NanoSafety Cluster

# Compendium of Projects in the European NanoSafety Cluster

**2017 Edition**

26th June 2017

Editor:

Iseult Lynch
University of Birmingham, United Kingdom

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# PREFACE

Welcome to the 2017 Edition of the Nanosafety Cluster compendium. This year's edition is the first that is **dominated by Horizon 2020 funded projects**, with only a few still-running FP7 projects, as 5 Fp7 projects finished in the first half of 2017, marked by a jointly organised conference in Malaga in February 2017. Thus, the 2017 edition contains information on 17 H2020 projects (11 that have featured in the 2016 Edition and 5 brand new projects. The recently ended FP7 projects provide updates here, as well as the currently running ones. Thus, the 2017 compendium contains from 17 H2020 projects and 10 FP7 projects.

As always, the compendium documents the status of important EU-funded projects on nanomaterial toxicity and exposure assessment and risk management, with increasing focus on safety-by-design considerations for nanomaterials, predictive toxicology approaches and high throughput / Tox21 type approaches. 6 new H2020 projects are presented and updates from 11 running H2020 projects are included.

The compendium is not intended to be a guidance document for human health and environmental safety management of nanotechnologies, as such guidance documents already exist and are widely available. Neither is the compendium intended to be a medium for the publication of scientific data and research results, as this task is covered by scientific conferences and the peer reviewed press. Instead, the compendium aims to showcase the exciting and important European-wide collaborative research being undertaken to ensure the safe implementation of nanotechnologies, and to act as a one-stop-shop for all stakeholders interested in acquiring an overview of current research activities.

The compendium also aims to bring the research community closer together and show the potential for synergy. It is a means to establish links and communication between them well before the publication of their results. It thus focuses on the communication of projects' strategic aims, extensively covers specific work objectives and the methods used in research, and documents human capacities and partnerships. As such, the compendium supports collaboration on common goals and the joint elaboration of future plans, whilst compromising neither the potential for scientific publication, nor intellectual property rights. Of course this publication alone will not be able to achieve these targets. However, we hope that it will help the research community to make significant progress towards them. The compendium will continue to be a dynamic, frequently updated, web-based document available free of charge to all interested parties.

Over the last number of years, the compendium has also provided an opportunity for the Nanosafety cluster Working Groups (NSC WGs) to provide an update on their activities. The NSC itself underwent a number of changes this year, including new leadership and a revision of the WGs topics to reflect the foci of the H2020 projects, and the increasing need for translation of research results to industry and regulators. Thus, only the active WGs report on their activities in this years edition, and next year will provide the work plans and progress of the streamlined and reinvigorated NSC WGs.

We hope that you find it useful, and please do feel free to cite it, and to contact the project coordinators and participants for more information or to collaborate on specific topics of interest. As ever, information sharing and fostering of collaborative activities are key goals of the Nanosafety Cluster. Feedback on the 2017 Compendium is most welcome, including ideas for additional information that could be included in future editions. Please email suggestions / ideas to: eunanosafetycluster@gmail.com.

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

# ACKNOWLEDGMENTS

I would like to thank the project coordinators / managers for their contributions in the creation of this publication. This compendium would not have been possible without their help. The compendium attests to the hard work, the outstanding ideas, the frustrations and successes, and the satisfaction of the researchers. Their commitment is the foundation of this publication. Projects appearing in this compendium are supported financially by the European Union and/or the Governments of the Member and Associated States. We gratefully acknowledge their continued support.

The editing of this year's Compendium was kindly supported by the ACEnano project (Grant Agreement 720952 under the EC's Horizon2020 Programme). Special thanks to Andrzej Fima for ongoing technical support.

*Iseult Lynch, Editor on behalf of the NanoSafety Cluster*

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# Foreword

Dear Readers of the Compendium, Dear Friends,

As Horizon 2020 becomes the dominant funder of the Nanosafety Cluster with just a few FP7 projects running into their final few months, nanosafety research remains a centrally important topic to ensuring Europe's vision of the knowledge economy and the safe implementation of nanotechnologies for the benefit of society. Indeed, nanotechnologies are one of the Key Enabling Technologies for investment of research effort, with safety and public acceptance of nanotechnologies at the heart of these efforts. Indeed, as will become apparent from the project descriptions in this 2017 Edition of the NanoSafety Cluster Compendium, there is an increased emphasis on advanced materials and materials as they exist in products and following environmental transformation & ageing, as well as on research with a regulatory and/or market focus, including Safety-by-design and Life Cycle Assessment approaches.

The recent calls for proposals focussed on high throughput, predictive toxicology and grouping approaches for risk assessment, and the results of these calls are expected imminently. The call topics for the second phase of Horizon2020 (2018-2020) are in advanced drafts and are also expected to be published shortly. Including governance of nanomaterials, an increased focus on nanobioinformatics, and calls for Innovation Hubs, for which nanosafety and nanocharacterisation are potential elements needed to support commercialisation of nano-enabled products.

This is the first volume of the NSC Compendium in which H2020-funded projects outnumber FP7 ones. Indeed only 4 FP7 projects are still running, with 6 having ended in the first half of 2017. 5 of the recently ended FP7 projects co-organised a nanosafety conference to disseminate their outputs in Malaga in February 2017, and the recordings are available here: http://www.nmsaconferencetalks.eu/lectures

The NanoSafety Cluster Compendium of projects is intended as to disseminate knowledge about European Commission funded research projects on various aspects of nanosafety to a wide variety of stakeholders, including international research communities, the regulatory authorities, parallel activities such as the OECD Sponsorship Programme, the EU DGs, industry and interested NGOs. It is intended to provide a concise snapshot of each project's aims, approaches and progress to date, thereby facilitating gap analysis, collaboration and provide a directory of European research and researchers. Given the scale of the 21 active projects outlined here, resulting from the active funding efforts of the Commission, Europe has taken a position of global leadership of nanosafety research, including establishing the Nanosafety Cluster itself, which has been recognised as an innovative mechanism for competitive/collaborative research during the recent review of FP7 nanosafety projects, and initiating the EU-US Communities of Research (CoRs).

The Nanosafety Cluster (NSC) was established as a mechanism for ongoing projects to benefit from one another, and from recently finished projects, through information sharing, as well as being a mechanism to collectively define strategic agendas for research, regulation and commericalisation. In February 2017, the leadership of the NSC passed from the Finnish Institute of Occupational Health (under the guidance of Kai Savolainen) to University of Birmingham under the leadership of Eva Valsami-Jones and a Coordination team consisting of Flemming Caseee (RIVM), Andreas Falk (BioNanoNet) and Iseult Lynch (UoB). As part of the changeover, the coordination team are utilising the opportunity to refresh some of the NSC activities, including re-invigorating the Working Groups by streamlining and merging these to remove inactive ones, and build on the strengths and initiatives of the productive ones. Thus, the new structure, presented in the WG section of the compendium reflects the focus of the current and forthcoming H2020 projects, and aims to ensure coherent integration of data and harmonisation of outputs in formats with high utility for industry, regulators and the general public. The WGs that have been active over the last yesr provide a final update here along with some recommendations for the future WGs, and subsequent editions of the compendium will include updates from the 6 new WGs. Participation in the WGs is open to all, so please do take the opportunity to get involved and sign-up to support specific tasks.

This compendium is a highly interesting read for those interested in knowing how European nanosafety research projects tackle 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 to allow readers / end-users / stakeholders to assess which projects might provide relevant information for them, or which might be relevant to collaborate with, as well as providing contact information of the coordinators of the projects. Please, make contacts, network, and increase collaboration further within Europe and globally.

I sincerely hope that the 2017 edition of the compendium again proves be an extremely useful source of information of European nanosafety research.

# Overview of research themes of the NanoSafety Cluster projects

| Project Acronym | ACEnano | Calibrate | CERASAFE | EC4SafeNano | eNanoMapper | Fibralspec | FutureNanoNeeds | GuideNano | Hisents | Lorcenis | ModCOMP | NanoDefine | NanoFARM | NanoFATE | NanoGenTools | NanoMILE | NANoREG | NaniREG2 | NanoSolutions | NanoToxClass | Necomada | npSCOPE | Pandora | ProSafe | SKHINCAPS | SmartNanoTox | SUN |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Start year** | 2017 | 2016 | 2016 | 2016 | 2014 | 2014 | 2014 | 2013 | 2016 | 2016 | 2016 | 2013 | 2016 | 2010 | 2015 | 2013 | 2013 | 2015 | 2013 | 2015 | 2017 | 2017 | 2016 | 2015 | 2015 | 2016 | 2013 |
| **End year** | 2020 | 2019 | 2018 | 2019 | 2017 | 2017 | 2017 | 2016 | 2020 | 2020 | 2020 | 2017 | 2019 | 2014 | 2019 | 2017 | 2016 | 2019 | 2017 | 2018 | 2019 | 2020 | 2020 | 2017 | 2019 | 2020 | 2016 |
| **Characterisation & measurement** | X | X | X | | | X | X | X | X | X | | X | X | X | X | | | X | X | | X | X | | | X | | |
| Physico-chemical properties | X | | X | | | | X | X | X | X | | X | X | X | X | X | X | X | X | | X | X | | | X | X | X |
| Analysis of "next generation" nanomaterials (2nd, 3rd or 4th generation) | X | | | | | X | X | X | | X | | X | X | | X | | | X | | | | | | | | | X |
| **Exposure assessment for humans and the environment** | X | | X | | | X | X | X | X | X | X | | X | X | X | | | X | | | | | | | | | X |
| Develop & validate exposure measurement and modelling methods | X | | X | | | X | X | X | X | | X | X | X | X | X | | X | X | | | | | | | | | X |
| Human Exposure: Application of measurement and modelling methods | | | X | | | X | X | X | X | | | X | X | X | X | | X | X | | | | X | | | | | X |
| Environmental Exposure Assessment | | | X | | | | X | X | | X | X | | X | X | | | X | | | | X | | | | | | X |
| **Interaction of NM with biological systems** | X | | | | | | X | | X | | | X | X | X | X | | | X | X | X | | | X | | X | X | |
| Interaction with physiological mechanisms | X | | X | | | | X | | X | | | | X | X | X | | X | X | | X | | | X | | X | X | |
| Toxicokinetics | | | | | | | | | X | | | | | X | | | X | | | X | | | | | | X | |
| Inter- and intraspecies variability | | | | | | | | | | | | | | X | X | | X | | | | | | X | | | | |
| Predictive models | | | | | | | | | X | | | | | X | X | | X | X | X | X | | | X | | | X | |
| Long term monitoring and assessment | | | | | | | | | | | | | X | X | | | X | | | | X | | | | | X | |
| **Human Health** | | | | | | X | | | X | | X | | X | | X | | | X | X | X | | X | X | | | | |
| Develop & validate testing & assessment strategy | | | | | | | | | X | | X | | | | X | | X | | X | X | | X | X | | | X | |
| Apply testing and assessment strategy | X | | X | X | | X | | | X | | | | X | | | | X | X | X | X | | X | X | | | | |
| Coexposures / Mixture toxicology | | | | | | | X | | | | | | X | | | | | | | | | | X | | | | |
| **Ecotoxicology** | | | | | | | X | X | | | | | X | X | X | X | | | X | | | X | X | | | | |
| Develop testing and assessment strategy | | | | | | | X | X | | | | | X | X | X | X | X | | X | | | X | X | | | | X |
| Apply testing and assessment strategy | | | | X | | | | X | | | | | X | X | | X | X | | X | | | X | X | | | | X |
| **Control measures at workplace** | | X | X | X | | X | | | | | X | | | | | | | X | | | X | | | | | | X |
| Develop & validate methods to evaluate control measures at workplaces | | X | X | X | | X | | | | | X | | | | | | X | X | X | | | | | | | | X |
| Apply methods to evaluate control measures at workplaces | | X | X | | | X | | | | | X | | | | | | X | X | | | X | | | | | | |
| Control banding approach | | X | X | | | X | | | | | X | | | | | | | X | | | | | | | | | |
| **Preliminary handling guidelines** | | X | | X | | X | | | | | X | | | | X | | | | X | | X | X | | | | | X |
| Collect available and ongoing approaches | | X | X | X | | X | | | | | X | | | | | | X | | X | | X | X | | | | | X |
| Evaluation and further development | | X | | X | | X | X | | | | X | | | | X | | X | | X | | X | X | | | | | X |
| **Information transfer** | | X | | X | | X | X | X | X | X | X | X | X | X | X | | | X | X | X | | | | X | X | | |
| Database generation | X | X | X | X | X | | X | X | | X | | X | X | X | X | X | X | X | X | | | | | | X | X | X | X |
| Public dialogue | | X | X | X | | X | | X | | X | X | X | X | X | | | | | | X | X | | X | X | X | | |
| Information to and training of workers, business and | X | X | | X | | X | X | X | | X | X | X | X | X | X | X | | | | X | X | | | X | | | X |
| **National and international collaboration** | | X | X | X | | X | | | X | X | X | X | X | X | X | | | X | X | | X | | X | X | | | |
| Development | X | | X | X | X | | X | X | X | X | X | X | X | X | | | X | X | X | X | | | X | | | X | |
| Testing | X | | X | X | X | X | X | X | X | | X | X | X | X | | | X | X | X | X | X | | X | | | X | |
| Validation | X | X | X | X | X | X | | X | X | | X | X | | X | X | | X | X | X | X | X | | X | X | | | |
| Standardisation | X | X | X | X | X | | | | | | X | X | | X | | | X | X | | X | X | | | X | X | | |
| Assessment activities | | X | | X | | | | X | | X | X | | X | X | X | | X | X | X | | X | | | X | | | |


# Introducing recently started Horizon2020 projects

# ACEnano

## Analytical and Characterisation Excellence in nanomaterial risk assessment: A tiered approach

Contract Agreement: 720952 Website: http://www.acenano-project.eu/
Coordinator: Èva Valsami-Jones, School of Geography, Earth & Environmental Sciences, University of Birmingham, B15 2TT Birmingham, United Kingdom, e.valsamijones@bham.ac.uk

*Table 1 Consortium List.*

| No. | Beneficiary name | Short name | Country |
|---|---|---|---|
| 1 | The University of Birmingham | UoB | United Kingdom |
| 2 | Universitat Wien | UNIVIE | Austria |
| 3 | Natural Environment Research Council | NEERC | United Kingdom |
| 4 | Douglas Connect Gmbh | DC | Switzerland |
| 5 | Postnova Analytics Gmbh | PNV | Germany |
| 6 | Centre Suisse D'Electronique et de Microtechnique SA – Recherche et Developpment | CSEM | Switzerland |
| 7 | Wageningen Research | RIKILT | Netherlands |
| 8 | Eidegenoessiche Technische Hochschule Zurich | ETH | Switzerland |
| 9 | Perkin Elmer Sweden AB | PE | Sweden |
| 10 | Sveriges Lantbruksuniversitet | SLU | Sweden |
| 11 | TofWerk AG | Tof | Switzerland |
| 12 | Helmholz-zentrum fuer Umweltforschung GMBH | UZF | Germany |
| 13 | Vitrocell Systems Gmbh | Vitrocell | Germany |
| 14 | Malvern Instruments Ltd | Malvern Inst | United Kingdom |
| 15 | Horiba UK Ltd | Horiba | United Kingdom |
| 16 | Biolin Scientific AB | BiSc | Sweden |
| 17 | Bundesanstalt fuer Materialforschung und –Pruefung | BAM | Germany |
| 18 | AB Sciex UK Limited | SX | United Kingdom |
| 19 | The Chancellor, Masters and Scholars of the University of Oxford | UOXF | United Kingdom |
| 20 | Nanofutures ASBL | NANOfutures | Belgium |
| 21 | Industry-University Cooperation Foundation of Hanyang University | HYU | South Korea |
| 22 | National Centre for Nanoscience and Technology | NCNST | China |
| 23 | Bundesinstitut fuer Risjobewertung | BFR | Germany |
| 24 | To21 Co Ltd | TO21 | South Korea |
| 25 | Oscube Ltd | OSC | South Korea |
| 26 | Joint Research Centre | JRC | Belgium |

## Contents

# 1 Summary

Project Duration: 4 years

Project Funding: €7M EU + Chinese & South Korean funding contributions = €10.5M total

Nanomaterials (NMs) are very diverse groups of materials with greatly varying properties. Nowadays, an increasing number of them are entering the market in every day products spanning from healthcare and leisure to electronics, cosmetics and foodstuff. However, the **novelty and variety in properties and forms of nanomaterials** makes the elaboration of a well-founded and robust legislative framework to ensure safe development of nano-enabled products particularly challenging.

At the heart of the challenge lies the difficulty in the reliable and reproducible characterisation of nanomaterials given their extreme diversity and dynamic nature, particularly in complex conditions, such as within different biological, environmental and technological compartments.

To resolve this, ACEnano project will bring together an impressive and substantial partnership of 26 experts from research and industry who will work together during the next four years to introduce **confidence, adaptability and clarity into nanomaterial risk assessment** by developing a widely implementable and robust tiered approach to nanomaterial physicochemical characterisation that will simplify and facilitate contextual (hazard or exposure) description and its transcription into a reliable nanomaterials grouping framework.

This will be achieved by the creation of the **"ACEnano virtual toolbox"** including reliable, innovative and optimised analytical techniques, instrumentation and equipment for the testing of nanomaterials properties/descriptors and a decision tree to guide users (specially SMEs) through selection of the most appropriate (combination of) methods to address their specific research or regulatory question and where to find it (ACEnano core labs, EU nanosafety cluster…).

# 2 Background

An increasing number of nanomaterials (NMs) are entering the market in every day products spanning from health care and leisure to electronics, cosmetics, energy, agriculture, food and transport. Nanotechnology is a truly enabling technology, with enormous potential for innovation. However, the novelty in properties and forms of NMs makes the development of a well-founded and robust legislative framework, capable of ensuring safe development of nano-enabled products particularly challenging.

At the heart of regulatory challenge for NMs lies the difficulty in the reliable and reproducible characterisation of NMs, given their extreme diversity and dynamic nature, particularly in complex environments, such as within different biological, environmental and technological compartments.

Three key actions can resolve this (Figure 1):

1) the development of a holistic analytical framework for reproducible NM characterisation, spanning from initial needs assessment through method selection to data interpretation and storage;


**Figure 1:** Illustration of how ACEnano will focus on bringing together the critical elements needed to deliver Analytical excellence and the end-user confidence needed to support risk assessment of nanomaterials, as well as associated issues.

2) the embedding of this framework in an operational, linked-up ontology ("common language") and data framework to allow identification of causal relationships between NMs properties, be they intrinsic, extrinsic or calculated, and biological, (eco)toxicological and health impacts; and

3) the full integration of training activities that will ensure technical expertise and underpinning data framework are fully delivered to the community in a tiered approach model.

The result will be an innovative and adaptive mechanistic 21st century risk assessment framework (based on grouping and read-across) that can keep pace with market and product innovation and facilitate prediction of NMs impacts and implementation of safe by design strategies and support regulation.

# 3 Scientific and technological challenges

The ACEnano vision is to introduce confidence, adaptability and clarity into NM risk assessment by developing a widely implementable and robust tiered approach to NM physico-chemical characterisation that will simplify and facilitate contextual (hazard or exposure) description and its transcription into a reliable NMs grouping framework. This will be achieved by the creation of a "conceptual toolbox" including a tiered approach to cost efficient NMs analysis that will facilitate decision-making in choice of techniques and SOPs, linked to a characterisation ontology framework for grouping and risk assessment. ACEnano will initiate activities to support data collection, management, interpretation and delivery to a data warehouse for safe use & storage. It will thus underpin the future of NM quality control, labelling and anti-counterfeiting. The benefits for Europe and beyond are enormous.

## 4 Objectives

ACEnano has the following specific objectives:

**I. Method Innovation.**

ACENano will innovate in a carefully selected and appropriate set of analytical techniques, instrumentation and equipment for the testing of NM properties/descriptors that are:

(i) most diagnostic to nanosafety,

(ii) linked to key descriptors (ensembles of particle sizes, complex shapes, surface area and surface chemistry, coating stability or multiple composition (multicomposites)),

(iii) facilitate assessment of the longer term fate of particles following their interactions within complex matrices, i.e. in living systems, or the environment; and

(iv) have a distinct potential to resolve analytical uncertainty and/or reduce testing costs.

Among the innovations that ACEnano will introduce are:

- removal of bottlenecks and minimisation of inconsistencies by streamlining and automating sample delivery (to analytical detection methods),

- provision of a one-stop solution to NM characterisation in an integrated modular screening regime (ACEnano toolbox), available as virtual tools for SMEs to decide which characterisation is needed, and with access provision via a group of core labs and the EU nanosafety clusters "access" programme.

There is tremendous potential impact in terms of decluttering and streamlining characterisation as a whole, and major impact for the nanosafety community, including industry and regulators, is foreseen by increasing the toolbox of reliable methods of NM characterisation ("ACEnano toolbox tier 1" as shown in Figure 2).

**II. Method Optimisation.**

Where an appropriate level of excellence is already available, to optimise the existing techniques/instrumentation, miniaturise and simplify where possible (to reduce costs and enhance accessibility for SMEs) and support their use by SMEs through training and documentation. Adapting those techniques which work well and have reached maturity would be financially prudent and will minimise needs for new equipment and/or training in the community ("ACEnano toolbox tier 2" as shown in Figure 2).

**III. Method benchmarking.**

To benchmark key components of the ACEnano toolbox and set criteria for future benchmarking of further components, thereby building the necessary confidence of all stakeholders. ACEnano will create a cohort of trained experts in 8 partner facilities, who will participate in all round robins and will deliver a series of training events to the nanosafety community.

The impact will be delivered via a series of improved methodologies / easily adaptable technological developments, where each addresses a current analytical need, accessible to the wider community as a one-stop tiered service, supported by a pyramid training, where the highest level of expert trainers will be delivered by ACEnano and their expertise will be cascaded to the community within the lifetime of the project ("ACEnano toolbox tier 3" as shown in Figure 2).

