to MNMs in the construction industry – SCAFFOLD deploys a specific task to build a proposal in this field – and b) the Safety of products (PPEs, construction products).

Standardisation is a highly relevant issue in this sector. In the European Committee for Standardisation (CEN), the construction sector covers around 3,000 work items on product standards and test methods. 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.

8 Citations


Clark, K et al (2012), Limitations and information needs for engineered nanomaterial-specific exposure estimation and scenarios: recommendations for improved reporting practices, Journal of Nanoparticle Research, August 2012, 14:970


http://ec.europa.eu/research/consultations/snap/consultation_en.htm


http://www.cen.eu/cen/Sectors/Sectors/Construction/Pages/default.aspx


9 Directory

Table 2 Directory of people involved in this project.

<table>
<thead>
<tr>
<th>First Name</th>
<th>Last Name</th>
<th>Affiliation</th>
<th>Address</th>
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Coordinator: Rainer Hagenbeck, Forschungszentrum Jülich GmbH, Germany

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Contents

1 Summary ................................................................................... 262
2 Background and Goals .................................................................. 262
3 Current Status of SIINN ERA-NET ........................................... 263
4 Summary of SIINN’s Key Expected Impacts ............................... 263
5 Organisation of SIINN ............................................................ 264
6 Directory .................................................................................. 265
7 Copyright .................................................................................. 267
1  Summary

Project Duration: 1 August 2011 – 31 July 2014
Project Funding: 1.5 Mio. EUR

The primary aim of the “SIINN” ERA-NET 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 the environment and 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.

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 worldwide 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 will, in its first three-year phase, concentrate 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 QNano infrastructure, the OECD Working Party on Nanomaterials (WPNM), the NANO Futures ETIP and NanoImpactNet.

Two transnational calls will be prepared during the lifetime of the SIINN ERA-NET allowing the scientists from the participating countries and regions to perform joint research activities. 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.

At the end of the project’s initial three years, SIINN will have established a coordinated, transnational programme 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.

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 on March 20, 2012. The meeting of the Steering Committee in November 2012 reviewed the progress and discussed the next steps.

At the end of 2012, after 17 months, 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 SIINN call.

Within WP2, a first 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 data platform will be available via the SIINN website from April 2013 on. The first draft of the Consolidated Framework for EHS is available for review purposes in the protected part of the SIINN website and will be made available to the projects funded by SIINN. 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 joint call for proposals. The first call was launched on March 20, 2012. Funding organisations from nine countries and regions have participated in the call. At the submission deadline (June 5, 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. Projects will start in spring 2013. The second call is now under preparation and is expected to be published in June 2013.

WP5 is concerned with communication and dissemination. The SIINN web page (www.siinn.eu) 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.

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, a joint transnational call has 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 call will be used as a starting point for the second call, which will be probably published in June 2013.

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.

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.

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.
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 and one member of the NMP High-Level Group on Nanotechnology serve in the Consultation Committee and assure a close link to related activities of the EU.

6 Directory

Table 1 Directory of people involved in this project.

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SMART-NANO

Sensitive MeAsuRemenT, detection, and identification of engineered NANOparticles in complex matrices

Contract Agreement: NMP4-SE-2012-280779  Website: www.smartnano.org
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<td>6</td>
<td>AHAVA Dead Sea Laboratories Ltd</td>
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<td>Israel</td>
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<td>7</td>
<td>Ruđer Bošković Institute</td>
<td>RBI</td>
<td>Croatia</td>
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<td>8</td>
<td>ABICH Srl</td>
<td>ABI</td>
<td>Italy</td>
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</tbody>
</table>

Contents

1 Summary ................................................................................... 269
2 Background .............................................................................. 269
3 Scientific and technological challenges ................................ 270
4 Objectives ................................................................................. 270
5 Organisation ............................................................................. 270
6 Expected Impact ....................................................................... 272
7 Directory .................................................................................... 272
8 Copyright .................................................................................. 273

1 Summary
Project Duration: 1 June 2012 – 31 May 2016
Project Funding: 3.5 Mio. EUR

The project SMART-NANO is aimed at developing an innovative, cost-effective technology platform that provides a total solution “from sample-to-result” for the detection, identification, and measurement of engineered nanoparticles (ENPs) in a wide range of matrices. The outcome of the project will be a miniaturized, modular, cartridge-based technology platform integrating all analytical steps needed for separation, detection, and quantification of ENPs in complex matrices. Parallel to the development of the technology platform, analytical methods and protocols will be developed and tested on the field, resulting in ready-to-use, cost-effective cartridges, for immediate, widespread use in real-life applications.