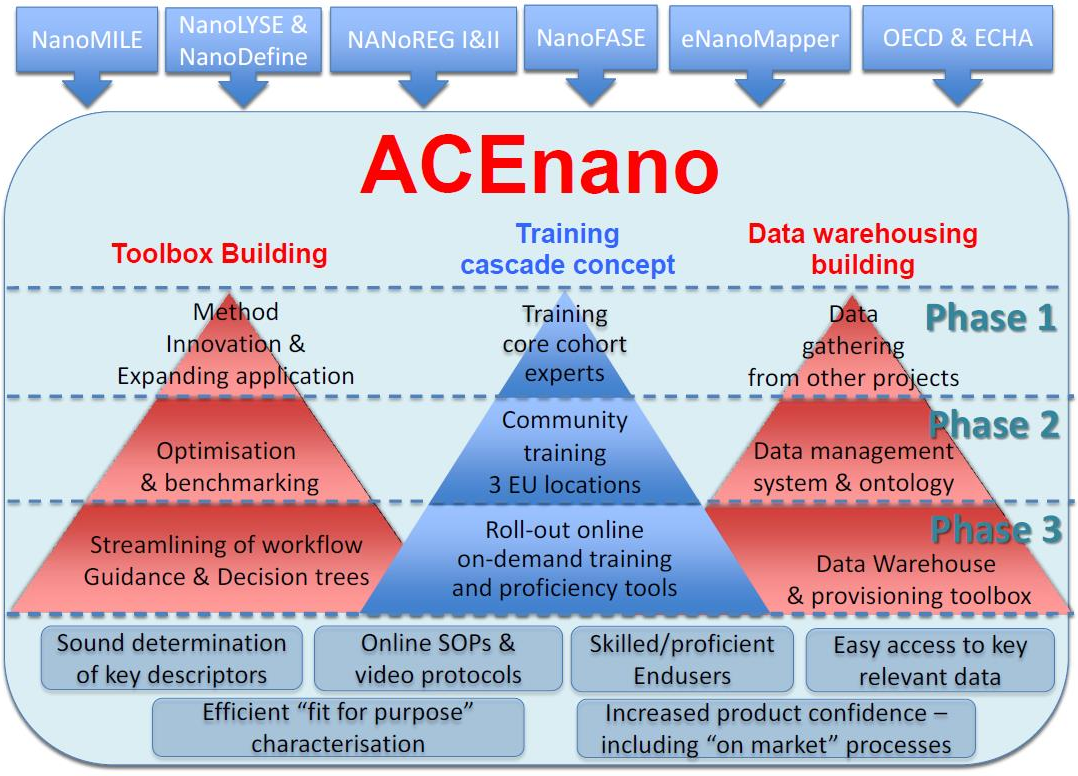



**Figure 2:** ACEnano tiered method toolbox building, training and data toolbox. Note input from other current FP7/H2020 projects.

**IV. Integration in mechanistic ontology framework.**

To link the methodological advancements of ACEnano to a mechanistic ontology framework, importantly considering fate and exposure as well as hazard, and create an interface between the ACEnano Data Warehouse and the ACEnano toolbox, thereby creating the ACEnano "virtual toolbox" and underpinning decision tree to guide users through selection of the most appropriate (combination of) methods to address their specific research or regulatory question. A robust and powerful data management system, embedded in the ISA-TAB-Nano specification and Elixir (the EU Life sciences data platform)-compatible, which would deliver centralised management of all ACEnano data, and harmonise other major existing data management systems and resources is central to the ACEnano concept.

This activity will deliver major impact in streamlining and facilitating data collection/management, harmonisation of reporting and delivery of integrated risk assessment tools to the nanosafety community, and in consolidating a common language within the nanosafety community and supporting / integrating existing platforms for communication.

**V. Quality Assurance & Risk Assessment Framework.**

To embed all above into a quality assurance and risk assessment framework to increase confidence in NMs characterisation, grouping, prediction of impacts and risk assessment that will facilitate uptake by industry and legislators. There is a clear potential for impact through this objective by ensuring continuity and longevity of the work beyond the duration of the project, and indeed offering approachable and well-considered solutions beyond the nanosafety community to industry, governments and NGOs and civil society.

**VI. Dissemination and Exploitation.**

To ensure dissemination and exploitation of the ACEnano project and its innovations and outcomes to the spectrum of stakeholders across industry (SME and enterprise, both NMs producers and users), the regulatory and policy communities including those responsible for implementation, and the scientific community.

# 5 Organisation

ACEnano will introduce confidence, adaptability and clarity into NM risk assessment by developing a comprehensive and structured toolbox of methods, for a thorough physico-chemical characterisation of NMs, either pristine or in biological/environmental media. The ACEnano ambition is to cover the full range of analytical methods currently used in nanosafety, but to develop an easy to follow tiered approach concept, as one of its deliverables (see WP descriptions). **The rationale for this broad based activity is that for a project of this scale and ambition, it is important not to leave major gaps the nanosafety community will need to address separately.** This is **feasible** because ACEnano has a broad expert partnership and builds on the state-of-the-art developed by recently completed and on-going EC funded projects such as NanoLyse, NanoDefine, QualityNano, NanoValid, NanoMILE, NanoFASE and NANoREG I & II.

ACEnano's ambition is to create a reliable, well-designed methods toolbox, with all the techniques addressed in Table 2, mirrored by a "*virtual toolbox*" that every lab working on nanosafety could have access to on their bench. To achieve this, firstly we will optimize our *actual* method toolbox, using our concept of a tiered approach. Three possible levels of development for any relevant method are envisioned (Figure 2):

- **Method innovation (WP1) (Table 2, blue),** where a method requires major innovation, a team of partners, involving the manufacturer's R&D lab will innovate to improve. The innovation themes in ACEnano are:

a) combine ("hyphenate") methods (e.g. AFM-IR-Raman, bringing together optical and chemical information) to improve diagnostic ability of each technique, so that maximum information can be gleaned in a one-stop analysis;

b) expand or improve method capability (e.g. multi-element SP-ICP-MS);

c) develop novel diagnostic and easy to use assays (e.g. solubility assay); and

d) miniaturise and develop bench top technologies (e.g. mini-Air Liquid Interface (ALI) exposure system).

These activities were carefully selected with costs in mind, for example by miniaturizing methods, or developing low-cost kits.

**Method optimization (WP2) (Table 2, green)**, where a method needs adjustment, i.e. works well but could be improved to fit better within the ACEnano toolbox by relatively incremental technological adjustments, for example harmonizing sample preparation with other methods, it will be worked on by one of the ACEnano labs, e.g. Correlative Reflectance Electron Microscopy, which the UoB team have demonstrated using gridded Matex dishes; the **advance is that the same sample** can be characterized by a number of methods: confocal, TEM, SEM, AFM. A further example involves the harmonization of sample introduction by introducing a simplified universal sample introduction system which is interchangeable between different techniques (e.g. DLS, NTA, FFF) so that there is no ambiguity in terms of batch to batch variation or effects from different sample preparation/introduction techniques.

**Benchmarking and streamlining (WP3) (Table 2, orange)**, where a method works well (e.g. surface area by BET), we will test internally (inter-laboratory comparisons by 8 partner labs, see Tables 2 & 3), benchmark, record & make available (e.g., as a video) for community training a set of online tools.

*Once ACEnano's method toolbox is assembled, a* ***virtual toolbox*** *(**WP4**) accessible through training, will be implemented via a pyramid training scheme (Figure 2). The highest level of expert trainers will be delivered by ACEnano, and expertise will be cascaded to the community within the project lifetime (Figure 2). The steps to deliver this are:*

- A selection of methods will be tested through inter-laboratory comparisons, optimized and once confidence in their precise delivery is established, they will be developed as a series of video linked publications, submitted to the journal JoVE, linked together in an "nanosafety toolbox" focus page similar to: focus.jove.com/imaging/.

**Table 2**: The ACEnano tool box approach in method development to address hazard (H), exposure (E) & fate (F).

| Key descriptor | Develop | Target | Tech TRL | Appli TRL | Instrumentation/ methodology* | ACEnano development and innovation | Partner |
|---|---|---|---|---|---|---|---|
| Size, shape, comp, coating | INNO | H/E | 8 | 3-5 | STXM , TXM | Localisation and quantification of NM content in individual cells | HYU, NCNST |
| Exposure | INNO | F / E | 6-8 | 5-6 | ALI | Miniaturise system for exposure; integrate ICP-MS nebuliser & imaging capactity. Low -cost benchtop instrument | VC, ETH, UoB |
| Size, comp | INNO | E, F | 5-8 | 3-5 | *single-cell-ICP-MS | Quantification of NM content in individual cells | PE, UoB, SLU |
| Size, comp | INNO | H/E | 5-7 | 4-6 | *SP-ICP-ToF-MS | Novel hyphernation - multi elemental analysis on single particle level, spectral libraries of NPs | UNIVIE, TOFW, RIKILT, ETH, UFZ, IPICYT |
| Size, comp | INNO | H/E | 3-4 | 3-5 | *LA-ICP-(ToF)-MS | Novel hyphernation - multi elemental analysis on single particle level, spectral libraries of NPs, laser ablation for surface analysis in/on biota and quatification of elements in there | UNIVIE, TOFW, RIKILT, UFZ, IPICYT |
| Size, shape, comp | INNO | H / E | 8 | 3-5 | AFM/Raman/confocal | Localisation & analysis of NMs in cells (especially for carbon-based / polymeric) | HRB, UoB, NERC, IPICYT |
| Comp, surface charge, coating | INNO | E | 7 | 4 | CE-MS | Corona characterisation | BS, UoB, IPICYT |
| Surface chem, coatings | INNO | E | 3-6 | 3 | HDC-RC-MS | On-line size separation & surface characterisation on size fractions of metal-oxide size NM; composition of organic NM | RIKILT, UFZ |
| Surface reactivity | INNO | H, F | 3-5 | 3 | reactivity assay on a chip | Miniaturised and automate surface reactivity measurements to microfluidic-chip assays . Three assays will be developed. The first to assess photocatalytic activity or ROS generation, while the second and third will assess hydrophobicity and solubility. Low-cost disposable assay | CSEM, UoB, RIKILT |
| Surface reactivity | INNO | H / F | 8 | 5 | QCM-D, TT, LB | NM interaction with natural and artificial cell membranes. | BS, CSEM, UoB, BAM, UFZ |
| Size | INNO | H, F | 3-5 | 3 | AF4, centrifugal FFF | Innovation: coupling with Raman; shape distribution. | JRC, PNV, UoB, SLU |
| | OPTI | | 4-6 | 3 | | Optimisation of sample delivery | |
| Size, shape, comp | OPTI | H / E | 8 | 4-7 | TEM (-EELS), CREM | Optimisation of Sample delivery, high throughput, cost reduction | UOXF, UoB, UNAM |
| Comp | OPTI | H / E | 8 | 5-8 | TG-IR GC/MS | Optimisation for carbon NMs & corona characterisation | PE, UoB |
| Size | OPTI | H, F | 8 | 4-7 | NTA (NanoSight) | Optimisation to reduce variation in NM size and concentration measurement | MVN, UoB, NERC, UOXF.DJ, RIKILT, |
| | BM | | | 5-8 | | Benchmark | UNIVIE, PNV, HYU |
| Size, surface charge | OPTI | H | 8 | 4-7 | DLS/ZP | Optimisation of sample delivery for e.g. zeta measurement | MVN, UoB, UNAM, UFZ, BAM, RIKILT, |
| | BM | | | 5-8 | | Benchmark | UNIVIE, PNV, BfR, HYU |
| Size, shape, comp | OPTI | H, F | 8 | 3-5 | AUC | Optimise for carbon NM characterisation. | UoB, UOXF, NERC |
| | BM | | | 5-8 | | Benchmark | |
| Comp, surf chem | OPTI | H | 8 | 5-8 | ToF-SIMS, XPS, AES, SEM, TEM, T-SEM | Increase throughput, reduce operating costs, improve specificity | UFZ, BfR, BAM, CSEM, JRC |
| | BM | | 8 | 5-8 | | Develop microfluidic device to enable high throughput analysis, cost reduction | |
| Surf chem, coatings, comp | BM | H | 8 | 5-8 | XPS/AES | Evaluation of application domain, cost reduction | see table 3 |
| Size | BM | H, F | 8 | 5-8 | disc centrifuge CLS/CPS | Benchmark | UoB, JRC |
| Size, comp | BM | H, F | 8 | 5-8 | SP-ICPMS | Expansion of application domain to complex matrices (e.g. plasma, tissues, food, soils, sediments), and smaller sizes of NM to be characterised therein | see table 3 |
| Size, comp | BM | H, F | 8 | 5-8 | FFF-ICPMS | Expansion of application domain | see table 3 |
| Size, shape, comp | BM | H, F | 8 | 5-8 | SEM (-EDX) | Evaluation of application domain, cost reduction | see table 3 |
| Structure, comp, size | BM | H | 8 | 5-8 | XRD | Evaluation of application domain | see table 3 |
| Surface area | BM | H | 8 | 5-8 | BET | Benchmark; Potential extension to biphasic or composites? | see table 3 |
| Size, comp | BM | H | 8 | 5-8 | UV-vis | Evaluation of application domain | see table 3 |

Key descriptors: Intrinsic: Size, composition (comp), shape, surface chemistry (surf chem), surface charge, surface area. Extrinsic: coating, surface reactivity, exposure. * Methods not suitable for carbon-NMs (CNT, graphene, etc.); All other methods apply to carbon & metal/metalloid NMs. *Core:* core partners for internal validation, see Table 2.

- The project will train a "core cohort" of experts, one at each lab shown in Table 3, who will train together in the priority techniques (shown in Table 3). They will then undertake three community training events in: Vienna (partner UNIVIE), Riga (supported by Advisory Board member Arnolds Ubelis) and Athens (supported by Costas Charitidis), to address the needs of Northern, Eastern and Southern Europe respectively). The training will then be made available, linked to the video-publications described, to the nanosafety community through the nanosafety cluster web page (as shown in the concept of Figure 2). Emphasis will be placed on SME and industry support and indeed a **"simple guide for SMEs"** will be developed.

**Table 3:** Partner participation in interlaboratory comparisons. Where 8 or more partners involved the project can deliver a full validation; where fewer partners available SOP development.

| Partner | DLS | NTA | FFF | UV-vis | XRD | BET | SEM | TEM/TSEM | AFM | ToF-SIMS | SP-ICP-MS |
|---|---|---|---|---|---|---|---|---|---|---|---|
| UoB | ✓ | ✓ | ✓ | ✓ | ✓ | ✓✓ | ✓ | ✓ | ✓ | | ✓ |
| UFZ | ✓ | | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓✓ | ✓ |
| BAM | ✓ | | ✓ | | ✓ | ✓ | ✓ | ✓ | | ✓ | |
| NERC | ✓ | ✓ | ✓ | ✓ | | | | | | | ✓ |
| UOXF.DJ | | ✓ | | ✓ | ✓✓ | | ✓ | ✓✓ | | | |
| RIKILT | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | | ✓✓ |
| UNIVIE | ✓ | ✓ | ✓ | ✓✓ | ✓ | ✓ | ✓ | | | | ✓ |
| PNV | ✓ | ✓ | ✓✓ | ✓ | | | | | | | ✓ |
| BfR | ✓ | | ✓ | ✓ | | | | | | ✓ | ✓ |
| MVN | ✓✓ | ✓✓ | | | | | | | | | |
| JRC | | | | | | | | | | ✓ | |
| HYU | ✓ | ✓ | | | | | | ✓ | | | ✓ |

✓: participates
✓✓: leads inter-laboratory activity

ACEnano's work programme is structured into 7 WPs interacting within a seamless and coordinated workflow to address the stated objectives of the project (Figure 4). The rationale for this structure relates to a logical path from discovery science to key innovations for instrumentation, analysis, training and data exploitation. It begins with early engagement with stakeholders to collaborate with project partners along a single research stream that intercepts five complementary activities: (1) Analytical innovation focussing on diagnostic capacity and method hyphenation (WP1); (2) Analytical optimisation, focussing on sample introduction and method harmonisation (WP2); (3) Method benchmarking against established methods and multi-tier training (WP3); (4) Enforcing and expanding nanoscience ontologies, by linking with methodology (WP4) and linking the ACEnano methodological innovation with quality assurance and pre-standardisation efforts (WP5). Each WP is subdivided into specific tasks that explore different aspects of these objectives.

WPs 1-3 will work in concert to drive analytical excellence forward through both purely technical innovation and also improved protocols and sample preparation for established methods. Figure 5 illustrates how multiple methods can be brought to bear on each of the most important key descriptors, and allow selection of the optimum method given the context of the parameter to be addressed in the media of relevance. The final step before fully optimised methods are sent to WP5 for Quality Assurance of the protocols, training program and material is to benchmark techniques that address the same key descriptors. This is needed in order to develop the guidance of how to select between them depending on both the question and context at hand (see Figure 4 for how methods align against some of the most important key descriptors).

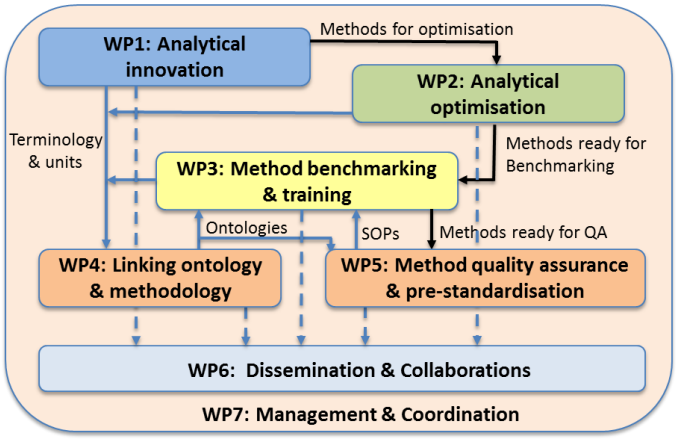



**Figure 4:** Diagram showing the interrelations of the workpackages (WPs) and the flow of methods, key information and documentation between WPs.

A significant innovation component of ACEnano is the active participation of analytical instrument manufacturers. Development and use of nano-enabled products is likely to be matched by a growing need for instrumentation that can detect NMs in complex (products, as well as biological and environmental) samples. Collaboration between industry and academic co-development of methods is evident throughout WPs 1-3, where essentially all methodologies are developed via cross sectoral partnerships. Even more interestingly, there are several examples of technologies being developed between clusters of industry and academic partners, such as the miniature ALI platform utilising the ICP-MS nebuliser to introduce NMs, and utilising microscopy cover slips to be interchangeable with industry standards. In many cases, there partnerships are between larger enterprises partners (e.g. Malvern) and SME partners (e.g. PNV) and are facilitated by the academic partnerships. Malvern linking their NanoSight device to PVN's FFF device can support increased sales for new detection and monitoring applications. Similarly development of the miniature ALI (mini-ALI) system will reduce the cost of the system, and coupling to existing neubilsers will further reduce costs and enhance consistency between characterisation of exposure measurements. ACEnano will be an ideal test-bed prior to commercial investment and market development. Exploitation activities (WP6) will also lead to development of consultancy services to support industry in product development and registration via REACH.

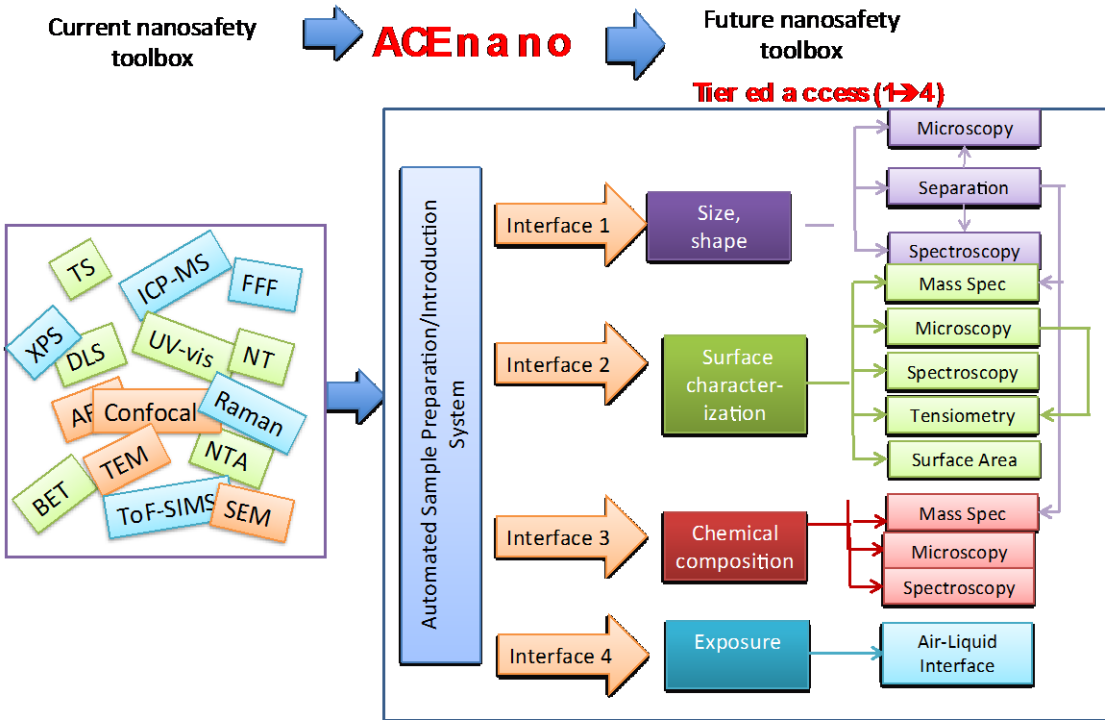



**Figure 5:** The method innovations in ACEnano: method alignment and simplification via shared sample introduction systems, shared sample preparation tools and microfluidic sample dispensation to reduce error and enhance method comparability.

## 6 Expected Impact

ACEnano is designed to provide innovative solutions for robust and reliable NM characterisation in support of improved nanorisk assessment and nanoregulation. Outputs are also expected to provide advances for related areas such as quality control, product traceability, labelling and counterfeiting, which will be highlighted below where appropriate.

The expected impacts from ACEnano will be as follows:

**Impact #1** - ***Enable identification of key descriptors that reveal correlations associated with health & environmental impacts and meaningful basis for grouping, read-across and QSARs purposes***

The primary aim of ACEnano is to eliminate resource and labour intensive, slow and inaccurate characterization methodologies that each require different sample preparation and delivery ormats. ACEnano will seek to supersede the current poorly optimized and ad hoc approach with a scheme that is scientifically sound, rationally selected, optimised for the question to be addressed and benchmarked. By enhancing the reliability and robustness of the methods, and ensuring they are utilized in appropriate manners to address relevant questions, ACEnano will dramatically increase the generation of sufficiently robust data to enable risk assessment. This will include identification of the key descriptors driving environmental fate and toxicity that can be feed into a regulatory framework in order to meet the data demands set by projects like GUIDEnano, SUN, CaLIBRAte, and the NANoREG 1+2 projects which are developing the Risk Assessment frameworks for nanosafety.

ACEnano's toolbox of methods has been selected to address the 9 key parameters / measurement challenges identified in Table 1. The project will identify those descriptors for which method innovation is required, and those which are already at advanced TRLs but which required optimisation and benchmarking efforts to expand their domains of applicability to a wider range of biological and environmental matrices.

The ACEnano characterization concept is built around a mechanistic understanding of NMs release processes, exposure and hazard for living systems. This knowledge will allow a focus on key parameters linked to exposure and toxicity (intrinsic, extrinsic or computed). ACEnano will deliver a robust and streamlined framework to measure these key properties to enable grouping and categorization. The efficiency gained via the tiered characterization concept and the ability to pinpoint the most diagnostic combination of tests for each particular NM or NM group, will introduce confidence as well as speed, and in turn reduce unnecessary costs and effort.

The ACEnano toolbox will be coupled with a science-based decision tree to support regulators and industry in the selection of relevant characterisation tools to address specific questions with a high degree of confidence and in a manner that is purposefully designed and uses the most appropriate tool to provide the highest quality data whilst utilising the minimum number of methods (reducing costs) and integrated sample preparation (reducing sample amounts) and utilising the simplest method suitable to provide the required information.

ACEnano's impact will thus be achieved through new characterisation tools & services that are robust, reliable & fit for purpose for risk assessment and regulation, enabling greatly increased confidence in datasets, and identification of quantitative NM structure-activity relationships (SAR) as the basis for grouping and read-across.

**Impact #2 - Increased confidence in nanosafety studies and findings through sound physico-chemical characterisation methods and standard operating procedures**

The ACEnano characterization concept is built around a mechanistic understanding of NMs release, fate, exposure and hazard to living systems. This knowledge will allow a focus on key parameters linked to exposure toxicity (intrinsic, extrinsic or computed). By tackling the 9 key parameters / measurement challenges identified in Table 1, ACEnano will innovate in terms of method and/or assay development for several parameters likely to be directly relatable/correlated to hazard and fate but independent of specific NM composition. Focusses will include key properties such as surface reactivity, hydrophobicity, dissolution as a complement to the traditional physical-chemical characterisation parameters normally investigated.

The ACEnano tiered characterization concept and ACEnano methods Toolbox will bring an enhanced efficiency in method selection. Further an ability to pinpoint the most diagnostic combination of tests for each particular NM or NM group and biological or environmental matrix through the ACEnano decision tree, will introduce confidence as well as speed to analysis. This will in turn reduce unnecessary costs and effort, whilst simultaneously increasing the confidence of industry and regulators in the quality, reliability and relevance of the data produced utilizing the ACEnano toolbox.

A further focus of ACEnano will be to tackle the reliability of measurements and NM characterization for risk assessment. This will be done in large part through harmonized sample preparation and increased speed of sampling to reduce sample evolution during measurement.

To support REACH and other relevant legislation and policy, ACEnano will embed a data management/modelling framework to meet the requirement of reliably establishing NM characterisation, and delivering reproducible answers for each nanoscale property considered. The data management tool that will be established will be valuable to industry both in the product development and regulatory compliance arenas.

The data management/modeling framework will be developed in collaboration with relevant EU projects, to ensure maximum interaction between the project's own modeling team and major external industrial and regulatory parties. The key deliverable will be a thoroughly tested framework, with established and demonstrated approaches identified that can be used to address key issue in the innovation chain for nanotechnology products. To ensure regulatory compliance and buy-in, the project will work with ECHA and EFSA, through its regulatory partners at all stages of development and implementation, as key identified stakeholders in the development on NM risk management frameworks in Europe.

**Impact #3 - Reduced costs related to the physico-chemical characterisation of nanomaterials in relevant environments**

As indicated under Impact #1, the primary aim of ACEnano is to supercede resource and labour intensive, slow and inaccurate characterization methodologies, that each requiring different sample preparation and delivery formats. Such sub-optimal existing methods will be replaced by a set of scientifically sound, rationally selected approaches that are each designed to be optimised for the question to be addressed. These improved methods will be benchmarked by ACEnano partners.

To increase overall reliability and throughput, ACEnano's industry partners will work to streamline their instrument offers to provide pared-down or cheaper and more accessible bench-top models of key equipment (e.g. Vitrocell Air-Liquid Interface (ALI); CSEM lab-on-a-chip) that can be run by non- expert users.

Another central theme of ACEnano's innovation is the hyphenation of methods and alignment of sample preparation. For example, ICP-MS nebulization can be utilised also for the ALI exposure chambers. This would support the provision of identical doses for characterization of NMs in the dispersion or for use for biological experiment. The later may include subsequent toxicity experiments; the growth of the cells for ALI on gridded Matek coverslips to facilitate post-exposure imaging via confocal, TEM, or a correlative/combination of methods. Further, the hyphenation of methods can also allow sample clean-up to be directly integrated with characterisation. Example here would include (LA)-SP-ToF-ICP-MS and TG-IR-GC/MS with the possibility that these combinations can speed up the process, reducing sample cost, and increasing confidence in the data.

Collectively these methods (the ACEnano toolbox) will support the generation of robust data sets for implementation of grouping approaches allowing prediction of impacts and risks.

**Impact #4 – Identification of synergies with applications of the methods in other areas such as quality control, product traceability, labelling and counterfeiting**

Methods developed/optimized within ACEnano will be assessed for potential uses in related markets/application areas. The ACEnano toolbox will provide greatly increased confidence for NMs quality control. Miniaturization and optimization will allow improved methods to be integrated as in-line measurements during NM production or purification at pilot-line scales. Similarly, method development and optimization will be an important enhancement to European capability for product traceability, labelling and counterfeiting, as increased in detection limits, and expansion of applicability domains to more complex matrices will allow detection and measurement at sites away from highly equipped laboratories.

As part of Horizon 2020 framework, ACEnano embodies the long term vision of the European Union for sustainable development, which is defined as a development that meets the needs of the present without compromising the ability of future generations to meet their own needs. In 2015, the United Nations adopted the global 2030 Agenda providing a set of 17 Sustainable Development Goals (SDGs). The ambition of ACEnano to set standards on characterization of nanomaterials finds echo in a large umbrella of SDGs. It includes participating in the preventive effort to substantially reduce the impact of nanomaterials on individual health (SDG 3), on water quality (SDG 6) and on the environment (SDG 14 and 15). Furthermore,

the international ACEnano framework is dedicated to promote development and dissemination of clean and environmentally sound technologies (SDG 9) and to share knowledge through multi-stakeholder partnerships (SDG 17).

## 7 Directory

*Table 4 Directory of people involved in this project.*

| First Name | Last Name | Affiliation | e-mail |
|---|---|---|---|
| Eugenia (Èva)<br>Iseult<br>Thomas | Valsami-Jones<br>Lynch<br>Carney | University of Birmingham | e.valsami-jones@vham.ac.uk<br>i.lynch@bham.ac.uk<br>t.carney@bham.ac.uk |
| Frank | Von der Kammer | University of Vienna | frank.von.der.kammer@univie.ac.at |
| Steve<br>Claus | Lofts<br>Svendsen | Natural Environment Research Council | stlo@ceh.ac.uk<br>csv@ceh.ac.uk |
| Thomas<br>Barry | Exner<br>Barry | Douglas Connect GmbH | thomas.exner@douglasconnect.com<br>barry.hardy@douglasconnect.com |
| Florian<br>Roland | Meier<br>Weiz | Postnova Analytics GmbH | florian.meier@postnova.com<br>roland.welz@postnova.com |
| David | Mueller | Centre Suisse d'Electronique et Microtechnique SA | David.MUeLLER@csem.ch |
| Hans<br>Ruud | Bouwmeester<br>Peters | Stichting Wageningen Research | hans.bouwmeester@wur.nl<br>ruudj.peters@wur.nl |
| Bodo | Hattendorf | Eidgenoessische Technische Hochschule Zuerich | bodo@inorg.chem.ethz.ch |
| Chady | Stephan | PerkinElmer UK Limited | Chady.Stephan@PERKINELMER.COM |
| Gert | Cornelis | Sveriges Lantbruksuniversitet | geert.cornelis@slu.se |
| Martin | Tanner | TOFWERK AG | m.tanner@tofwerk.com |
| Stephan | Wagner | Helmholtz-Zentrum für Umweltforschung | stephan.wagner@ufz.de |
| Christoph<br>Tobias | Schlager<br>Krebs | Vitrocell Systems GMBH | <br>t.krebs@vitrocell.com |
| Patrick<br>Jo | Hole<br>Sullivan | Malvern Instruments Ltd | Patrick.Hole@malvern.com<br>jo.sullivan@malvern.com |
| Adam<br>Pierre | Holland<br>Burgos | HORIBA Scientific | adam.holland@horiba.com<br>pierre.burgos@jobinyvon.co.uk |
| Michael<br>Deborah | Rodal<br>Rupert | Biolin Scientific AB | Michael.Rodal@biolinscientific.com<br>Deborah.Rupert@biolinscientific.com |
| Wolfgang | Unger | Bundesanstalt für Materialforschung und –prüfung | wolfgang.unger@bam.de |
| Jim<br>Andy | Thorn<br>Chetwynd | AB Sciex UK Limited | Jim.Thorn@sciex.com<br>andrew.chetwynd@sciex.com |
| Colin | Johnston | University of Oxford | colin.johnston@materials.ox.ac.uk |
| Raquel | Garcia | NANOfutures ASBL | rgg@prodintec.com |
| Tae-Hyun | Yoon | Industry-University Cooperation Foundation of Hanyang University | thyoon@gmail.com |
| Chunying | Chen | National Center for Nanoscience & Technology | chenchy@nanoctr.cn |
| Jutta<br>Harold | Tentschert<br>Jungnickel | Bundesinstitut für Risikobewertung | Jutta.Tentschert@bfr.bund.de |
| Heekyung | Bae | TO21 Co. Ltd | heekyung.bae@to21.co.kr |
| Gabriela | | OSCube | gabriela@oscube.co.kr |
| Douglas | Gilliland | Joint Research Centre | douglas.gilliland@ec.europa.eu |

## 8 Copyright

# CERASAFE

## Safe production and Use of Nanomaterials in the Ceramic Industry

Contract Agreement: 16 Website: www.cerasafe .eu
Coordinator: Mar Viana (mar.viana@idaea.csic.es), Institute of Environmental Assessment and Water Research (IDAEA-CSIC), C/ Jordi Girona 18, 08034 Barcelona, SPAIN.

*Table 1 Consortium List.*

| No. | Beneficiary name | Short name | Country |
|---|---|---|---|
| 1 | Agencia Estatal Consejo Superior de Investigaciones Científicas | CSIC | Spain |
| 2 | Universidade de Lisboa | UL | Portugal |
| 3 | Nova.ID | Nova.ID | Portugal |
| 4 | ITC-Universitat Jaume I | ITC | Spain |
| 5 | Babes-Bolyai University | BBU | Romania |
| 6 | National Institute of Health Dr. Ricardo Jorge | INSA | Portugal |
| 7 | University of Littoral Côte d'Opale | ULCO | France |

## Contents



## 1 Summary

Project Duration: January 2016 to December 2018

Project Funding: 751.510€ (SIINN-ERANET)

CERASAFE proposes an integrated approach to environmental health and safety (EHS) in the ceramic industry. It aims to characterise nanoparticle release scenarios during industrial processes in this sector, and to assess exposure by addressing the release mechanisms, toxicity, and physico-chemical properties of intentionally and unintentionally produced nanoparticles, as well as mitigation measures to minimise exposure. CERASAFE is also developing an online tool to discriminate engineered nanoceramic particles from background aerosols, innovating in the field of characterization methods relevant for EHS issues. The project will establish a set of Good Manufacturing and Use Practices for nanoceramic materials. Results will be collected in public database (e.g., NECID) complemented with risk assessment and including recommendations for industry, users and stakeholders to ensure worker safety during nanoceramic materials manufacturing, application and processing.

## 2 Background

Applications of nanoscience and nanotechnology have a significant impact on sustainable development, influencing virtually all industrial sectors. In particular, the manufacture of engineered nanomaterials, such as nanoceramics, is starting to be effective within resource saving, reduced consumption of materials, and the possibility of substituting current materials (Rickerby and Morrison, 2007). However, the increasing demand for appropriate risk assessment of nanotechnologies affects every organization involved in development and manufacture of engineered

nanomaterials (Friedrichs and Schulte, 2007). Therefore, these organizations must adopt responsible risk assessment, as well as risk management strategies, in order to protect both their staff and end-users from the potentially hazardous effects thus arising. Understanding the relationship between airborne nano-sized particulate matter and human health, under different environmental conditions is of great importance for improving exposure estimates and for developing efficient control strategies to reduce human exposure and health risk and for establishing, evaluating and improving regulations and legislation aiming to guarantee the safe production of manufactured nanomaterials. The current lack of specific online instrumentation for the targeted detection of ENMs in real time has been identified as a key knowledge gap regarding this (Savolainen et al., 2013).

Workers may be exposed to nano-scaled materials while manufacturing these materials, formulating them into products, transporting or handling them in the storage facilities, and even during industrial processes suffering from unintentional nanoparticle release. Because higher concentrations of nano-scale materials and higher frequency of exposure to them are more likely to happen in workplace settings, occupational exposures require special attention (Aitken et al., 2004; Hristozov and Malsch, 2009). While consumer exposure to airborne nanomaterials cannot generally be neglected, it is much more unlikely than worker exposure, because consumers will commonly only get into contact with nanomaterials that are tightly embedded, e.g. in surface coatings, whereas workers handle them in aerosol or powder form. The focus of CERASAFE is therefore on worker exposure, including exposure to process-generated nanoparticles from industrial processes (Figure 1). High-energy processes such as laser treatments and plasma projection also have a high potential for nanoparticle formation and release (Fonseca et al., 2015). Considering manufacturing processes, in machining (i.e., cutting, drilling and grinding), small sized chips are produced, while in production a large amount of nanoparticles is lost to working environment as observed in manufacturing of functionally graded materials by friction stir processing nanoparticles of ceramics are used as alumina and silicon carbides (Gandra, Miranda et al, 2011).

Figure 1. Example of worker exposure to process-generated nanoparticles in the ceramic industrial sector.

Over the past half century, ceramics (both conventional and advanced, Figure 2) have received significant attention as candidate materials for use as structural materials under conditions of high loading levels, high temperature, wear, and chemical attack that are too severe for metals (Thurnauer, 1954). However, inherent brittleness of the ceramics has prevented their wide use in different applications. Significant scientific efforts have been directed towards making ceramics more flaw-tolerant, one of the most effective being the distribution of multiple phases in a ceramic composite at the nanoscale (Richerson, 1992). Owing to prevalence of nanoscopic features, such composites are referred to as nanoceramics or, even, ceramic nano composites, when considering the combination of nanoceramic materials with metals (Ajayan, et al., 2003). Nanoceramics are ceramic materials comprised of particles usually less than 100 nanometers in diameter. Note that even when the grain size in the microstructure is larger than 100 nm, the material may be derived from nanopowders. The main property of nanoceramics is their larger surface area, with clear benefits in applications where a high reaction rate is needed. Due to their particle size, nanoceramics can display improved insulating or conducting properties (Dubey and Tomar, 2009).

*Figure 2. Examples of uses for conventional (left) and advanced (right) ceramics.*

Nanoceramics have applications in many present and emerging technologies. Due to their insulating and/or conducting properties, nanoceramics have uses in the construction of the next generation of high-speed computer chips, and in other electronics items. For the same reasons, they also have applications in power generation. In medicine, nanoceramics are starting to be used to develop new bone implants and artificial organs, since working at the nanoscale allows device manufacturers to fine-tune how the implant will interact with the human body, thus improving the chance of the body to accept it. In the dental medicine the translucency of the ceramic crown and bridge frameworks is increased by using zirconia nanopowders generating small grained and dense microstructures. Apart from these, nanoceramics have applications in space exploration, optics, weapons manufacturing, construction, consumer goods and transport.

In sum, CERASAFE will address detection, exposure and risk assessment of nanoparticles emitted into the workplace during nanoceramic manufacturing and application processes, as well as during unintentional nanoparticle release scenarios which may or may not involve nanoparticles as input materials. Examples of nanoparticle generating processes targeted are plasma thermal spraying, laser ablation, laser sintering of tiles, and physical vapour deposition.

## 3 Scientific and technological challenges

Production of nanomaterials and, in particular, the manufacture and use of nanoceramics, cannot be considered safe without a thorough investigation regarding exposure and toxicity of nanoceramic materials, which is unavailable so far. This requires a better knowledge on workers' exposure in the nanoceramics manufacture, handling and processing, which will firstly require the understanding of exposure scenarios. Once the potential release scenarios are understood, worker exposure needs to be

characterized following clearly defined protocols. Although as of now there are no legally binding protocols, the discussion is fairly advanced. It is known that the main limitations of occupational exposure assessments are the concentrations of ENPs in the working settings are seldom properly measured, the occupational exposure pathways are not well studied, the lack of ENP-specific online detection methods, and that official data on the number of workers exposed to ENPs are not available (Hansen, 2009; Brun et al., 2008). Nevertheless, there are 400,000 workers worldwide in the field of nanotechnology (Rocco et al., 2010), and by 2020 there will be approximately 6 million workers employed in nanotechnology industries worldwide. Furthermore, unintentional NP release from industrial processes not dealing with ENPs as input materials should also be thoroughly evaluated. CERASAFE is addressing these issues specifically for exposure scenarios in the ceramic industrial sector.

Another important building block for linking exposure and toxicological studies is a thorough physical and chemical characterization of the nanomaterials (Dahmann, 2013). Because of the large number of ENMs available, this kind of characterization is frequently not available. Furthermore, characterization is generally done offline in the laboratory, and real-time online detection methods are not available and are considered a research gap to be filled (Savolainen et al., 2013). Regarding NMs risk assessment, in vitro cytotoxicity studies and biodistribution data are essential to identify their potential hazard. The available data on toxic effects of the selected test nanoceramics is still scarce. These gaps in knowledge regarding biological interactions of nanoceramics will be addressed by performing in vitro and in vivo studies to provide insights on MNMs toxicity profile, namely on those related with oxidative stress, inflammatory and genotoxic responses.

Finally, mitigating exposures is of utmost importance. This includes filtering of the exhaust fumes and the use of efficient personal protective equipment where necessary. However, it is essential to base these measured on best practices approaches tailored to the specific needs of each industrial plant and process, and this is a generally lacking in real-world industries. To overcome this, CERASAFE will provide guidance on best practices for the ceramic industry.

## 4 Objectives

The project objectives are:

1. Identification of exposure scenarios during the nanoceramic value chain, mainly:
   a. during production and application of engineered nanoceramics, specifically aluminium-doped zinc oxide, La-based, $BaSO_4$, $TiO_2$, ceramic pigments, and silica nanoparticles
   b. during processes applied in the ceramic industry with potential for unintentional NP release, regardless of whether nanoceramics are used as input materials (laser ablation, plasma thermal projection, laser sintering, physical vapour deposition and inkjet printing)
2. Characterisation of worker exposure for the identified exposure scenarios
3. Identification of relevant nanomaterials and full physical chemical characterisation
4. Toxicity assessment for all materials identified as relevant
5. Development and testing of an online tool for real-time detection of nanoceramics in the workplace environment
6. Development of exposure mitigation strategies and evaluation through experiments and simulations in real-world workplace environments
7. Development of Good Manufacturing and Use Practices (GMUPs) for the safe production and use of nanoceramics.

Thus, the project's strategy will be based on a combination of:

- comprehensive experimental workplace exposure measurements,
- nanomaterial characterisation (toxicological, physico-chemical) in laboratory,
- development of online tool, and
- assessment of exposure mitigation strategies.

Its final result will be a set of guidelines to ensure safe manufacturing and use of nanoceramics and materials in the ceramic industry. Therefore, CERASAFE will characterise exposure and release scenarios under real-world conditions and produce applied guidance for industries in the ceramic sector, based on robust experimental data on nanoparticle release, physico-chemical and toxicological characterization.

## 5 Organisation

The project is divided into 8 work packages (WP) as illustrated in Figure 3. WP1 deals with project management. WP2 refers to exposure scenarios, both during manufacturing of nanoceramic materials and during processes in the ceramic industry with potential for nanoparticle emissions. The particles fabricated (intentionally) or generated (unintentionally) in the exposure scenarios will be characterised in WP3 (physico-chemical properties) and in WP4 (toxicological properties). The work in WP5 (development of an online tool for real-time detection of nanoceramics in workplace air) will run in parallel to the exposure assessments in WP2. Based on the results from WP2, WP3 and WP4, exposure mitigation strategies will be developed in WP6, which will input into WP7 to define an integrated approach and a set of guidelines for Good Manufacturing and Use Practices for nanoceramics. Finally, the project's results will be disseminated in the framework of WP8.

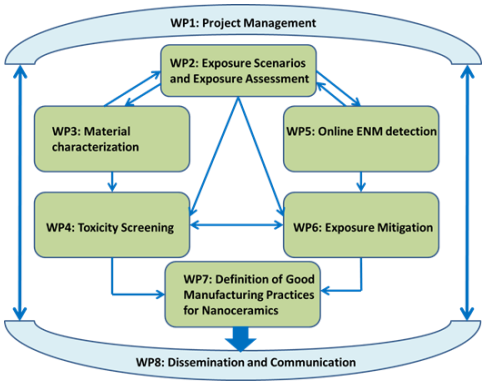



*Figure 3. Overview of the CERASAFE work-packages and their interrelationships.*

## 6 Expected Impact

- A full workplace exposure assessment concerning manufacture of nanoceramic materials in at least two real-world industrial workplaces.
- A full workplace exposure assessment concerning application of ceramic materials in industry such as plasma projection, laser sintering, laser ablation and/or inkjet printing in at least two real-world workplaces.
- A full physical-chemical characterization of at least 3 types of NPs and nanopowders (Sb, Sn nanoparticles), both as raw materials and also after use in industrial workplaces.
- Toxicological characterization of at least 3 types of nanoparticles and nanopowders (Sb, Sn nanoparticles), both as raw materials and also after use in industrial workplaces. Identification of correlations between in vitro and in vivo data and establishing of a ranking of hazard for the tested nanoceramics
- Novel tool/approach for online detection of nanoceramics in workplace air.
- Evaluation of the effectiveness of local exhaust and filtration systems and personal protective equipment in use in at least 4 real-world industrial workplaces (2 manufacturing, 2 applications).
- Setting-up of a publicly available database on occupational exposure and toxicity, for the materials and processes evaluated.
- Generation of a document on Guidance on Good Manufacturing and Use Practices (GMUPs) for the safe production and use of nanoceramics.