The SMART-NANO consortium is formed by 8 partners (3 academic and 5 SME’s) from 8 different countries. The balance between industrial and academic partners is one of the key strengths of the project.

2 Background

Nanotechnology is having a large impact in many industrial sectors and the use of nanoparticles is continuously increasing in many fields, from paints, automotive components, consumer products (such as cosmetic sunscreens and anti-odorant) up to biology and medicine. It is estimated that 3-4 new products containing some form of nanomaterial enter the market every week and that by 2014 1.6 trillion Euros of manufactured goods will be based on nanotechnology.

The recent introduction of legislation on the labelling requirements of cosmetic products containing nanoparticles and the on-going activities in the legislative arena at EU level (see for example the “Commission Recommendation on the definition of nanomaterial” –L 2011/696/EU-) on the labelling of ENP-containing consumer products clearly call for cost-effective, practically implementable, robust, highly sensitive, and specific methods for the detection and measurement of nanoparticles, all these attributes being required for their widespread use and acceptance as standards in the community.
3 Scientific and technological challenges

The detection, quantification and identification of ENPs is complicated by the sheer variety of complex matrices in which engineered nanoparticles (ENPs) are embedded, ranging from food, to clothing and cosmetic products. For a proper assessment of the fate and potential safety risks associated with nanoparticles, ENPs also need to be detected downstream in matrices as different as waste water sludge, biological systems and soils. The biggest challenge that researchers face today is thus to develop a technology platform that can be used in a wide range of application scenarios (in terms of embedding matrices and wide range of concentrations) with just minimal adjustments and optimizations for new applications.

As of today, there are technologies that partially address the three areas of detection, identification and quantification, but usually they have not been optimized for the detection of ENPs nor have they been tightly integrated with each other. An example of required technology innovation is size measurement of nanoparticles in the single digit nanometer range (1-10nm), which still suffers from a severe lack of technological progress. More importantly, there is a lack of integration between the different technologies with respect to sample preparation, ENPs isolation, pre-concentration and separation as well as their measurement and identification. These shortcomings render the routine use of current analytical methods difficult, particularly in the case of samples containing nanoparticles of different size and/or chemical composition. Moreover, the many required process steps are time consuming and introduce a large variability of the results obtained. The lack of methods for detection and characterization of nanoparticles in complex matrices has been recently highlighted as the main factor holding up the successful transfer of nanotechnology to nanomedicine.

The SMART-NANO project aims at developing a key technology platform able to address the measurement requirements of the most common applications with ready-to-use application-specific “from sample-to-result” (i.e. integrated) cartridges and protocols. The technology platform is based following a modular and scalable approach, which gives the flexibility to target new applications with minimal optimization.

4 Objectives

The SMART-NANO consortium has selected four key technological advances that will yield innovative, practically implementable, and cost-effective measurement approaches for the identification, detection and quantification of ENPs. These advances are:

- To increase the sensitivity, specificity and versatility of nanoparticle detection instruments using newly developed and optimized pre-separation and separation steps.
- To increase the sensitivity and quantification of nanoparticle detection by next generation dynamic light scattering.
- To provide robust, highly sensitive, miniaturized, and application-specific separation and detection cartridges.
- To validate the SMART-NANO detection platform and demonstrate its application for cost-effective measurements of nanoparticles in complex matrices such as biological systems, consumers products and in the environment.

The integration of all of these advances into a single technology platform delivering all steps “from sample-to-results” (i.e. sample preparation, ENP isolation, pre-concentration, separation, quantification and identification) represents the key breakthrough targeted by SMART-NANO.

5 Organisation

In order to successfully achieve the ambitious objectives of the project, an efficient, well-defined but flexible management structure was defined, shown in Fig 1. The management structure also ensures an effective communication with other ENP-related activities at the EC level.

The SMART-NANO project is organized in 8 Work Packages (WPs), which are strongly interlinked as shown below.
The four first Work Packages (WP1-4) target the key technological advances described in the previous section. These four innovation driven WPs yield modules that are integrated to build the SMART-NANO application-specific “from sample-to-results” cartridges. In order to maximize progress and accomplishments within the given schedule and the available resources, these four WPs are designed to progress simultaneously and are interlinked with each other only at the microfluidics interface level. This will reduce the project risk while maintaining a high level of resilience and flexibility in the proposed platform. Integration of the different cartridge modules into the SMART-NANO platform is accomplished in WP5, while WP6 is dedicated to the design and optimization of workflow and protocols for specific applications. WP7 and WP8 are focused on impact and dissemination activities and project management and will run for the whole duration of the project.

The various Work Packages and their respective goals are described in more detail in the following table.

<table>
<thead>
<tr>
<th>WP Title</th>
<th>Objective</th>
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<tbody>
<tr>
<td>1 Ecologically-sound ENPs Isolation Cartridge Modules</td>
<td>Establishment, testing, and benchmarking of an ecologically-sound technology for the sample preparation for the analysis of ENPs in complex materials and in highly diluted samples. Two approaches will be followed: CO2-based isolation and chemical isolation.</td>
</tr>
<tr>
<td>2 Innovative Cost-effective ENP Separation Cartridge Module</td>
<td>Development of macroscopic (first) and microscopic (later) separation modules combining two known flow field-flow fractionation sub-technologies (SPLITT and asymmetrical AF4). Upgrade of the standard instrumentation environment to allow the integration of the microscopic separation module.</td>
</tr>
<tr>
<td>3 On-chip in-line ENP Detection, Measurement and Quantification Cartridge Module</td>
<td>Integration of UV and light scattering detection with the microfluidic-based cartridges. This integration will lead to an increased sensitivity of the system, a reduced dilution of the sample, and a lower cost of the whole instrumentation. In addition, the development and testing of calibrating solutions containing ENP of different sizes will allow the size measurement of the most common NP based on their elution time.</td>
</tr>
<tr>
<td>4 Innovative ENP Hyper-Sensitive Size Measurement and Identification Modules</td>
<td>Development of a method for the highly sensitive size measurement and identification of ENPs. The hypersensitive size detection will be achieved by homodyne optical fiber dynamic light scattering (HDLS), and the hypersensitive identification by single nanoparticle ICP-MS.</td>
</tr>
<tr>
<td>5 Cartridge &amp; Instrument Integration</td>
<td>Develop and test the integration of the different modules that have been developed in WP 1-4 at the cartridge and instrument levels.</td>
</tr>
<tr>
<td>6 Applications Specific Cartridges Protocol Optimization</td>
<td>Optimize protocols for ENPs detection and identification to meet future industry needs. Analyse the level of applicability of the technology platform for a successful implementation in the industry, provide added value to the end user, and meet relevant regulatory demands.</td>
</tr>
<tr>
<td>7 Exploitation, Impact and Dissemination Activities</td>
<td>Activities for promotion and dissemination of the scientific and technological results achieved during the project, in and beyond the industrial reference European market.</td>
</tr>
<tr>
<td>8 Project Management and Consortium Coordination</td>
<td>Ensure that the project is executed successfully, i.e., that the objectives of the project are reached within the project’s scope, schedule and resources, and ensure maximum impact of SME-related activities.</td>
</tr>
</tbody>
</table>
6 Expected Impact

SMART-NANO is expected to have a strong impact in different areas:

Scientific and technical impact
The innovative, cost-effective technology platform providing a total solution for the detection, identification and measurement of ENPs in complex matrices, will certainly generate high scientific and technical impact and innovations, some of which will be patentable inventions. It is critical, therefore, to provide a strong link between the dissemination activities and the intellectual property strategy plan to protect the results appropriately for commercial exploitation.

Commercial impact
There will be a strong commercial impact from SMART-NANO as the outcome of the project will be a validated platform for exploitation and distribution by the SME partners. Moreover, Agilent Technologies has expressed strong interest in the outcome of the project as this could potentially enable smaller and more affordable ICP-MS systems.

Policy impact
We expect the scientific evidence gathered during the application phase of the project will be translated into guidelines and recommendations for control of ENPs in consumer products, food, environment, in situ in biota. Our dissemination strategy views the policy impact as a key factor in the subsequent success of raising public awareness and commercial development of the outputs from the project. We will engage with the policy makers to inform governmental environment, food and health agencies at an EU-wide and national level.

Societal impact
The information generated by SMART-NANO will have a significant impact on the safety of the European population as analyses from a broad range of ENPs in consumer products, food, environment and in biota. To enable this, awareness of the uses and benefits of the technology has to be created amongst the industrial and governmental stakeholders. Developing a good relationship with the accredited laboratories end-users will also provide a valuable feedback opportunity during the commercial development of the technology platform.

7 Directory

Table 2 Directory of people involved in this project.

<table>
<thead>
<tr>
<th>First Name</th>
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