The project outcomes will be made publicly available through:

- The CERASAFE website (www.cerasafe.eu)
- Reports and database: the assessments and characterisations will be published in the form of reports and through the website and database, taking into account potential confidentiality constraints.
- Scientific papers, conferences and databases (e.g., NECID; MARINA).
- International workshop: a workshop will be organised in collaboration with NanoSafe (Grenoble) or SENN (Helsinki).
- Guidance document: a document on Guidance on Good Manufacturing and Use Practices (GMUPs) will be made available through the website, disseminated through the international workshop and distributed broadly, i.e. OECD, NanoSafetyCluster, etc.

## 7 Directory

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

| First Name | Last Name | Affiliation | Address | e-mail |
|---|---|---|---|---|
| Mar | Viana | IDAEA-CSIC | Barcelona, Spain | mar.viana@idaea.csic.es |
| João | Gomes | UL | Lisbon, Portugal | jgomes@deq.isel.ipl.pt |
| Rosa | Miranda | Nova.ID | Lisbon, Portugal | rmiranda@fct.unl.pt |
| Eliseo | Monfort | ITC | Castellón, Spain | emonfort@itc.uji.es |
| Simion | Simon | BBU | Cluj Napoca, Romania | simons@phys.ubbcluj.ro |
| João Paulo | Teixeira | INSA | Porto, Portugal | joao.teixeira@insa.minsaude.pt |
| Eugene | Bychkov | ULCO | Dunkirk, France | bychkov@univ-littoral.fr |

## 8 Copyright

# MODCOMP

## Modified cost effective fibre based structures with improved multi-functionality and performance


Contract Agreement: 685844
Coordinator: NTUA

Website: http://modcomp-project.eu/

*Table 1 Consortium List.*

| No. | Beneficiary name | Short name | Country |
|---|---|---|---|
| 1 | NATIONAL TECHNICAL UNIVERSITY OF ATHENS | NTUA | Greece |
| 2 | TWI LIMITED | TWI LIMITED | United Kingdom |
| 3 | INEGI - INSTITUTO DE CIENCIA E INOVACAO EM ENGENHARIA MECANICA E ENGENHARIA INDUSTRIAL | INEGI | Portugal |
| 4 | THE UNIVERSITY OF BIRMINGHAM | UoB | United Kingdom |
| 5 | THALES SA | TRT | France |
| 6 | INSTITUTO TECNOLÓGICO DE ARAGÓN | ITA | Spain |
| 7 | GLOBALSAFEGUARD LTD | GSG | United Kingdom |
| 8 | ANTHONY, PATRICK & MURTA EXPORTAÇÃO LDA | AP&M | Portugal |
| 9 | NCC OPERATIONS LTD | NCC | United Kingdom |
| 10 | OPEN SOURCE MANAGEMENT LTD | OSM | United Kingdom |
| 11 | INNOVATION IN RESEARCH & ENGINEERING SOLUTIONS | IRES | Belgium |
| 12 | POLITECNICO DI TORINO | POLITO | Italy |
| 13 | SWEREA SICOMP AB | SICOMP | Sweden |
| 14 | AERNNOVA ENGINEERING DIVISION S.A.U. | AERN | Spain |
| 15 | FRENI BREMBO SPA | BREMBO | Italy |
| 16 | YUZHNOYE DESIGN OFFICE NAMED AFTER MIKHAIL YANGEL | YUZ | Ukraine |
| 17 | EUROMOBILITA S.R.O. | EUMO | Czech Republic |

# Contents



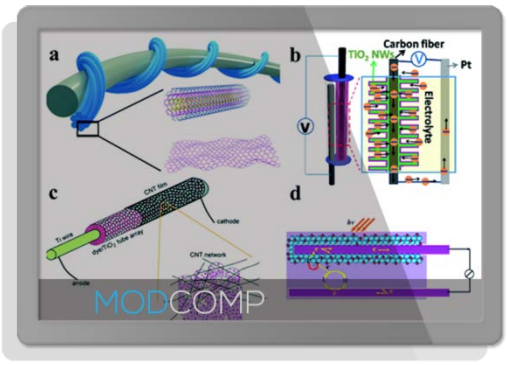
## 1 Summary

Project Duration: 48 months

Project Funding: 7,980,036.25 €

Current technological demands are increasingly stretching the properties of traditional materials to expand their applications to more severe or extreme conditions, whilst simultaneously seeking cost-effective production processes and final products. The aim of this project is to demonstrate the influence of different surface enhancing and modification techniques on carbon fibre (CF)-based materials for high value and high performance applications. These materials are a route to further exploiting advanced materials, using enabling technologies for additional functionalities, without compromising structural integrity. CF based materials have particular advantages due to their lightweight, good mechanical, electrical and thermal properties. Current generation CFs have extensively been used in a multitude of applications, taking advantage of their valuable properties to provide solutions in complex problems of materials science and technology. The limits

of the current capability of such materials, however, have now been reached.




**MODCOMP develops the next generation of CF-based materials for structural and electronics applications.**

## 2 Background

MODCOMP aims to develop novel engineered fibre-based materials for technical, high value, high performance products for non-clothing applications at realistic cost, with improved functionality and safety. Demonstrators will be designed to fulfill scalability towards industrial needs and focus on TRL5/TRL6. End users from a wide range of industrial sectors (transport, construction, leisure and electronics) will adapt the knowledge gained from the project and test the innovative high added value demonstrators. An in-depth and broad analysis of material development, coupled with dedicated multi-scale modelling, recycling and safety studies will be conducted in parallel for two types of materials (concepts):

- ✓ CF-based structures with increased functionality (enhanced mechanical, electrical and thermal properties).
- ✓ Carbon nanofibre (CNF)-based structures for new flexible electronics applications.

Meanwhile, standardization, up-scaling, cost-effectiveness and production of reference materials will also be considered.

## 3 Scientific and technological challenges

**New approaches to improve functionality are important.**

Limited multi-functionality has often prevented composites from being more widely adopted. MODCOMP will provide new approaches to improve functionality in particular electrical and thermal properties as well as structural performance of fibre-based materials, by integrating nanostructures and/or using environmentally-benign surface treatment technologies.

**Fibre based material for high value, high–performance products at reasonable prices with improved functionality and safety, represent a challenge for materials science and engineering.**

Although CFs are much more expensive than glass fibres, they are often used in lightweight, high-strength composites where there are limited alternatives. Their superior mechanical properties combined with multi-functionality make them the primary option. MODCOMP proposal will focus on the desire for fibre-based materials for high value, high performance products by demonstrating the successful development of functionalized fibre-based composites. The reduced cost of CFs will be addressed by optimizing the fraction of fibres present in the composite without a detrimental reduction in performance. This will be achieved by the introduction of small quantities of nano-fillers, which can significantly improve the overall functionality of the final product with a competitive total price.

**Sustainability, recyclability, safety, energy.**

Techniques will be favored which allow environmentally friendly processing, based on reduced process temperatures and less environmentally challenging chemical routes. MODCOMP will promote safe-by-design approaches of carbon-nanostructure integrated fibre-based materials, aiming at control/tailoring of structural integrity, multifunctionality and sustainability at scales through nano- to macro-.

**Cost effectiveness and commercial potential of innovativeness compared to state-of-art.**

Increased mechanical performance by inclusion of nanomaterials will result in the reduction of the number of micro fibres needed in a composite, contributing to a substantial reduction in the manufacturing costs (~20%) and further reduced weight.

**Market estimate.**

The global composite market is predicted to expand in the coming years. MODCOMP will develop demonstrators of fibre-based materials with reduced environmental impact and cost but enhanced processing flexibility in order to provide an alternative material for a wide range of industrial applications. Currently increasing number of space-rocketry structures are being manufactured from CFs.

**Proof of concept.**

The output of the project will be the generation of high performance fibre-based material prototypes that address to the end user requirements, which have demonstrated improved performance compared with currently traditional materials. In order to validate these new multifunctional materials for ease of processing/ production and fabrication of complete products, this project will demonstrate its mechanical stability, fabrication and validation procedures.

## 4 Objectives

Current technological demands are increasingly stretching the properties of traditional materials to expand their applications to more severe or extreme conditions, whilst simultaneously seeking cost-effective production processes and final products. The aim of this project is to demonstrate the influence of different surface enhancing and modification techniques on carbon fibre (CF)-based materials for high value and high performance applications. These materials are a route to further exploiting advanced materials, using enabling technologies for additional functionalities, without compromising structural integrity. CF based materials have particular advantages due to their lightweight, good mechanical, electrical and thermal properties. Current generation CFs have extensively been used in a multitude of applications, taking advantage of their valuable properties to provide solutions in complex problems of materials science and technology. The limits

of the current capability of such materials, however, have now been reached. MODCOMP will develop the next generation of CF-based materials for structural and electronics applications. The benefits of fibre-based materials have clearly been shown in aerospace applications which require lightweight, high strength, high stiffness, and high fatigue-resistant materials.
This vision will be realized through MODCOMP via the following specific objectives.

1. Develop CF-based composites with multi-functionalities (i.e. a combination of enhanced mechanical properties, electrical conductivity, thermal stability, flexibility) by the incorporation of nanomaterials.
2. Develop cost-effective manufacturing processes which consider sustainability and recycling/energy (new (bio) - precursors/ bioresins and life cycle), and safety (safe-by-design and toxicology).
3. Evaluate new configurations in lighter structural composite taking advantage of nanotechnologies to sustain damage from lightning strike for Zone 2A of the Horizontal Tail Plane.
4. Look for the optimum processes and the best multiscale reinforcement combinations using synergistically experimental testing and analytical together with computational modelling techniques.
5. Use the CF-based materials developed from MODCOMP to re-design caliper and steering knuckles for brake systems with reduced weight (>20%), increased stiffness (>20%) and enhanced performance (faster response to driver actions and improved safety).
6. Demonstrate innovative secure storage modules, flat pack shelter system and training sailing craft with reduced cost and high performance (corrosion, UV and abrasion resistance, rigidity and increased thermal properties).
7. Promote the exploitation of industrial-preferred composite materials and standardize activities throughout the production chain.
8. Disseminate outputs for raising the profile of the new MODCOMP technologies.
9. Identify new potential market for the developed fibre-based composite materials in avionics, construction, and electronics industry.
10. Evaluate the industrial impact of MODCOMP-concept with respect to economic as well as technical aspects.
11. Open a new field of innovation based on materials and technologies development and push for the industrial leadership of Europe in strategic domains (electronics, aerospace… ).

## 5 Organisation

MODCOMP is a well-balanced consortium of multidisciplinary experts from 6 universities and research institutes and 11 industries, whose 6 are SMEs, from 10 countries who will contribute to reach the ambitious aim of the project. All partners have been selected in view of their individual expertise and complementarities. Moreover, they have previous successful experience in multilateral collaborative research, and all of them have successfully worked previously in EU-funded research projects. Their resources will be assembled to form a coherent and synergistic project. In addition to the skills and capabilities of each of the MODCOMP partners, each partner also represents a network of contacts, technology and market overview that in some cases is much greater than the consortium itself. An important outcome of MODCOMP will be the consolidation of these networks.


## 6 Progress and Outcomes to date

During the first 12M of MODCOMP project, safety issues and risk assessment regarding the use on nanomaterials in the innovative processes have been taken into account. Questionnaires including safety principles have been distributed by partner IRES to all partners dealing with manufacturing. In parallel, a Safe-by-Design Survey took place, trying to implement all relevant principles for the installation, maintenance and harmonization of the manufacturing procedures. Finally, a first risk assessment has been executed for the widely used carbon (nano-) materials, carbon fibres (CFs) and carbon nanotubes (CNTs), by using the Nano module 1.0 from Stoffenmanager 6 software. This module allows to qualitatively assess occupational health risks from inhalation exposure to Manufactured Nano Objectives (MNO). The results revealed that CFs are of low risk, however special caution is needed when handling CNTs, since they were high ranked in the risk classification.

Stoffenmanager 6®

| Nano component | Hazard class | Time weighted exposure class | Time weighted risk score | Task weighted exposure class | Task weighted risk score |
|---|---|---|---|---|---|
| CF | E | 1 | III | 1 | III |
| CNTs | E | 1 | I | 2 | I |

## 7 Expected Impact

The main breakthrough of MODCOMP project is to contribute to the EU economic growth through the generation of knowledge for the development of novel fibre-based high performance components economically affordable, with improved functionality. Demonstrators

will be designed to fulfill scalability towards industrial needs and focus on TRL5/TRL6. End users from a wide range of industrial sectors (transport, construction, leisure and electronics) will adapt the knowledge gained from the project and test the innovative high added value demonstrators. The developed materials can position Europe in a forefront position in the supply of raw-materials to produce multifunctional composites. These materials are expected to have a great significance in the market, since the properties that are currently obtained with conventionally used resin modification strategies are limited by the available processing technique.

## 8 Directory

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

| First Name | Last Name | Affiliation | Address | e-mail |
|---|---|---|---|---|
| Costas<br>Elias<br>Kate<br>Panagiotis | Charitidis<br>Koumoulos<br>Trompeta<br>Kainourgios | NTUA | Heroon Polytechneiou 9 Str., 15780 Athens - Greece | charitidis@chemeng.ntua.gr<br>elikoum@chemeng.ntua.gr<br>ktrompeta@chemeng.ntua.gr<br>pkainourgios@chemeng.ntua.gr |
| Amit<br>Craig | Rana<br>Jones | TWI | Granta Park, Great Abington, Cambridge CB21 6AL, UK | amit.Rana@twi.co.uk<br>craig.jones@twi.co.uk |
| Nuno<br>Raquel | Rocha<br>Santos | INEGI | Campus da FEUP, Rua Dr. Roberto Frias, 400, 4200-465, Porto | nrocha@inegi.up.pt<br>rmsantos@inegi.up.pt |
| Hanshan<br>Xiaoying | Dong<br>Li | UOB | Edgbaston B15 2TT Birmingham - UK | h.dong.2o@bham.ac.uk<br>x.li.1@bham.ac.uk |
| Paolo | Bondavalli | THALES | Rue de Villiers 45, 92200 Neuilly Sur Seine – France | paolo.bondavalli@thalesgroup.com |
| Agustin | Chiminelli | ITA | María de Luna, nº 7, 50018 Zaragoza, Spain | achiminelli@itainnova.es |
| Glen | Monaghan | GSG | Todburn Moor Farm Cottages 2, NE65 8RX Longhorsley, Northumberland, UK | info@globalsafeguard.com |
| Guy | Simmonds | APM | Sitio Da Falfeira EN 120, 8601 903 Lagos - Portugal | simmonds.guy@gmail.com |
| David<br>Ioannis | Fishpool<br>Papasideris | NCC | Bristol & Bath Science Park, Emersons Green, Bristol, BS16 7FS, UK | david.fishpool@nccuk.com<br>ioannis.papasideris@nccuk.com |
| Emmanouel | Sofianopoulos | OSM | Cowley Road, CB4 0WS Cambridge - UK | emmanuel@osm.eu.com |
| Deppie<br>Athanasios | Brasinika<br>Morozinis | IRES | Boulevard Louis Mettewie 71/50, 1080 Brussels, Belgium | ceo@innovation-res.eu<br>amorozinis@innovation-res.eu |
| Pietro<br>Mauro<br>Matteo<br>Alberto | Asinari<br>Giorcelli<br>Fasano<br>Tagliaferro | POLITO | Corso Duca degli Abruzzi 24, 10129 Torino – Italy | pietro.asinari@polito.it<br>mauro.giorcelli@polito.it<br>matteo.fasano@polito.it<br>alberto.tagliaferro@polito.it |
| Guan | Gong | SICOMP | Box 7047, SE-164 07 KISTA, Sweden | guan.gong@swerea.se |
| Yasser | Essa | AERN | Leonardo da Vinci, nº 13, Parque Tecnologico de Alava, 01510 Miñano (Álava), Spain | yasser.essa@aernnova.com |
| Giorgio<br>Luca | Valota<br>Menapace | BREMBO | CURNO (Bergamo) - Via Brembo, 25 Italy | Giorgio_Valota@brembo.it<br>Luca_Menapace@brembo.it |
| Iryna<br>Yevheniia | Husarova<br>Tishchenko | YUZ | Krivorozhskaya 3, 49008, Dniepropetrovsk - Ukraine | space@yuzhnoye.com<br>tishchenkoyevheniia@gmail.com |
| Ales<br>Petra | Loncaric<br>Gamberger | EUMO | Taborska 922, 29301 Mlada Boleslav – Czech Republic | ales@euromobilita.com<br>Petra@euromobilita.com |

# 9 Copyright

# NanoFARM

## Effect of Nanopesticides in the environment

Contract Agreement: SIINN/0001/2014 Website: https://research.ce.cmu.edu/nanofarm/
Coordinator: Gregory Lowry (glowry@cmu.edu), Carnegie Mellon University

*Table 1 Consortium List.*

| No. | Beneficiary name | Short name | Country |
|---|---|---|---|
| 1 | Carnegie Mellon University | CMU | USA |
| 2 | University of Kentucky | UKy | USA |
| 3 | Universidade de Aveiro | UAVR | PT |
| 4 | University of Vienna | UNIVIE | A |

## Contents



## 1 Summary

Project Duration: 36 months (march 2016-february 2019)

Project Funding: NSF, FCT and BMVIT/FFG

NanoFARM is a research consortium whose mission is to provide information to aid in safe development of effective and sustainable nano-agrochemicals. We're made up of researchers from Carnegie Mellon University, Aveiro University, University of Kentucky, and University of Vienna.

Manufactured nanomaterials (MNMs) hold promise for increasing the sustainability of agriculture. Nanofertilizers and nanopesticides can improve the efficiency of agrochemical use and decrease energy and water requirements for food production. However, nano-enabled agrochemical formulations need to be developed safely. This requires a fundamental understanding of the factors influencing the fate and effects of nano-enabled agrochemicals.

NanoFARM research will determine how agricultural MNM properties and applied concentration affect their:

- Persistence in the environment;
- Bioaccumulation by tomato and wheat plants and trophic transfer to terrestrial organisms;
- Toxicity and multigenerational effects on soil organisms and potential for ecological impacts;
- Analytical methods will be developed to track and characterize MNMs in soil at realistic concentrations, and
- Test guidelines will be developed for assessing the bioavailability of nano-enabled agrochemicals using simple protocols and standardized reporting formats.

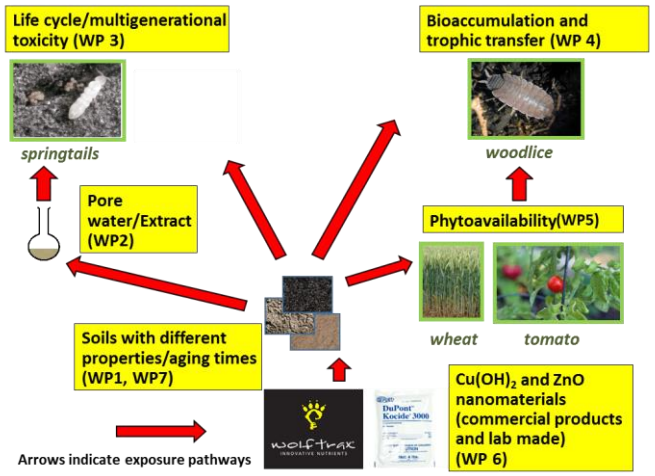
## 2 Background

The proposed study is aligned with Topic 4 "Environmental Impacts of MNMs" and Topic 1 "Exposure Assessment". It addresses multiple aspects of each of those topical areas. In particular, we address long-term exposures and bioaccumulation of MNMs in biotic and abiotic compartments of agricultural soil systems. We focus on use "hot spots" and both the use phase and "end-of-life" aspects of MNMs in agricultural chemicals since the "end of life" for these products is aging in those soils. Importantly, we track changes in the product over time and assess impacts of concentration on fate and effects. We develop and test methods to track MNMs in soils, and to distinguish them (and metal ions derived from them) from natural background. We identify the factors that most impact distribution in soil, bioavailability, plant uptake, and toxicity into appropriate environmental species directly from soils, and for real products. This allows us to make links between data collected in well-controlled laboratory systems, to more complex environment while maintaining sufficient control over experimental parameters to determine how MNM properties and product formulations impact their fate and effects. We

develop methods to detect Cu- and Zn-based MNMs in soils and soil pore waters, using methods that preserve the native speciation of the materials. The knowledge gained here will be used to prevent adverse multigenerational impacts during long-term exposure from use of MNM-based fertilizers and pesticides through better product designs that can improve efficacy and decrease unwanted environmental effects. Finally, we develop a robust assay for the prediction of bioavailability and toxicity of Cu- and Zn-based MNMs in agrochemicals. The proposed work also addresses Topic 3 "Effects of MNMs on human health" by assessing the factors the result in uptake of MNMs into plant tissues, the speciation of the MNMs in those tissues, and therefore the potential for human exposure to MNMs through the food supply. We address elements of Topic 2 "toxicity mechanisms" through genomic testing and assessment of multigenerational long-term effects using "realistic" MNMs in real exposure scenarios.

## 3 Scientific and technological challenges

While development of nano-enabled agricultural chemicals is proceeding apace, properties of these manufactured nanomaterials (MNMs) in the environment make predicting their fate in soils impossible using the risk assessment framework for traditional chemicals. This project will modify the existing fate modelling paradigms by systematically determining how agricultural MNM properties, applied concentration, spatial distribution and temporal behaviour in different soil types, influence MNM uptake by important crop plants, toxicity, multigenerational effects on soil organisms, bioaccumulation/trophic transfer, and potential for ecological impacts. We will also develop necessary methods to track and characterize MNMs in soil at realistic concentrations, and develop a simple test for measuring dissolution rate and distribution between soil pore water and solids to predict the observed fate and effects, key assays for validating theoretical relationships developed herein.




## 4 Objectives

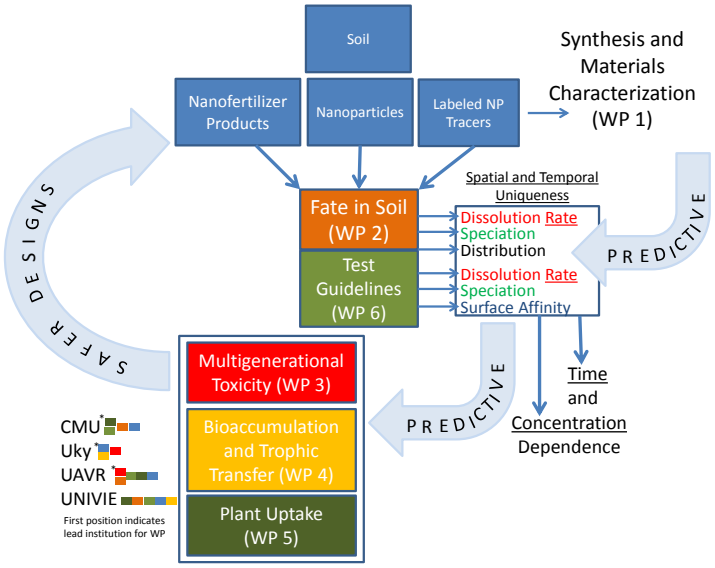
The goal of this project is to address essential gaps in knowledge about how soil properties, MNM properties, MNM concentration, and reaction kinetics affect the spatial and temporal behaviour of metal-oxide MNM-enabled pesticides and fertilizers in soils. Specifically, we will assess how these variables influence 1) transformation and distribution in soils (WP 2), toxicity and multigenerational effects on soil organisms (WP 3), bioaccumulation/trophic transfer (WP 4), phytoavailability (WP 5), and therefore the potential for ecological impacts or human exposures from consumption of key food crops (Tomato and Wheat). This project will provide guidance on modifying existing OECD assays for chemical fate in soils to measure MNM dissolution rate and distribution in soils to predict the observed fate and effects (WP 6) and develop novel methods to track and characterize MNMs in soils at realistic concentrations (WP 7).

## 5 Organisation

Integrated research tasks are used to determine the MNM properties that most affect their fate and effects in agriculture soils. Coordinated studies will use well-characterized soils and nanoparticles, along with soil extraction procedures to provide consistent and comparable datasets. The developed extraction procedures will serve as the basis for OECD test guidelines for MNMs used as agrochemicals.

Several work packages will run in an integrated way and shown below. This integration is provided within WP7- Characterization of NMNs in complex matrix (soil, soil pore waters, tissues) which is not shown in the diagram. This work package led by UNIVIE is integral to ALL work packages as it provides analytical support of the experiments, especially at low MNM concentration. The block in the lower left of the figure shows the lead and participating institutions for each work package.

The NanoFARM project addresses essential gaps in knowledge about how the soil properties, MNM properties, applied concentration, and aging time, affect the spatial and temporal behaviour of Cu- and Zn-based oxide/hydroxide-based pesticides and fertilizers in soils, and how the spatial and temporal behaviour affects toxicity, bioavailability and trophic transfer to selected plants and soil organisms. Specifically, we assess how MNM properties, product formulation, and soil conditions influence 1) transformation and distribution in soils (WP 2), toxicity and multigenerational effects on soil organisms (WP 3), bioaccumulation and trophic transfer (WP 4), and uptake by plants (WP 5), and therefore the potential for ecological impacts or human exposures due to consumption of food from key food crops (tomato and wheat). In addressing these gaps, we will modify existing OECD assays for chemical fate in soils (WP 6) to measure dissolution rate and distribution between pore water and soils to predict the observed fate and effects measured in WPs 2, 3, 4, and 5, and develop novel methods to track and characterize MNMs in soils at realistic concentrations (WP 7).




**WP 1. Synthesis and material and soil characterization**. The goal of WP 1 is to ensure that all consortia members will have fully characterized MNMs, MNM-enhanced products, and soils. Deliverables include fully characterized starting materials and all characterization data, characteristics of soils made available to all WPs, and delivery of labelled material to all WPs.

**WP 2 Assessment of reactivity and chemical availability of MNMs in agricultural soil.** The goal of WP 2 is to determine the effects of MNM properties and soil properties on the fate and spatial distribution of MNMs in soils and pore. Results will be used to determine mechanisms of MNM toxicity and bioavailability. Deliverables include primary data, and peer reviewed publications on chemical availability of MNMs in soils, how soils properties impact this availability, and factors influencing attachment of each MNM to soils.

**WP 3 Toxicity and mutigenerational effects of MNMs soils containing MNMs and MNM-enhanced products.** The goal of this WP is to assess the toxicity of Cu- and Zn-based MNMs to *Folsomia candida*, *Porcellionides pruinosus* and *Caenorhabditis elegans* and multigenerational effects (*F. candida* and *C. elegans*). This WP will provide the toxicity data and foundation for the bioaccumulation studies (WP4). Deliverables include primary data on toxicity, and peer-reviewed publications on the toxicity of the MNMs, impact of the product matrix on toxicity, and multigenerational effects due to prolonged exposures.

**WP 4 Bioaccumulation and trophic transfer of MNMs in invertebrates.** The goal of this WP is to develop a valid approach to assess the bioaccumulation potential of MNMs in soils by plants and invertebrates. The data will be used to validate the test protocol to measure distribution of MNMs between soil and pore water and the dissolution rate. Deliverables include the data on bioaccumulation to other WPs, and peer reviewed scientific papers on bioaccumulation of MNMs from soils in *P. pruinosus*, biodynamic modelling of trophic transfer *P. pruinosus* and *M. sexta,* and on biodistribution of MNMs and metals in tissues of these organisms.

**WP 5 Key properties of soil environment and MNM which determine phytoavailability.** The goal of this WP is to determine how concentration, and the properties of the MNM, soil, and product matrix impact the phytoavailability of MNMs. Deliverables include data on the tissue concentrations of MNMs and metals in different plant components, and peer-reviewed publications on uptake of MNMs in to tomato and wheat plants from soil exposures, uptake of MNMs from MNM-enabled products applied to soils, and uptake of MNMs in plants via foliar applications.

**WP 6. Development of a testing guideline for predicting bioavailability and toxicity**.The goal of this WP is to assess the ability of simple assays with LUFA (model) soils to predict MNM fate(WP2), toxicity (WP 3), bioaccumulation (WP4), and phytoavailability (WP 5) of MNMs. Deliverables include four peer-reviewed publications on methods and guidance to the OECD about test guideline for predicting fate and effects of MNMs in soils.

**WP 7. Characterization of NMNs in complex matrix (soil, soil pore waters, tissues).** The goal of this WP is to develop the necessary analytical tools for the characterization and tracing of Cu- and Zn-based NMNs in complex samples as soils and tissues at realistic environmental concentrations. Deliverables include new methods for characterizing MNMs in soils and other relevant biological matrices (e.g. plant tissues), methods to quantify Cu & Zn MNMs in model soils and tissues with SP-ICPMS and FFF-ICPMS, and data analysis for selected samples from other WPs.

## 6 Expected Impact

The project outcomes are highly aligned with the goals of the 3rd SIINN Call. In particular, the proposed study will provide general principles that affect bioavailability and toxicity of MNMs in soils, expressed in mathematical terms. We assess the effects of concentration (dose) and MNM properties on fate and toxicity, which are relevant to a broad range of systems. We assess the behaviour of "real" MNMs within their product formulation and at realistic applied doses to soils. We provide guidance for OECD tests for assessing MNM dissolution rate and distribution between pore water and soil, and to track MNMs in soils will find high utility for other materials and systems. The proposed framework will assess the ability to use extrinsic environmental properties in soil to predict behaviors of MNMs in soils. Moreover, it will relate these properties back to the intrinsic properties of the MNMs (e.g. size and crystal structure) to provide information for better selection of MNM properties for products. Finally, we will determine if MNMs made from soft metal cations can be considered as a "group" to classify MNMs in terms of their environmental fate in soils, and determine methods to efficiently analyse them in soils and other matrices, even at high bulk soil background concentrations by separation and single particle ICP-MS.

# 7 Directory

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

| First Name | Last Name | Affiliation | Address | e-mail |
|---|---|---|---|---|
| Gregory | Lowry | CMU | USA | glowry@cmu.edu |
| Xiaoyu | Gao | CMU | USA | xiaoyug@andrew.cmu.edu |
| Eleanor | Spielman-Sun | CMU | USA | espielma@andrew.cmu.edu |
| Jason | Unrine | UKy | USA | jason.unrine@uky.edu |
| Olga | Tsyuskso | UKy | USA | olga.tsyusko@uky.edu |
| Jieran | Li | UKy | USA | jli295@uky.edu |
| Anye | Wamucho | UKy | USA | |
| Susana | Loureiro | UAVR | PT | sloureiro@ua.pt |
| Rui | Morgado | UAVR | PT | ruimorgado@ua.pt |
| Maria | Pavlaki | UAVR | PT | maria.pavlaki@ua.pt |
| Joana | Neves | UAVR | PT | jtneves@ua.pt |
| Sónia | Rodrigues | UAVR | PT | smorais@ua.pt |
| Sónia | Lopes | UAVR | PT | sonialopes@ua.pt |
| Nuno | Cruz | UAVR | PT | |
| Frank | von der Kammer | UNIVIE | A | frank.von.der.kammer@univie.ac.at |
| Melanie | Kah | UNIVIE | A | melanie.kah@univie.ac.at |
| Adam | Laycock | UNIVIE | A | adam.laycock@univie.ac.at |

# 8 Copyright

# NECOMADA

**N**ano-**E**nabled **C**onducting **M**aterials **A**ccelerating **D**evice **A**pplicability.

Supporting high speed roll-2-roll integration of hybrid and large area electronics

Contract Agreement: 720897 Website: http://www.necomada.eu

Coordinator: Neville Slack (Neville.slack@uk-cpi.com), CPI, NetPark, Sedgefield, UK

*Table 1 Consortium List.*

| No. | Beneficiary name | Short name | Country |
|---|---|---|---|
| 1 | Centre for Process Innovation (Formulation & Printable Electronics) | CPI | UK |
| 2 | Henkel Electronic Materials | HEM | BE |
| 3 | PragmatIC Printing Limited | PragmatIC | UK |
| 4 | Teknologisk Institut | DTI | DK |
| 5 | Contitech Elastomer Beschichtungen GmbH | ContiTech | DE |
| 6 | Nanogap Sub nm Powder | Nanogap | ES |
| 7 | Thomas Swan & Co Limited | Swan | UK |
| 8 | BSH Electrodomesticos | BSH | ES |
| 9 | Henkel KGaA | Henkel | DE |
| 10 | Crown Packaging Manufacturing UK Limited | Crown MP | UK |
| 11 | Fraunhofer Gesellschaft Zur Foerderung Der Angewandten Forschung | FhG | DE |
| 12 | Tyoeterveyslaitos | FIOH | FI |
| 13 | NXP Semiconductors | NXP | BE |

## Contents



## 1 Summary

Project Duration: 36 Months (1-01-2017 to 31-12-2019)

Project Funding: 8,101,378.75€ (6,820,289€ EC H2020).

The NECOMADA collaboration addresses key material challenges inherent in realising of the "Internet of Things" (IoT): which is forecast to deliver a potential economic value of €4.3-€12.2tn per year by 2025. The integration of electronics into everyday items and the environment in which we live and work will require a new series of value chains and effect a paradigm shift in both Business to Business (B2B) and Business to Consumer (B2C) relationships. At a price point of €1-2¢ per integrated solution: IDTechEx forecast a €16.58bn market by 2026. As such this emerging technology area provides a very real opportunity for the European Economic Area (EEA) to regain its historic global manufacturing leadership through the effective integration of new materials, production tools and user interfaces. NECOMADA will establish new manufacturing platforms where cost, productivity and capacity are effectively mapped to specific application performance requirements. It is recognised that a significant proportion of the primary innovators delivering key enabling technologies in this space are SMEs; many of these are pre-revenue start-ups and university spinouts. As such, the use of European pilot line resources to help establish the industrial eco-systems, R&D investment and new supply chains to support market entry in the planned 5-7 year timeframe is essential.

The ambition of NECOMADA is two-fold. Firstly the intention is to develop materials and methods that are beyond state of the art, which will enable the manufacture of very low cost RFID inlays to be realised opening up the market and possibilities for the Internet of Things (IoT). The second ambition is to deliver an open access pilot line that will be able to develop, evaluate and produce nanoparticles, inks and adhesive materials and devices beyond the project. This legacy will enable other manufacturers from around Europe to develop new materials and processes to further drive down costs and enable new applications to be realised.

**The project will target the incorporation of advanced functional materials to deliver customised conductive inks and flexible adhesives compatible with high volume manufacturing platforms. Specifically the development of these enabling** materials will support high speed roll to roll integration of hybrid and large area electronics to address internet of things opportunities.

The consortium will integrate materials development with end application requirements in terms of technical performance (thermal/electrical conductivity, processing conditions, materials integrity and adhesion) and unit cost of production to facilitate market adoption. The project will utilise and build on existing CPI pilot facilities (R2R print line) to demonstrate technology integration, manufacturability and produce components for end user evaluation to enable the direct comparison of production techniques. It will deliver a supply chain to support future commercialisation: incorporating materials suppliers of inks and adhesives, supporting RTO in Formulation and nano-particle production, established high fidelity print equipment manufacturers, electronic device manufacturers, established pilot line facilities and potential end users from the apparel, packaging and healthcare sector – relating to the internet of things (Fig 1).

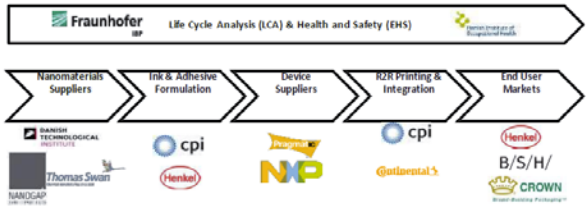



Fig 1 – Necomada Pilot line Supply Chain

## 2 Background

**Nanomaterials**

The global nanomaterials market was valued at $3.4bn in 2014 and is expected to reach $11.8bn by 2020 showing a CAGR of 23.1%. Specifically IDTechEx estimates the global market for conductive nanosilver inks to grow to $80M by 2026. Whilst the nanosilver ink market is still in its infancy, NanoGap is a leading player, engaged with most ink formulators, and has an estimated 10% current market share. Today NanoGap's customers and collaborators have around 50% of this current market sub-sector. For nanowires, Cambrios and Innova Dynamics recently went out of business, leaving Seashell and C3 nano as the main competitors. For nanoparticles supply is more fragmented. Ferro is a large player, but produces more micro scale than nano-enabled products. Some companies manufacture both particles and inks (eg. Agfa-Gevaert Corporation), but it is expected that many would formulate with third party materials if the right product was available at the right price. IDTechEx forecasts the cost of silver nanoparticles to decrease by 60% between 2010 and 2022 in line with market development. Similarly IDTechEx believes that the cost of copper nanoparticles will decrease by 75% over the period 2012-18.

With respect to carbon-based products: the graphene market is embryonic. Most inks today are metallic (e.g., silver) and command a high cost. This means that graphene can deliver value when compared with other printed antennas. Its main limitation is high sheet resistance, which limits reading range. IDTechEx anticipate a market for graphene enabled tags of 4% of 37.76bn with a market value of ≥$6.1M by 2018. They forecast the overall graphene market growth to $390M by 2024. Independent assessment by BCC Research forecasted the first commercially significant sales of graphene products in 2015 growing to $675M by 2020, reflecting a 58.7% CAGR between 2015 and 2020. Thomas Swan forecasts that their market share for a range of graphene and graphene enabled products will reach €5-10M by 2020.

The NECOMADA project will enable Swan to demonstrate its carbon based and 2D materials (single and multi-walled carbon nanotubes, & graphene) in value added applications and to connect with consumer markets via collaboration with the multi-national end-users in the project. Swan has an established position in high specification single wall nanotubes and has the ability to manufacture graphene products
with high electrical & thermal conductivity. Carbon nanotubes enable a paradigm shift in conductive ink technology because of their high conductivity and nanoscale size. This combination enables the production of new inks that create the needed conductive paths at much smaller feature sizes than traditionally possible. Another mid- to long-term application of SWCNTs is to enhance copper based ultra-conductive compounds. A study by the International Copper Association (ICA) in 2009 found that 0.1%wt carbon nanotubes could enhance the ambient temperature electrical conductivity of copper by 30%.

**Conductive Inks & Adhesives**

The market for conductive inks was estimated to be worth $2.86bn in 2012 and the overall flexible circuit market $10.8bn. This market is forecasted to rise to $3.36bn in 2018. Currently silver flakes inks (several microns in size) serve the largest and most mature markets for numerous applications. A recent report estimates nanomaterials based inks (silver and copper) will be worth $679M and $56M respectively by 2018 (or approximately ~22% of the total conductive inks market share). A more recent report from IDTechEx has re-estimated the global market for conductive nanosilver inks as growing to $80M by 2026. This reflects the significant decrease in market growth achieved for PV in the 2012-16 period. Customers require easy access to low cost, reliable and reproducible conductive inks and printing technologies crucial for the European printing and electronics manufacturing industries to compete effectively on quality and specialisation. The demand for new electronic platforms is increasing rapidly and customising form factors and substrates is important (especially flexible ones for consumer packaging, wearable electronics and sensors). Necomada will allow Henkel Belgium to develop and

commercialise nano based materials for antenna circuitry & IC attach applications. These materials will be suitable for the high speed manufacture of flexible devices designed to meet the cost targets and production speed of the growing NFC based applications.

**IC Devices**

In 2012, IDTechEx estimated that 2.88bn tags were produced and sold with prices varying from $0.06 to $30, depending on application requirements. In typical tags the costs structure is: antenna 0.1-8¢, IC 3-30¢; substrate 0.1-2¢ and conversion 1-5¢. RFID tag sales were predicted to grow from ~5bn to 37bn units between 2012 and 2018 (with the proportion of active tags remaining less than 1% of the market). Customers require extensive discussions with prospective customers have identified a wide range of market opportunities that would benefit from the unique form factor and/or cost advantage of the PragmatIC FlexIC versus conventional Silicon ICs. NECOMADA targets a dramatic simplification of the integration process, both increasing throughput and significantly reducing cost. Together these two innovations can enable a huge disruption within the existing RFID market, in addition to developing many new applications and supporting the development of the IoT. The majority of these applications are highly price sensitive consequently this new capability will support a substantial growth in volumes. Examples of such potential market sectors include beverages, FCMG and apparel.

**Electronic Packaging**

Opportunities for the introduction of electronics into the packaging market are stated where ever possible in terms of specific market surveys covering the sectors, e.g. Metal Can Coatings, Fast Moving Consumer Goods (FMCG) and Domestic Appliances. Where specific data are not available for electronic packaging the market opportunity is extrapolated from published analysis of the overall sectors.

The global metal packaging market is projected to reach $135.69bn by 2020, with a CAGR of 3.0% from 2015 to 2020. This is because continued strong growth of end-use industries in developing countries including India, China, and Brazil is expected. (China and India, together with Brazil have changed their buying patterns by purchasing more packaged food to preserve food and reduce food waste.) The North American region accounted for around 34.4% of the total market share in terms of value, followed by the European and Asia-Pacific regions.

The global FMCG market: IDTechEx estimates that the Global market for electronic smart packaging based on EAS and RFID will increase from $75M in 2014 to $1480M in 2024. IoT products and services have been estimated to be worth €3bn by 2020. It is envisaged that 99% of physical objects will eventually become part of this connected network. Within the Smart Packaging environment, today RFID/NFC is almost exclusively used for Electronic Article Surveillance. In the future exponential growth is forecast for RFID/NFC at the primary packaging/ item level. In order for this to be achieved, the industry has to be able to supply low cost tags at high production volumes.

Global Domestic Appliances market: BSH claimed €12.6bn share of the Global Domestic Appliances market in 2015. The global household appliances industry is expected to reach an estimated value of $324.2bn by 2019 and 1.5bn units by 2020. Based upon an estimated market share of 12.5%, this equates to a direct potential demand for 190M NFC tags per annum by BSH group. Today Asia pacific represents the largest market worldwide with the region registering 6.3% CAGR in the period 2013-20. During the last years the Home Appliances field has evolved considerably. BSH is the market leader in Europe and number two in the world, new companies from emerging countries are arising based on low cost production. In order for BSH to maintain or increase its competitiveness, it needs to be flexible, innovatory and deliver cost reduction.

# 3 Scientific and technological challenges

- The materials challenges to overcome in enabling the low cost, high volume manufacturing process required for the ubiquitous adoption of M2M devices.
- Need for conductive adhesives that can bind a wide range of components, from bare die and packaged IC, to printed electronic components such as FlexIC together with flexible substrates in Roll 2 Roll processes.
- The need for the adhesives to meet volume and pricing demanded by the market.
- The need for new, lower cost conductive inks used to print the electronic circuits and antennae.

# 4 Objectives

The overall NECOMADA objective is the delivery of conductive inks and adhesives compatible with Roll to Roll (R2R) application on flexible substrates via a pilot line featuring high speed printing and/or pick and place conversion. Such printed flexible electronics will e nable the manufacture of inter alia devices for machine to machine communication devices such as Near Field Communications (NFC) and Radio-Frequency Identification (RFID).

The project targets substantial reduction in device costs compared with the current state of the art (SoA). This will be achieved through the combination of formulated structured nanomaterials (conductive inks/adhesives) together with R2R printing, coating and component pick and place to demonstrate at pilot scale the potential for low cost, robust, high volume device manufacture.

| # | Objective | Measure | Impact |
|---|---|---|---|
| 1 | Develop nano-enabled materials (structured conductive inks) to meet physical demands of high speed R2R processing & forward conversion | No mechanical failure during simulated R2R processing | Adequate R2R processability |
| 2 | Develop nano-enabled materials (structured conductive adhesives) to meet the physical demands of R2R processing & forward conversion. | R2R- elongation to break, adhesion, mechanical strength, modulus, thermal stability, processing conditions | Adequate materials performance in application |
| 3 | Develop nano-enabled structured conductive inks (RFID antenna) to meet electrical conductivity specification. | Electrical conductivity of $10^{-2}\Omega$cm, providing low resistance at dry thicknesses between 0.2-2µm | Adequate materials performance in application. |
| 4 | Develop nano-enabled structured conductive adhesives to meet an electrical conductivity specification | Volume resistivity $<5x10^{-3}\Omega$cm & cure-speed<1sec | Adequate materials performance in application |
| 5 | Demonstrate this manufacturing approach across a minimum of three devices applicable to a variety of market requirements. | Performance and cost | Market acceptability and cost performance |
| 6 | Validate capability to volume manufacture radio frequency (RF) antenna & integrated NFC tags | Unit cost: RF antenna at ≤€0.5¢ NFC tags <€2¢ (FlexIC unit <€0.5¢) | Market cost point acceptability |
| 7 | Establish pilot production capability through integration of existing R2R coating on flexible substrates with high speed precision pick & place | line speed 20m/min and 330units/min | Cost reduction |
| 8 | Undertake techno-commercial assessment (device performance/cost, manufacturability, life cycle management, business/supply chain models) of validity of the manufacturing approach across market sectors (FMCG, Domestic Appliances and metal coatings). | Performance, cost, manufacturability, regulatory acceptability, market routes | Overall market acceptability & customer engagement |

The capture of future value within the European Economic Area is a key consideration and hence the NECOMADA consortium constitutes the basis of a robust, pan-European future supply chain

– aligning enabled materials development and supply with infrastructure providers, ICT integrators and end markets.

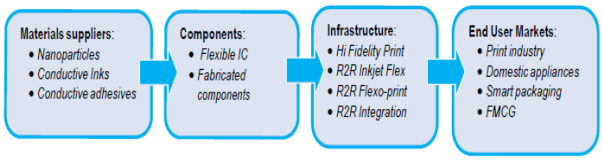



Fig 3 – NECOMADA value chain

## 5 Organisation

To deliver the NECOMADA vision, this 13 partner consortium brings together innovative SME's, technology providers, integrators, SoA pilot lines and prototyping facilities with market interfaces across a range of applications. This 36 month project, coo-ordinated by CPI-Formulation is structured as a series of 12 interdependent Work Packages (WP1-12) as illustrated by the PERT diagram below (Fig 4).

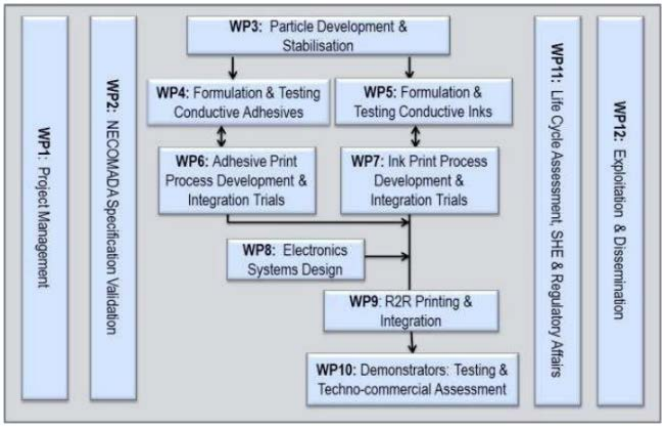



Fig 4 – PERT of NECOMADA project

The work programme can be sub-divided into four sections of work:-

(i) Nanomaterials, formulation and properties (WPs 3-5)
(ii) Print deposition and process optimisation (WPs 6 & 7)
(iii) Device design, integration and assembly (WPs 8-9)
(iv) Demonstrators evaluation and techno-economic assessment (WP10)

These are supported by the Project Management (WP1), Specification Validation (WP2), Life Cycle Assessment, SHE &Regulatory Affairs (WP11), and Exploitation & Dissemination (WP12) activities which span the project duration.

## 6 Expected Impact

- Advanced functional materials, in particular conductive inks and adhesives, are forecasted to be key enablers of the "Internet- of-Things" (IoT) via printable electronics, thereby embedding electronic intelligence within physical objects.
- The value of embedded intelligence is today >€300bn2, including electronics in cars >60M, computing/mobile devices 2.3bn and microcontrollers 15bn. Penetration into areas such as clothing (>80bn items) and disposables (5-10tn items) is expected to open-up a €100bn opportunity in the next 5-10 years.
- A key enabler in these types of applications is the use of Radio Frequency (RF) functionality, in particular for Near Field Communication (NFC). This is driven by the growing prevalence of NFC in smart phones and retail/security infrastructure, as well as the benefits of using NFC for both power & communication. NFC technology may be delivered via printed electronics with novel functionality and form factor. Extension of the NFC technology at a lower cost base will provide Radio-Frequency Identification (RFID) for supply-chain management & secure track-and-trace.
- The latter is a requirement for major retailers, consumer-goods companies and government institutions. Visiongain20 estimates the overall smart packaging market [Quick Response (QR) coding & RFID] at >€4bn in 2013. IDTechEx21 forecasts growth in electronic packaging from €75M in 2013 to >€1.45bn by 2024, representing >14bn units.
- This market is highly price sensitive, hence the reduction in the average cost of electronics from €5¢ to <€1¢ envisaged within the NECOMADA Project is expected to accelerate growth.
- The advanced functional materials needed within NECOMADA are conductive inks/adhesives for print/application to flexible substrates. Such structures permit the adoption of automated R2R printing and assembly processes to the manufacture of ultra-low cost devices and hence the delivery of the IoT.

## 7 Directory

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

| First Name | Last Name | Affiliation | Address | e-mail |
|---|---|---|---|---|
| Neville | Slack | CPI - Formulation | UK | Neville.slack@uk-cpi.com |
| Lois | Hobson | CPI - Formulation | UK | Lois.hobson@uk-cpi.com |
| Colin | Graves | CPI - Formulation | UK | Colin.graves@uk-cpi.com |
| Alex | Borak | CPI - Formulation | UK | Alex.borak@uk-cpi.com |
| David | Johnson | CPI - Formulation | UK | David.johnson@uk-cpi.com |

| First Name | Last Name | Affiliation | Address | e-mail |
|---|---|---|---|---|
| Mike | Clausen | CPI – Printable Electronics | UK | Mike.clausen@uk-cpi.com |
| Dave | Barwick | CPI – Printable Electronics | UK | Dave.barwick@uk-cpi.com |
| Nicola | Broughton | CPI – Printable Electronics | UK | Nicola.broughton@uk-cpi.com |
| Cindy | Doumen | HEM | BE | Cindy.doumen@henkel.com |
| Inge | Van der Meulen | HEM | BE | Inge.vandermeulen@henkel.com |
| Richard | Price | Pragmatic | UK | richard@pragmaticprinting.com |
| Darshana | Kariyapperuma | Pragmatic | UK | dash@pragmaticprinting.com |
| Brian | Cobb | Pragmatic | UK | bcobb@pragmaticprinting.com |
| Catherine | Ramsdale | Pragmatic | UK | cramsdale@pragmaticprinting.com |
| Zachary | J Davis | DTI | DK | zjd@teknologisk.dk |
| Martin | Sondergard | DTI | DK | maso@teknologisk.dk |
| Bjorn | Heise | ContiTech | DE | Bjoern.heise@ec.contitech.de |
| Armin | Senne | ContiTech | DE | Armin.senne@contitech.de |
| Allen | Reid | NanoGap | ES | a.reid@nanogap.es |
| Noelia | Duran | NanoGap | ES | n.duran@nanogap.es |
| Paul | Ladislaus | Thomas Swan | UK | pladislaus@thomas-swan.co.uk |
| Dimitris | Presvytis | Thomas Swan | UK | dpresvytis@thomas-swan.co.uk |
| Sergio | Alonso | BSH | ES | Sergio.alonso-ext@bshg.com |
| Jorge | Alaman | BSH | ES | Jorge.alaman@bshg.com |
| Olaf | Lammerschop | Henkel KGaA | DE | Olaf.lammerschop@henkel.com |
| Eileen | Rasslenberg | Henkel KGaA | DE | Eileen.rasslenberg@henkel.com |
| Cormac | Neeson | Crown Packaging Manufacturing | UK | Cormac.neeson@eur.crowncork.com |
| Stefan | Albrecht | Fraunhofer FhG | DE | Stefan.albrecht@ibp.fraunhofer.de |
| Jan Paul | Lindner | Fraunhofer FhG | DE | Jan.paul.lindner@ibp.fraunhofer.de |
| Hannes | Kreig | Fraunhofer FhG | DE | Hannes.kreig@ibp.fraunhofer.de |
| Helene | Stockmann-Juvala | FIOH | FI | Helene.stockmann-juvala@ttl.fi |
| Anna-Kaisa | Viitanen | FIOH | FI | Anna-kaisa.viitanen@ttl.fi |
| Tomi | Kanerva | FIOH | FI | Tomi.kanerva@ttl.fi |
| Radu | Surdeanu | NXP | BE | Radu.surdeanu@nxp.com |
| Axel | Nackaerts | NXP | BE | Axel.nackaerts@nxp.com |

# 8 Copyright

# npSCOPE

## The nanoparticle-scope : a new integrated instrument for accurate and reproducible physicochemical characterisation of nanoparticles

Contract Agreement: 720964 Website: http://www.npscope.eu/
Coordinator: Tom Wirtz (tom.wirtz@list.lu), LIST, L-4362 Esch-sur-Alzette, Luxembourg

*Table 1 Consortium List.*

| No. | Beneficiary name | Short name | Country |
|---|---|---|---|
| 1 | LUXEMBOURG INSTITUTE OF SCIENCE AND TECHNOLOGY | LIST | LU |
| 2 | CARL ZEISS MICROSCOPY GMBH | ZEISS | DE |
| 3 | HELMHOLTZ-ZENTRUM DRESDEN-ROSSENDORF EV | HZDR | DE |
| 4 | PHOTONIS NETHERLANDS BV | PHOTONIS | NL |
| 5 | EIDGENOESSISCHE TECHNISCHE HOCHSCHULE ZUERICH | ETHZ | CH |
| 6 | FRIEDRICH-ALEXANDER-UNIVERSITAET ERLANGEN NUERNBERG | FAU | DE |
| 7 | VLAAMSE INSTELLING VOOR TECHNOLOGISCH ONDERZOEK N.V. | VITO | BE |
| 8 | MJR PHARMJET GMBH | MRJ | DE |
| 9 | INSTITUT NATIONAL DE LA RECHERCHE AGRONOMIQUE | TOXALIM | FR |

## Contents



## 1 Summary

Project Duration: 48 Months (2017-01-01 to 2020-12-31)

Project Funding: 6.661.600 €

The npSCOPE project aims at developing a new integrated instrument (the nanoparticle-scope) optimised for providing a complete physico-chemical characterisation of nanoparticles both in their pristine form or embedded in complex matrices such as biological tissues. Using sophisticated correlative data processing methodologies and algorithms based on statistical methods in conjunction with appropriate visualisation methods of the results, the npSCOPE instrument will allow rapid, accurate and reproducible measurements.

The instrument will be based on the Gas Field Ion Source as a key enabling technology, which we will combine with a number of new developments in the field of electron and ion microscopy. We will progressively ramp up the TRL of the instrument and associated methodologies to reach TRL 7 by the end of the project. The new technology, and all related processes and methodologies, will be validated via round-robin studies performed independently by several partner institutions, crosschecked with conventional analysis technologies to demonstrate the advancements and capabilities of the npSCOPE technology and benchmarked in representative case studies. Given the low sample quantities needed and the strong potential of the instrument to generate high-quality physico-chemical data on nanomaterials, both ex situ and in situ, npSCOPE will allow a major step forward in defining key descriptors for read-across, grouping, in silico modelling and creating meaningful relationships with biological activity data for QSAR purposes.

To reach these objectives, the project consortium will be composed of research centres internationally recognised for innovative instrument developments, well-established instrument

manufacturers and experts in nanotoxicology in various fields of application to demonstrate and validate the applicability of npSCOPE for the risk assessment of nanomaterials in consumer products.

## 2 Background

Nanomaterials have become omnipresent in our daily life, including food, cosmetics, textiles, electronics devices, materials engineering, energy production, surface cleaning, aerospace, and medical applications. The current trend in nanotechnology is the modification of materials so that they present specific desired properties such as reduction/increase of redox potential, anti-fouling properties, no ion leaching etc. for the use in different industrial sectors and to serve different purposes such as increased hardness/strength, in cosmetic applications, as coatings, as antimicrobial protection etc. All introduced modifications generate a new material, with potential unknown health risks for humans and the environment. Therefore, it is important to develop adequate physico-chemical characterisation of nanomaterials. Because of this identified need, a number of international organisations and EU projects are working to establish the essential measurement requirements and list of physico-chemical data that enable adequate understanding of nanoparticle properties in both environments, ex vivo and in vitro, in relation to health, safety and environmental aspects of nanostructured materials.

For raw materials as well as for nanoparticles embedded in complex matrices such as products and biological environments, the nanoparticle characterisation needs can be classified according to the three key questions "what does the nanomaterial look like?", what is the nanomaterial made of?" and "what factors affect how the nanomaterial interacts?" To address these questions, a number of techniques - each one having its advantages and its limitations - are currently being used, as summarised in Figure 1.

Until today, this multi-technique approach has been performed on separate (expensive) instruments. Our concept consists in providing the answers to ALL of the three key questions in ONE SINGLE instrument. A new integrated microscopy tool is advanced from existing technologies and adapted specifically for the purpose of providing quick and concise analyses of nano-particulated material that can even be embedded in complex and biological matrices. Thereby the npSCOPE instrument aims to provide a new integrated tool for nanoparticle toxicology studies that can provide more efficient, comprehensive and accurate data in one instrument.

## 3 Scientific and technological challenges

Concretely, the concept of npSCOPE is to scan the sample to be investigated with a finely focused helium ion beam as this beam can produce three different datasets (Figure 1):

- Transmitted helium ions, providing STEM type information able to address the questions about the particles' size, the size distribution, the particles' morphology, the agglomeration state, the surface area, the surface per volume (curvature)
- Secondary ions, providing mass spectrometry information to determine the bulk and surface chemical composition (for secondary ion generation the GFIS can also be operated with $Ne^+$ ions rather than $He^+$ ions to further enhance the yield and hence the detection limits)
- Secondary electrons, providing HIM type information able to address the questions about the particles' localisation, their size and size distribution

Using sophisticated correlative data processing methodologies and algorithms based on statistical methods in conjunction with appropriate visualisation methods of the results, the npSCOPE concept will allow providing rapid, accurate and reproducible measurements both of pristine nanomaterials and nanomaterials in complex matrices. It is important to note that ex-situ multi-technique combinations do not allow the same performances as such an approach is hampered by several limitations. First, exposure to air while transferring the sample between instruments can introduce severe artefacts due to surface oxidation and

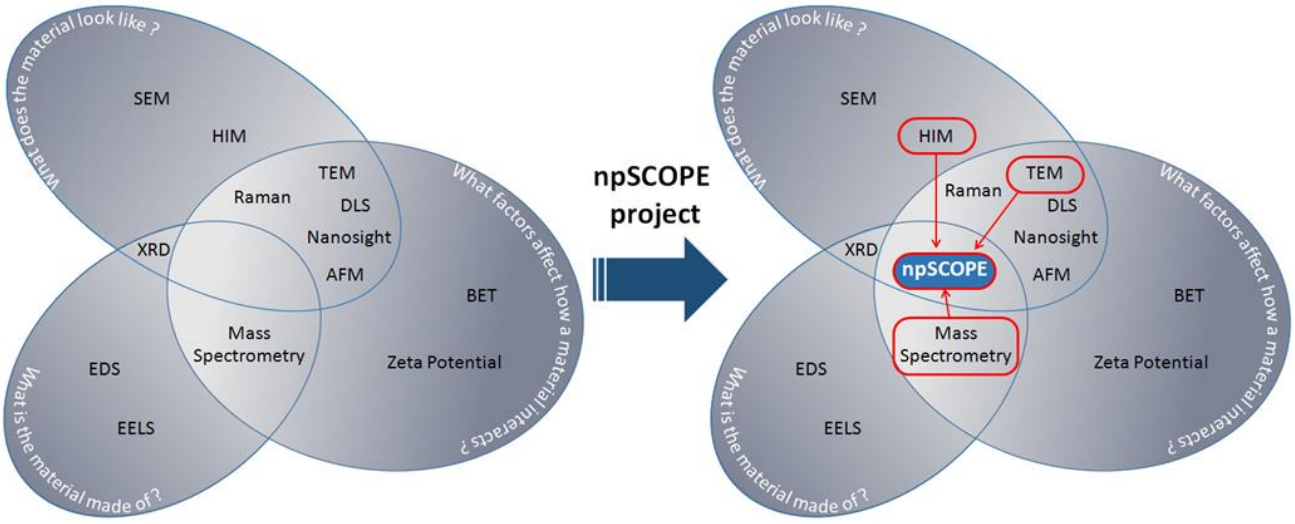



*Figure 1: (left) Overview of the technique used to characterise nanoparticles: different techniques are required to answer the three key questions. (right) The correlative approach of the npSCOPE instrument using a focused helium ion beam to obtain three different datasets in one single instrument will address the three question blocks simultaneously.*

surface reorganization, up to completely changing the surface structure. This is a significant problem especially when reactive ion beams are used for the Secondary Ion Mass Spectrometry (SIMS) analysis. Second, the overlay of images obtained ex-situ is hampered by arbitrary differences in scale and image orientation, non-linear distortions in the images arising from variable magnifications or non-square or non-uniform raster scans and of course by problems in precise re-localization of analyzed zones after transferring the sample between the standalone instruments. All of these problems are much more easily dealt with in a single instrument as relative orientations are fixed and non-linearities may be calibrated and corrected for the two techniques that will be combined. Third, performing a correlative approach using separate standalone instruments is inherently less flexible and more time consuming, as switching from one technique to another requires changing the instrument, so that experiments where two different techniques are used multiple times in a sequential approach become nearly impossible if the combination is not in-situ.

## 1. The instrument

The main components of the npSCOPE instrument will be the following:

**(i) Gas Field Ion Source (GFIS)**

The Gas Field Ion Source (GFIS) developed is the unique key enabling technology of the npSCOPE instrument. The GFIS provides ***spot sizes of 3 Å*** while maintaining an ion current that is appropriate for imaging and analytics thanks to its super-high brightness ($4\times10^9$ $Acm^{-2}sr^{-1}$) combined with an energy spread <1 eV.

While the GFIS has been only used until now for basic imaging purposes by detecting secondary electrons emitted from a sample exposed to the $He^+$ or $Ne^+$ beam, we will develop powerful STIM and SIMS capabilities to fulfil the list of specifications defined for the npSCOPE instrument. **ZEISS** will adapt the GFIS column interfacing for the specific requirements of the npSCOPE instrument. In addition, the beam blanker of the column will be designed in a way to allow beam pulsing with short rectangular pulses in view of ion energy-loss spectroscopy.

***(ii) Compact high-performance mass spectrometer for SIMS***

The npSCOPE instrument will be equipped with a novel compact high-performance mass spectrometer coupled to high-efficiency secondary ion extraction optics in order to perform mass spectrometry of material sputtered from the sample (SIMS). While the $He^+$ or $Ne^+$ ions ("primary ions") penetrate into the sample, they lose all or a part of their energy during collision cascades with the target. Some of these collision cascades will lead to the ejection of one or several target atoms. Amongst the emitted particles, the ions ("secondary ions") are of interest in SIMS. These ions are extracted using an electric field and accelerated into a mass spectrometer, mass filtered and counted by detectors. SIMS is an extremely powerful technique for analysing surfaces owing in particular to its excellent sensitivity, high dynamic range, very high mass resolution and ability to differentiate between isotopes.

**(iii) Detection system for scanning transmitted helium microscopy (STIM)**

A detection system for scanning transmitted helium ions (STIM) will be developed by HZDR and PHOTONIS. We will take advantage of the fact that He atoms are the second lightest atoms after H so that a very large fraction of the incident $He^+$ ions are transmitted through a thin sample and can be used for high resolution imaging purposes. The STIM set-up will provide good elemental contrast due to mass dependent scattering, a high lateral resolution even for heavy elements, and an excellent signal to noise ratio. A first order approximation for the achievable lateral resolution can be obtained from the He exit positions on the backside of the sample. For light elements the beam broadening is minimal and most of the He leaves the samples only 0.1 nm away from the beam axis. For heavy elements the situation is less optimal but calculations clearly show that a resolution of at least 2 nm should be reachable.

The STIM detector will be used in various ways. First ***BF*** *(bright field)* ***and DF*** *(dark field)* ***imaging*** will be performed to obtain ***mass thickness contrast that will allow assessing composition and 3D morphology***. As the BF/DF border will be adjustable on the detector developed by **PHOTONIS**, the resolution and the mass sensitivity can be changed independently. Secondly, ***channeling pictures and information on the crystal structure*** will be obtained taking advantage of the high pixel density and the spatially resolved read-out system of the detector (resolution ~0.05°), combined with the capability of tilting the sample.

A special possibility arises from the fact that the envisioned design of the npSCOPE allows for additional space below the sample. This will allow ***energy loss spectroscopy of the transmitted ions*** by measuring their time of flight (ToF).

**(iv) Secondary electron detectors**

The npSCOPE instrument will be equipped with two secondary electron detectors. The first one will be a conventional Everhart-Thornley type detector mounted above the sample plane to perform ***high-resolution SE imaging*** while raster scanning the sample surface with the ion beam (as is done in a conventional HIM). A second SE mode detector will be included in the new STIM detector for ***high signal to noise ratio backside images***, which are in particular useful for the ***localization and characterization of nanoparticles*** in the sample.

**(v) Cryo-stage**

The npSCOPE instrument will be equipped with a cryo-stage compatible with SIMS and STIM requirements, enabling ***accurate and reproducible characterisation of nanoparticles in biological tissues***. Without careful cryo-preparation and the use of a cryo-stage during analysis, cell structures in biological samples are often altered. The use of a cryo-stage also helps to reduce ion beam damage to the sample during the measurement.

***(vi) Analysis chamber equipped with a rapid load-lock system***

The npSCOPE instrument will be equipped with an analysis chamber specifically designed to fit the needs of npSCOPE:

- Compatibility with the GFIS column (working distance, differential pumping to avoid contaminations migrating to the GFIS tip)
- Compatibility with the SIMS system (working distance, space for the SIMS extraction optics)
- Compatibility with the STIM detection system (availability of sufficient room below the sample to allow for reasonable working distances that can go up to 300 mm for the detection of diffraction patterns)
- Compatibility with the SE detectors (working distance, space)

- Compatibility with the cryo-stage (integration of a cryo-shield protecting the near-sample region from being exposed to "hot" surfaces)
- Base pressure < 10-8 mbar
- Mounting on an anti-vibration frame to allow for sub-nm resolution imaging

The samples will be transferred into this analysis chamber using a specifically designed load-lock system with intermediate vacuum sample storage chamber.

## 2. Workflows and automation

Analysis work flows and correlative image analysis and spectroscopy software will be developed for automation:

**(i) Workflows**

For high-quality correlative microscopy results, which will be the overarching approach on the npSCOPE instrument, the workflow needs to be strategically managed to minimize or eliminate artefacts. The optimal sequence of imaging, e.g., STIM followed by SIMS or vice versa needs to be analyzed in a sample-specific manner (see for example, Figure 2). Other artefacts connected to the workflow can also hamper accurate characterization of samples. Contaminants can find their way to the sample due to the use of inappropriate sample preparation methods. Moreover, ion beam induced damage formation can also lead to image artefacts. Furthermore the images acquired by various techniques and imaging modes need to be accurately aligned and overlaid. Errors due to distorted raster scans can further compound the difficulty. The solution to these issues is non-trivial because of the instrument-specific limitations and image-distortions. Hence, both hardware and software solutions capable of automatic and preferably, real-time rectification will be desirable. One of the strategies to counter some of these issues is by using standard samples of known characteristics for instrumental calibration. Additionally, strategies to deal with non-linear and inhomogeneous shrinkages of biological samples during imaging need to be developed. We will therefore develop specific strategies to address these issues.

**(ii) Correlative image analysis and spectroscopy software for automation**

Dedicated ***algorithms and software for rapid and automated data processing*** will be developed by project partner **FAU**. A current problem when attempting to combine images obtained by different techniques is the lack of appropriate software, especially for multispectral data such as form the npSCOPE SIMS module. Different physical information volumes (and voxel or pixel sampling sizes) combined with different contrast mechanisms can make automatic registration of images unreliable. The use of multiple techniques may lead to a more robust solution, but it can be harder to find that solution. Different imaging modalities may have different types of artefact (such as shadows, or other topographic effects) which can be particularly confusing when datasets are combined. Another challenge is the complexity of the data, which can be overwhelming. Segmentation of the constituent images (before registration) or of the combined image can help the user, with automation or partial automation by means of principal component analysis or k-means clustering. The STIM capability of the npSCOPE instrument not only allows a tomographic reconstruction of the sample, but can also determine the surface topography which helps to interpret 2D and 3D SIMS data. The npSCOPE software will therefore include components to facilitate the registration, segmentation and scientific usage of

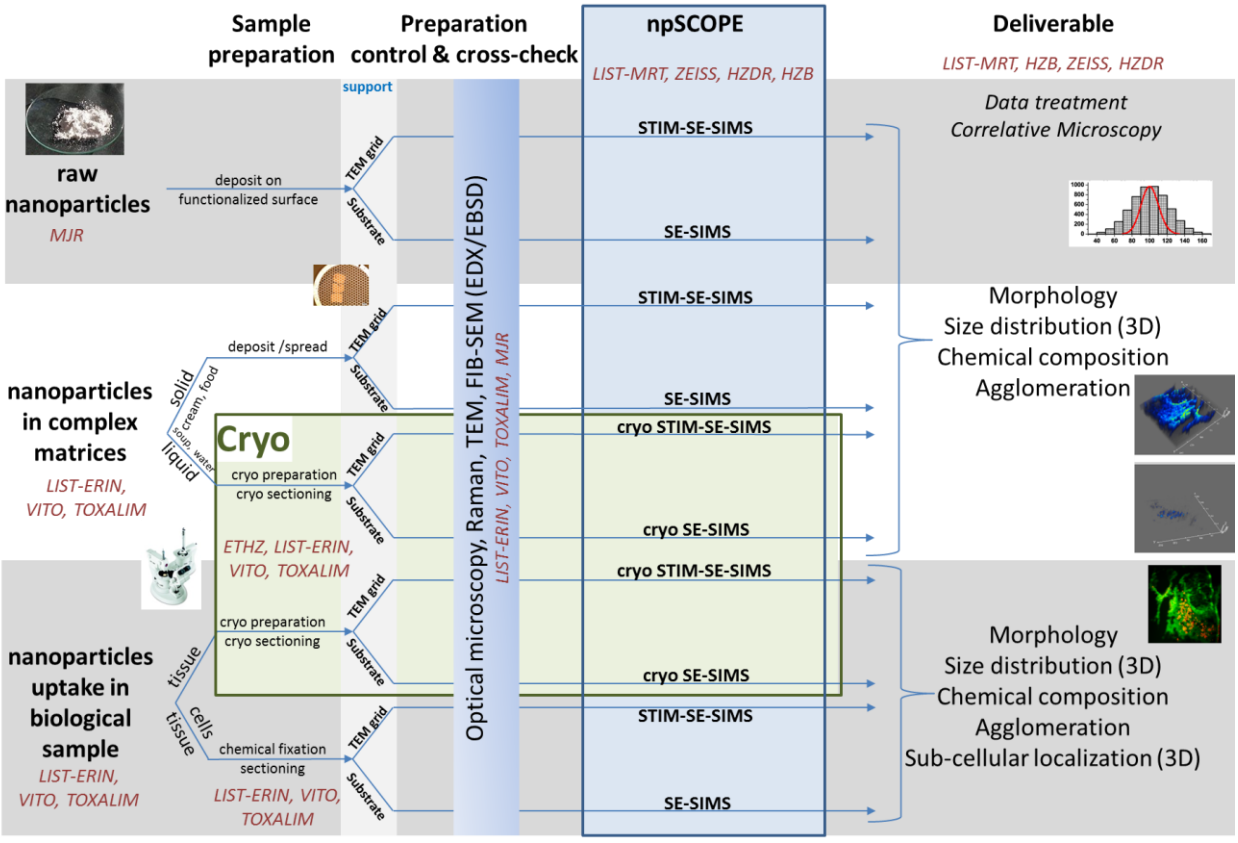



*Figure 2 : Illustration of likely sample analysis workflows.*

datasets from SE, SIMS and STIM obtained with the npSCOPE protoytpe, as well as e.g. Raman, TEM, EDX and SE/SIMS/STIM from the instruments used within the project for validation and round-robin studies.

The software tools and data formats will be compatible with existing solutions and will, where possible, use open standards for file formats (OMERO, bio-formats, OME-tiff, NetCDF, HDF5…), as well as open source software for image analysis. The final implementation of the analysis protocols into an user-friendly operating software for the npSCOPE instrument will be done in close cooperation between the research and industry partners.

**3. Calibration standards and test-cases**

**Calibration standards and test-cases for performance assessment are a further point.**

In order to calibrate the npSCOPE instrument and to provide the end users with the means to assess, at any time, the performances of the instrument in different application fields, the npSCOPE consortium will select a certain number of industry relevant nanomaterials with different physico-chemico properties and will develop test-case biological samples, based on SOPs, covering different application fields.

1. Calibration standards: Nanomaterials representative of industrial materials will be selected and used to calibrate the instruments in its different components and to verify its performances over time.
2. Test-cases representative of different application fields will be developed together with the respective SOPs, in order to allow future users of the npSCOPE instrument to produce reference biological materials to be used to verify the performances of the instrument.

# 4 Objectives

The npSCOPE project will develop a new instrument that couples the extraordinarily high resolution of the recently commercialised helium-ion microscope with sensors for composition (a mass spectrometer) and 3D visualisation (transmitted ion detector) in order to more fully characterise individual nanoparticles and their interaction with their environment (tissue, cells, etc.) and to better understand the risks they might pose to human health or the environment. It is well known that the risks posed by particles depend not only on their composition and surface chemistry, but also upon their shape. The prototype instrument (nanoparticle 'scope → npSCOPE) will be thoroughly benchmarked against existing techniques, and its performance validated using representative materials and calibration standards. Software and sample-handling techniques and hardware will be developed to allow high-throughput analysis (for useful statistical descriptions of real-world samples of many nanoparticles), and to facilitate the use of correlative microscopy techniques for a complete understanding of the context of nanoparticles in biological materials. These techniques and protocols will be tested on a suite of well-characterised representative materials and matrices.

In the last twenty years, engineered nanomaterials have become the focus of intensive research and financial investment in a multitude of areas. However, the same properties that make nanomaterials desirable in these various applications have the potential to alter biological properties, and thus to have an impact on the environment, health, and safety. Nano-specific health risks could result from altered environmental fate, bio-availability, bio-persistence, etc. when compared with the larger or bulk forms of the same material, thus the risks are not simple to extrapolate from similar bulk materials. As nanomaterials and products incorporating these materials are more and more widely adopted, it becomes more and more important to evaluate their potential environmental and human health risks - preferably as early on as possible in the value chain and certainly throughout their life cycle. Therefore (nano)toxicologists need to have a precise and complete idea of the characteristics of the particles, both ex situ and in situ, that could interact with the biological system of concern.

Both for raw materials and in biological environments, nanoparticle characterisation needs can be classified according to 3 domains:

1. What does the material look like?
   - Particle size / size distribution (3 dimensions).
   - Grain, particle, film morphology (shape, roughness, topography, crystal structure).
   - Agglomeration state/aggregation.
2. What is the material made of?
   - Overall composition (chemical composition).
   - Surface composition.
   - Purity (including levels of impurities).
3. What factors affect how a material interacts with its surroundings?
   - Surface area, surface per volume (curvature).
   - Surface energy, reactivity, hydrophobicity, porosity, crystallinity.
   - Surface charge.

In addition, for biological systems, the context in which the particle is found is critical (e.g. in which organs does it accumulate, or in which parts of a cell).

**The overarching objective of the npSCOPE project is to develop a single instrument in combination with dedicated methodologies that will answer these three questions in a rapid, accurate and reproducible way, both for nanoparticles in their pristine form and embedded in complex matrices (**Error! Reference source not found.**). While a particular focus will be on inorganic nanoparticles, as these account for the most abundant group as outlined above, we will also investigate the potential of npSCOPE on organic nanoparticles using two test cases.**

The npSCOPE instrument will provide a full dataset about an investigated sample containing nanoparticles with the following specifications:

- Size distribution of the nanoparticles: range from 0.5 nm to several hundreds of nm.
- Morphology of the nanoparticles: 3D resolution of 0.5 nm.
- Semi-quantitative chemical composition of the nanoparticles with excellent detection limits: from $10^{-3}$ (i.e. 0.1 at%) for nanoparticles having a volume of 100 $nm^3$, to ppm (i.e. $10^{-4}$ at%) for nanoparticles having a volume of $10^5$ $nm^3$, parallel detection of all elements.

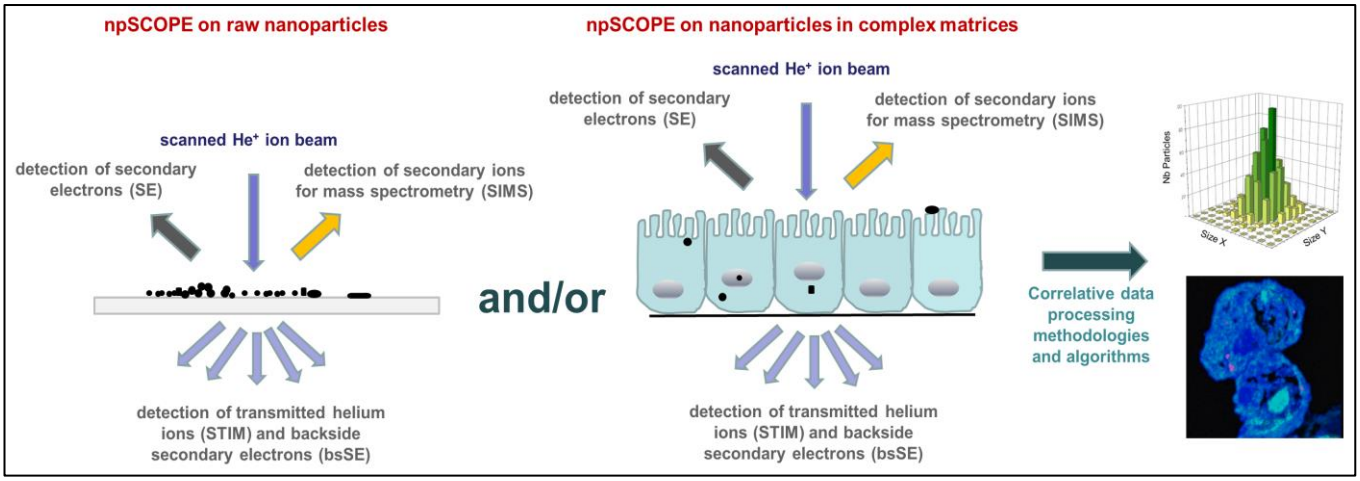



Figure 3 : In situ correlation of STIM, SIMS and SE datasets to provide rapid, accurate and reproducible data of the physico-chemical properties of industrial nanomaterials, both in their pristine form and in complex matrices.

- The number of nanoparticles in the investigated matrix: automatic and accurate determination of the concentration of nanoparticles (i.e. number of nanoparticles per unit volume).
- Precise localisation of the nanoparticles (e.g. in biological samples): 3D resolution of 0.5 nm.
- Interaction of the nanoparticles with their environment: detection of changes in surface composition, detection of changes in morphology, etc.

This overarching objective of the npSCOPE project can be differentiated into the following sub-objectives:

1. Development of hardware based on the Gas Field Ion Source (GFIS) as a unique key enabling technology to enable an original in situ real-time combination of Scanning Transmission Helium Ion Microscopy (STIM), Secondary Electron (SE) imaging and Secondary Ion Mass Spectrometry (SIMS) in one single platform. A cryo-stage compatible with the above described characterisation techniques will be part of the final solution. The GFIS can be operated with $He^+$ and $Ne^+$ ion beams and has a high brightness of $4 \cdot 10^9$ $A \cdot cm^{-2} \cdot sr^{-1}$ with an energy spread of less than 1 eV, enabling very small spot sizes ($He^+$ spot sizes of 3 Å have been demonstrated) while maintaining an ion current that is appropriate for imaging and analytics.
2. Development of protocols for sample preparation and instrument operation, and correlative methodologies and software tools that will allow the automatic and accurate correlation of high sensitivity and high resolution chemical data with morphological information obtained at 0.5 nm resolution.
3. Development of analytical standards, and standardized exposure scenarios as representative test-cases for validation and cross-checking and benchmarking purposes of the npSCOPE instrument and, in general, for analytical nanomaterial characterization technologies.
4. Development of go-to-market strategies for the npSCOPE instrument taking into account performance criteria, cost, easiness to operate, level of automation, intellectual property and freedom to operate aspects.

## 5 Organisation

npSCOPE consists of four technical work packages WP1 through WP4, and two non-technical work packages - WP5 for dissemination/exploitation and WP6 for management/coordination. The relationship between these work packages, the EC and other stakeholders is sketched in Figure 4.

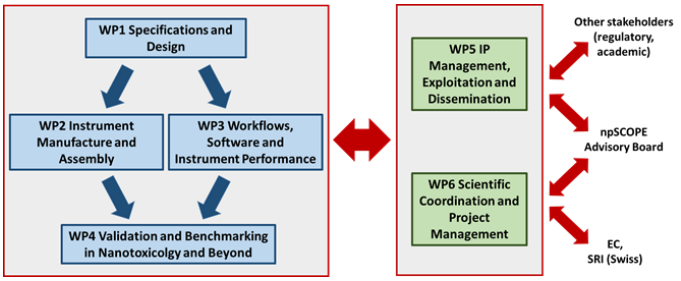



Figure 4 : Schematic of the projects components (work packages) and how they are linked to each other and to the funding agencies, the project's advisory board and to other stakeholders.

It is planned that the npSCOPE project will be completed within 4 years of its start. The specification and detailed design phase of WP1 is expected to last 12 calendar months, finishing with the design blueprints for the instrumentation. Building the npSCOPE instrument and upgrading some existing instruments (for their later use within the round-robin studies performed within WP4) will be performed in WP2, which will run until month 28 and finish with the functional testing of npSCOPE instrument. WP3, which will focus on workflows, software and validation of the performance specifications of the npSCOPE instrument will start at an early stage so that workflow and software development can be done in parallel to the instrument designing and building effort. WP3 will run until month 45 to allow that the software and algorithms can be continuously optimised and upgraded based on the findings of WP4. Two major steps in WP3 are the milestones "Alpha version of analysis software" to facilitate the round-robin and benchmarking work, and "All performance specifications of the npSCOPE instrument met" fixed at month 32. WP4 is the work package with the largest effort in terms of manpower, consisting of testing, validating and benchmarking the npSCOPE instrument

and methodologies using test cases in nanotoxicology, as well as - to a lesser extent – demonstrating applications beyond nanotoxicology as suggested by the expected impacts in the call. The dissemination / exploitation (WP5) and management / coordination (WP6) work packages last for as long as the project.

## 6 Expected Impact

The development of the npSCOPE will reinforce the leading position of Europe in the field of nanotechnology and in the field of advanced instrumentation.

***Identification of key descriptors that can be used to reveal correlations associated with health and environmental impacts and meaningful basis for grouping, read-across and QSARs purposes***

Several attempts have been made to try to group nanomaterials and to model their toxicity using QSAR approaches. The COST action MODENA, of which **LIST** was a member, analysed over 170 datasets in order to develop coherent approaches for the modelling of toxicity of nanomaterials (report under preparation). Recently one OECD working group on the categorisation of manufactured nanomaterials highlighted that nanomaterials should be considered separately from chemicals and that different parameters should be considered for categorization and (Q)SAR modelling. Parameters such as size, shape, aggregation state, fate exposure, etc. should all be considered in order to develop a successful approach. The technology that will be developed within the npSCOPE project, in combination with standardized test-cases and analytical representative materials that will be produced, will allow addressing most of the critical points highlighted by the OECD working group. One barrier to developing Quantitative Structure-Activity Relationships (QSARs) that would allow the prediction of risks from nanoparticles has been the difficulty of properly characterising the materials used, both in their original form and in tissue samples or organisms after exposure. The npSCOPE instrument will go some way towards reducing this barrier by making a fairly comprehensive characterisation possible on a single instrument that can handle several sample formats, including cryo-prepared tissue sections, and perform both imaging and chemical mapping at nanometre scales. The ability to measure many particles in tissue sections (using automated sample analysis with load-lock chamber, customised ample stage and new software) will allow a better estimate of the true nanoparticle dose in particular organs or cell-types to be made; the estimation of effective doses has been one of the difficulties facing QSAR development. The npSCOPE approach will build upon the recommendations for read-across made by the European Chemicals Agency and a recent OECD survey.

Given the low sample quantities needed and the strong potential of the platform to generate high-quality physico-chemical data on nanomaterials, both *ex situ* and *in situ*, a major step forward in defining **key descriptors** for **read-across**, **grouping**, ***in silico* modelling** and creating **meaningful relationships with biological activity data** is expected. In particular, parameters describing the nanomaterials' interface with the environment, as well as the subcellular localisation and quantification of intracellular dose are currently difficult to describe in combination with basic physico-chemical parameters, so that npSCOPE will establish an important currently missing link. The resulting **QSAR models** for the production of new **safe-by-design** nanomaterials will represent a major improvement for R&D strategies from an industrial perspective, with a considerable reduction of the cost and time needed to ensure the safety of the new products. Another major advantage of the npSCOPE instrument as compared to the state-of-art approach, which relies on *ex situ* multi-technique analysis on separate standalone instruments, is that the newly developed instrument will allow a correlative *in situ* approach using one single ion beam, hence significantly increasing the reproducibility and the accuracy of the data acquisition and processing. The use of this innovative approach will allow an **increased confidence** in the determination of physico-chemical features, thus providing **more reliable and consistent data** for **safety evaluation of nanomaterials**.

The performance specifications of the npSCOPE instrument, in particular 0.5 nm resolution imaging combined with simultaneous elemental/chemical information of major elements and trace elements, together with representative nanomaterials for its calibration and verification, will make it a very suitable tool for **quality control** of nanomaterials during their development, production, processing, transport and storage, and **detection of counterfeiting** applications. Information on nanomaterials' stability over their life time is important to avoid loss of desired product properties or development of undesired functionalities. Another application area of the platform concerns reliable traceability of nanomaterials in consumer products, which allows for correct product labelling (cf. cosmetics directive, novel foods regulation). The obtained technical expertise as well as the developed detection systems and data analysis strategies are **not restricted to nanoparticle characterisation**. Many biological and technological processes rely on the unambiguous, efficient and reliable analysis of microscopic samples, sample-structure and sample-composition. Research areas such as 2D materials and traditional materials science (materials for nuclear applications, inorganic and polymer nano-composite materials, etc.) will **benefit from the developed analysis equipment**.

***Increased confidence in nanosafety studies and findings through sound physico-chemical characterisation methods and standard operating procedures***

Nanosafety studies must not only assess the effects (toxicity) of nanomaterials upon the environment and human and animal health, but for these results to be useful and transferable the nature of the nanomaterials and the dose must be understood. Because a variety of instrumental techniques and sample preparation techniques have been used to carry out physico-chemical characterisation (but not always enough or the appropriate techniques), there has been some scatter and uncertainty in interpretation of nanosafety studies. The npSCOPE project addresses this problem in two ways. Firstly, by using a common instrument platform to carry out a number of high resolution imaging and characterisation tasks on the *same sample(s) and particles*, with no need for sample transfer between instruments or institutions (especially important for cryo-sections). Secondly, by developing a set of protocols and workflows for dosing, sampling, sample preparation, imaging and image analysis and by benchmarking and validating these protocols and workflows using well-characterised materials, repeated experiments and independently repeated measurements. The npSCOPE project has selected several application area and exposure scenarios. e.g. for ingested food additives (or nanoparticles from packaging or appliances), or for dermal exposure to cosmetics. This project will help safety authorities by

improving the analytical information available for materials that are already on the market, while providing relevant test-cases based on standard methodologies (*in vitro* and *in vivo*) for further reliable and reproducible toxicity testing.

The expected outcome is to propose standard conditions for the physico-chemical characterisation of representative nanoparticles (using multi-technique analysis in one platform) in realistic conditions for tissue translocation of nanoparticles. If these approaches are widely adopted, we are certain that increased confidence in study results will follow. The increased confidence (through reproducible and validated results) in studies and findings will apply not only to the authors and readers of such studies, but will eventually be apparent to the wider public as consensus in the field emerges on the risks of some categories of nanomaterials.

***Examples of synergies with other applications of the method***

***Nanomedicine*** will rely on the fact that both translocation and subsequent potential toxicity in tissues and cells are highly dependent on the surface properties and coatings of nanoparticles. e.g. nanomaterials may be 'protected' in part of the body whereas intake by particular cells elsewhere may be favoured, thus increasing oral bioavailability of nanomedicines. Future requirements for the clinical ***quality control*** of nanosized delivery systems (including metal nanoparticles) will depend on the accuracy of physico-chemical characterization of nanoparticles, which will be improved by npSCOPE's outputs.

The npSCOPE technology can provide a valuable tool for **quality control and process analysis in technical nanosystems** such as fuel cells, batteries, solar cells or semiconductor nanostructures; including the analysis of undesired contaminations. The high lateral resolution in 3D and the possibility to analyze light elements (H, Li), make the npSCOPE instrument a powerful tool to solve issues such as lithium diffusion in battery applications, catalyst dispersion or interfacial diffusion which is crucial for the effective proliferation of an electric economy and for the implementation of many renewable energy technologies, or reliability of the materials.

# 7 Directory

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

| First Name | Last Name | Affiliation | Address | e-mail |
|---|---|---|---|---|
| Akif Emre | TÜRELI | MJR | Überherrn, Germany | e.tuereli@mjr-pharmjet.com |
| Arkady | KRASHENINNIKOV | HZDR | Dresden, Germany | a.krasheninnikov@hzdr.de |
| David | DOWSETT | LIST | Belvaux, Luxembourg | david.dowsett@list.lu |
| Elisa | BOUTET | TOXALIM | Toulouse, France | elisa.boutet-robinet@inra.fr |
| Eric | HOUDEAU | TOXALIM | Toulouse, France | eric.houdeau@inra.fr |
| Fabrice | PIERRE | TOXALIM | Toulouse, France | fabrice.pierre@inra.fr |
| Falk | LUCAS | ETHZ | Zürich, Switzerland | falk.lucas@scopem.ethz.ch |
| Gregor | HLAWACEK | HZDR | Dresden, Germany | g.hlawacek@hzdr.de |
| Inge | NELISSEN | VITO | Mol, Belgium | inge.nelissen@vito.be |
| Jean-Nicolas | AUDINOT | LIST | Belvaux, Luxembourg | jean-nicolas.audinot@list.lu |
| Julien | VIGNARD | TOXALIM | Toulouse, France | julien.vignard@inra.fr |
| Lasse | KLING | FAU | Nürnberg, Germany | lasse.kling@mpl.mpg.de |
| Michael | STEIGERWALD | ZEISS | Jena, Germany | michael.steigerwald@zeiss.com |
| Miriam | LUCAS | ETHZ | Zürich, Switzerland | miriam.lucas@scopem.ethz.ch |
| Nazende | GÜNDAY TÜRELI | MJR | Überherrn, Germany | n.guenday@mjr-pharmjet.com |
| Nicolas | BLANC | ETHZ | Zürich, Switzerland | nicolas.blanc@scopem.ethz.ch |
| Olivier | BOUTON | LIST | Belvaux, Luxembourg | olivier.bouton@list.lu |
| Olivier | DE CASTRO | LIST | Belvaux, Luxembourg | olivier.decastro@list.lu |
| Patrick | PHILIPP | LIST | Belvaux, Luxembourg | patrick.philipp@list.lu |
| Peter | GNAUCK | ZEISS | Jena, Germany | peter.gnauck@zeiss.com |
| Peter | ROZEMA | PHOTONIS | Roden, Netherlands | p.rozema@photonis.com |
| Rachid | BARRAHMA | LIST | Belvaux, Luxembourg | rachid.barrahma@list.lu |
| Russ | MELLO | ZEISS | Jena, Germany | russ.mello@zeiss.com |
| Sebastian | TACKE | ETHZ | Zürich, Switzerland | sebastian.tacke@scopem.ethz.ch |
| Serge | DUARTE PINTO | PHOTONIS | Roden, Netherlands | s.duartepinto@photonis.com |
| Sévérine | IFFLAND | LIST | Belvaux, Luxembourg | severine.iffland@list.lu |
| Silke | CHRISTIANSEN | FAU | Nürnberg, Germany | silke.christiansen@mpl.mpg.de |
| Tom | VENKEN | VITO | Mol, Belgium | tom.venken@vito.be |
| Tom | WIRTZ | LIST | Belvaux, Luxembourg | tom.wirtz@list.lu |
| Tommaso | SERCHI | LIST | Belvaux, Luxembourg | tommaso.serchi@list.lu |
| Uwe | MICK | FAU | Nürnberg, Germany | uwe.mick@mpl.mpg.de |
| Véronique | BECKER | LIST | Belvaux, Luxembourg | veronique.becker@list.lu |

# 8 Copyright


# Updates from Horizon2020 projects that are currently running

# caLIBRAte

## Performance testing, calibration & implementation of a next generation system-of-systems risk governance framework for nanomaterials

Contract Agreement: 686239 Website: http://www.nanocalibrate.eu
Coordinator: Keld Alstrup Jensen (kaj@nrcwe.dk), NRCWE, DK-2100 Copenhagen, Denmark

*Table 1 Consortium List.*

| No. | Beneficiary name | Short name | Country |
|---|---|---|---|
| 1 | DET NATIONALE FORSKNINGSCENTER FOR ARBEJDSMILJO | NRCWE | DK |
| 2 | DANMARKS TEKNISKE UNIVERSITET | DTU | DK |
| 3 | EIDGENOESSISCHE ANSTALT FUER WASSERVERSORGUNG ABWASSERREINIGUNG UND GEWAESSERSCHUTZ | EAWAG | CH |
| 4 | EIDGENOSSISCHE MATERIALPRUFUNGS- UND FORSCHUNGSANSTALT | EMPA | CH |
| 5 | TYOETERVEYSLAITOS | FIOH | FI |
| 6 | FUNDACION GAIKER | GAIKER | ES |
| 7 | KAROLINSKA INSTITUTET | KI | SE |
| 8 | ACONDICIONAMIENTO TARRASENSE ASSOCIACION | LEITAT | ES |
| 9 | MISVIK BIOLOGY OY | MISVIK | FI |
| 10 | ASSOCIAZIONE ITALIANA PER LA RICERCA INDUSTRIALE - AIRI | AIRI | IT |
| 11 | NATURAL ENVIRONMENT RESEARCH COUNCIL | NERC | UK |
| 12 | NANOTECHNOLOGY INDUSTRIES ASSOCIATION | NIA AISBL | BE |
| 13 | DIALOGIK GEMEINNUETZIGE GESELLSCHAFT FUER KOMMUNIKATIONS- UND KOOPERATIONSFORSCHUNG GmbH | DIA | DE |
| 14 | RIJKSINSTITUUT VOOR VOLKSGEZONDHEIDEN MILIEU | RIVM | NL |
| 15 | NEDERLANDSE ORGANISATIE VOOR TOEGEPAST NATUURWETENSCHAPPELIJK ONDERZOEK | TNO | NL |
| 16 | TTY-SAATIO | TUT | FI |
| 17 | UNIVERSITA CA' FOSCARI VENEZIA | UNIVE | IT |
| 18 | Steinbeis Advanced Risk Technologies GmbH | R-Tech | DE |
| 19 | RESEARCH TRIANGLE INSTITUTE | RTI-HS | USA |
| 20 | DUKE UNIVERSITY | DUKE | USA |
| 21 | GREEN DECISION SRL | GD | IT |
| 22 | INSTITUT NATIONAL DE L ENVIRONNEMENT ET DES RISQUES | INERIS | FR |
| 23 | NATIONAL HEALTH LABORATORY SERVICES | NIOH | ZA |
| 24 | HELSINGIN YLIOPISTO | UH | FI |

## Contents

## 1 Summary

Project Duration: 42 Months (2016-05-01 to 2019-10-31)

Project Funding: 9.828.106,25€ (7.999.687,50€ EC H2020).

The overall objective in the caLIBRAte project is to establish a state-of-the-art risk governance framework for assessment and management of human and environmental risks of MN and MN-enabled products. The framework will be a web-based "system-of-systems" (SoS) linking different tested calibrated models and methods for:

1) Screening of apparent and perceived risks and trends in nanotechnology

2) Control banding, qualitative and fully integrated predictive quantitative human and environmental risk assessment, which are operational at different information levels

3) Help-tools including multi-criteria decision support

4) Risk surveillance, -management and -guidance documents.

The models will enable risk assessment of MN and MN-enabled products during both innovation and post product-launch. This will be achieved by aligning models according to the information and safety assessment needs along the principle innovation steps in a "Cooper Stage-Gate®-like" product innovation model ; considering the principles of the iNTeg-Risk ERMF (Emerging Risk Management Framework) and the ISO31000 risk governance framework (Fig. 1).

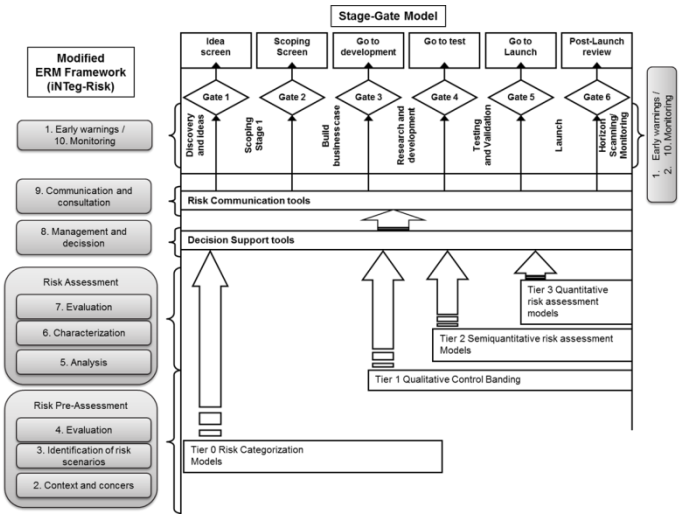



Figure 1. Principle sketch of the caLIBRAte nano-risk governance framework illustrating the anticipated alignment of different models to support the "Cooper Stage-Gate®-like" product innovation model and the principles of the iNTeg-Risk ERMF (Emerging Risk Management Framework) and the ISO31000 risk governance framework.

The project has particular focus on optimization, calibration and demonstration of existing models and methods that support the risk governance framework. Testing and calibration will be made on existing as well as new data generated in the project covering both material characterization, human and environmental exposure, and (eco-)toxicology from both in vitro and in vivo studies. Next generation computational risk assessment and communication models are anticipated in the framework. The framework will finally be demonstrated by industrial case studies.

Stakeholders will define the user requirements of the framework and will receive training in the framework at the end of the project.

## 2 Background

There is a high drive in the scientific and industrial communities towards development of manufactured nanomaterials (MN) and their potential application in intermediates and final products. The innovation and development of MN include both physico-chemical modification of known 1st generation MN towards higher generation MN and development of entirely new MN species of different compounds (e.g., fullerene, graphene and nanotubes).

New MN, discovery of new properties or new applications usually gains high industrial interest. However, as it has been realized, obstacles may arise when launching new MN or new uses of MN in products due to a general uncertainty regarding their safety and validity of the existing human and environmental risk assessment methods. It has also been observed that these uncertainties are challenges in risk transfer to insurance and the regulatory approval of MN and associated products. Apparent or perceived risks and uncertainties are likely to result in loss of downstream users and consumer acceptance. To avoid such issues some companies and associations (e.g., several organic food associations) have decided not to use MN or MN-enabled products. All together such "nanosafety issues" can cause loss of investment and fretfulness in industrial investments in nanotechnology product development. Therefore there is real risk of losing the large European investment in nanotechnology as a key-enabling technology.

The risk uncertainties related to MN and MN-enabled products can be significantly reduced if results from risk assessments are taken into consideration already during product development. However, to make this approach feasible, it is necessary to partially replace the existing data-based risk assessment with prospective risk assessment models for MN. If successful, this approach can also reduce the safety assessment costs considerably.

Due to lack of exposure data and incomplete documentation of MN hazards, such modelling-based risk assessment need to strongly rely on precautionary or predictive model estimates. However, before true benefits of this paradigm can be reached, confidence must be established in the results generated by the models. Lessons from experimental tests have shown that there is a need for MN-specific models and better prediction of exposure and hazard levels with considerations on associated uncertainties. For environmental risk assessment, a similar situation exists.

An impressive number of exposure, fate and hazard and risk models for MN have already been developed or are in development. However, their application domains and readiness levels vary considerably. Moreover, the models are not comparable in regards to their output and not truly connected as full risk governance framework. Therefore, the final uncertainty on the predicted risks is not easily understood. This is a major obstacle for confident and clear communication with the stakeholders. Therefore, there is a high need for a coherent nanospecific risk governance framework to enable a transparent and reliable risk assessment of the many different types of MN and MN-enabled products in the nanotechnology sector.

## 3 Scientific and technological challenges

- The conventional risk assessment approach is not suited for risk assessment of NM and to ensure the safe production and use of newly developed materials and products in the fast moving market of nanomaterials.
- The challenge is to build a state-of-the art and flexible risk banding tool to keep pace with developments in innovation and risk research by harvesting and implementing results from concluded, ongoing and planned research in next generation risk governance frameworks.
- The risk analysis is still technically and methodologically limited and associated with a high uncertainty. This uncertainty must be reduced by testing and calibration of the risk assessment modeling procedures
- Stakeholders' concerns, including those of the insurance sector, and risk perception should be understood and communicated.
- Risk acceptance is strongly affected by a clear understanding of the risks, the benefits and the uncertainties perceived on equity and trust.

## 4 Objectives

The key objective of the caLIBRAte project is to funnel and merge the state-of-the-art in nanosafety research with state-of-the-art in risk governance and communication sciences to establish a versatile SoS risk governance framework for assessment and management of human and environmental risks of MN and MN-enabled products. The overall ambition is to move nano-risk governance for MN and MN- enabled products from theory to demonstrated praxis. The aim is that the quality and trust in the nano-specific risk assessment and management models exceed the current level of most existing REACH tools to ensure a hitherto unseen risk assessment support to nanotechnology innovation. The outcome of this approach should be safer and state-of-the-art-assessed MN and MN-based products with the chance for faster implementation and better competitiveness and profit of MN as a key-enabling technology.

To establish the nano-risk governance framework, a number of achievements and interim critical objectives must be reached:

1. Stakeholders representing nanotechnological industries, regulatory entities, insurance companies and the public must be involved to define and align and implement the risk governance system according to their specific needs. Liaisons must be made with parallel ongoing environmental health and safety research projects to enable timely access to data and new models and methods as well as nano-innovation projects to learn of their experience and potential risk need for risk governance.
2. A new Nano-Risk Radar web-tool must be developed as part of the caLIBRAte project to enable horizon scanning of "hot spots" and emerging real and perceived risks based on a.o. web-based analysis of scientific literature, reports, expert groups, and the public; including sentiment analysis.
3. Further development of human exposure, hazard and risk assessment models to establish integrated qualitative to quantitative occupational and consumer risk assessment models according to different user- and stakeholder-specific requirements. This includes further development of innovative hazard grouping and predictive hazard assessment methods (physicochemical modelling, in vitro, HTS [high-through-put screening], omics technologies, bioinformatics, PBPK [Physiologically based pharmacokinetic] modelling) and integrate these into the different hazard and risk assessment methods applicable for specific high-level users and stakeholders.
4. Compilation and further development of a set of tools to establish integrated predictive environmental risk assessment models considering alignment with user and stakeholder-specific requirements. Development and integration of new innovative hazard/risk methods such as (HTS, omics, bioinformatics, and migration, transformation and fate modelling) is intended. A goal is to move environmental risk assessment from "generic" MN assessments to become more predictive and MN specific.
5. Further development, integration, testing and refinement of procedures and decision support tools applicable for evaluation of risk assessment and data quality, uncertainty, risk perception etc. to ensure an open and evidence-based mitigation and risk communication with relevant stakeholders and the public.
6. Establishment of a comprehensive data repository with data from previous national and EU-funded research projects to identify data and information gaps regarding the input requirements in the specific risk scanning and risk assessment models. Additional data must be generated to ensure performance and calibrate testing of hazard, exposure, and risk assessment tools.
7. Establishment and further development of existing and new state-of-the-art value-chain case-studies to finally enable demonstration of the risk governance framework in as many risk governance aspects as possible.
8. Methods and models considered for the nano-risk governance framework must be evaluated in regards to their data requirements, use domains and applicability for intended use, and finally performance tested and calibrated. The entire risk governance framework must be demonstrated using comprehensive case studies.
9. The nano-risk governance framework must be established as a web-based system-of-systems linking the tested and calibrated risk screening, risk assessment, decision support and management models to support the iNTeg-Risk ERMF and ISO31000 risk governance framework for each steps along the "Cooper-like" stage-gate innovation chain, including mitigation and risk communication models as needed.
10. Training in the purpose and use of the nano-risk governance framework and the knowledge generated must be completed for the different stakeholder groups ranging from academia over industry, insurers, regulators/administrators to consumers and NGOs (non-governmental organizations) to maximize acceptance of the method.

## 5 Organisation

The project is organized into 12 work-packages (WPs) with distinct objectives to establish the caLIBRAte risk governance framework. As shown in Fig. 2, WP1 to WP4 are concerned with development and refinement of the different models to establish the risk

governance framework (a nanorisk radar (WP1; Lead R-Tech), human risk assessment models (WP2; Lead TNO), environmental risk assessment models (WP3; Lead EMPA), risk management and decision support models (WP4; Lead DIALOGIK). Two WPs (WP5; Lead RIVM and WP6; Lead LEITAT) are dedicated collection and generation of data to facilitate model testing, calibration and demonstration of the framework. WP5 builds a data inventory on physicochemical characteristics of MN and MN-enabled products, release rate data and (eco-)toxicological test results. A new data suite on a minimum of 10 new MN and MN-enabled products is anticipated in the project. WP6 collects and further generates exposure and environmental release data along the life-cycle value chain to generate up to five comprehensive and data-rich case studies for demonstration of the risk governance framework. WP7 (Lead NRCWE) is a central and performs assessment, sensitivity and performance testing and to the extent possible calibration of the models with data from WP5 and WP6. WP7 also performs the final demonstration of the risk governance framework, which is established in WP8.

WP9 (Lead NIA) is responsible for the project dissemination and implementation of the nano-risk governance framework and exploitation. WP10 (administrative coordination) WP11 (scientific coordination) and WP12 (ethical management) are all lead by NRCWE. WP12 shall ensure that all documentation requirements related to the testing and stakeholder mapping are respected.

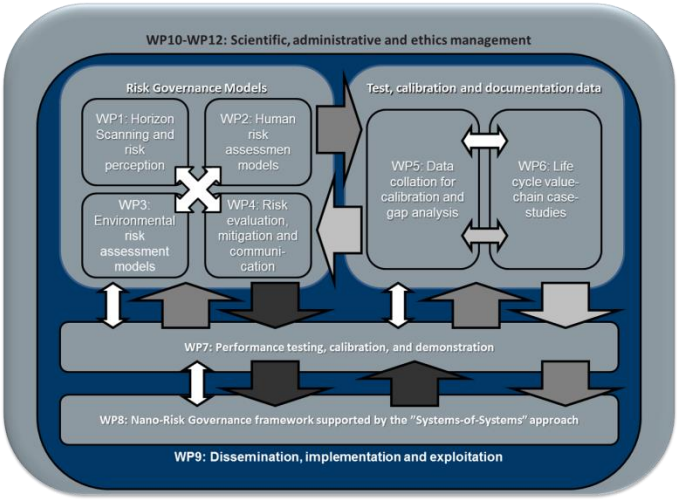



Figure 2. Overview of the work-packages in caLIBRAte and their general interrelationships for communication (white arrows), results (light gray arrows) and model exchange (dark grey arrows)

## 6 Expected Impact

- A risk governance framework for MN and MN-enabled products entering the market consisting of tested and calibrated risk prioritization, control banding and risk assessment and -management models and tools for horizon scanning, risk appraisal, risk transfer and guidance for risk communication.
- Comprehensive data-libraries with physicochemical, release, and (eco-)toxicological data on MN and MN-enabled products as well as industrial value-chain case studies with exposure and environmental release data, leveraging and building upon previous work.
- Worked case-study examples demonstrating the use of the caLIBRAte nano-risk governance framework in specific industrial settings to show the feasibility of the developed approaches with outcomes as guidance, good practices and tools for risk management and risk communication.
- Improved understanding of the needs and the risk perception, -behaviour and -management within different stakeholder groups covering industry, regulators/administrators insurance, and consumers to improve the risk management and dialogue efforts with key stakeholders including regulators and insurers.
- Increased competitiveness of MN producers and downstream user companies' by simplifying their risk governance processes for introducing new MN and MN-products on the market.

## 7 Progress and Outcomes to date

The caLIBRAte project has now passed the first year into the action. Significant progress has been achieved during the first year.

WP 1, 2, 3, 4, 8 and 9 has collaborated to performed targeted interviews, general stakeholder consultations, and completed a first round of a Delphi survey to enable us to align the risk-governance framework and underlying models and guidance to stakeholder needs. WP1, 4 and 8 also successfully organized a face-to-face stakeholder consultation meeting on overall risk governance needs in connection with the Society of Risk Analysis Policy Forum: Risk Governance for Key Enabling Technologies, in Venice, March 1-3, 2017.

WP2 and WP3 collected a large number of candidate models for human and environmental risk assessment. These models were analysed in regards to their input and output requirements and the results were compared with information from stakeholders on data availability and needs along the stage-gate innovation funnel. The models that fitted best the stakeholder needs were aligned to match risk assessment criteria defined for each of the stage-gates and proposed for initial testing in WP7.

In WP5 and WP6 comprehensive reviews of data sources have been made and collation of extensive data sets is ongoing. caLIBRAte collaborates closely with NANOREG 2 to gather and qualify the physicochemical and toxicological data from previous and parallel on-going projects. In WP5, synthesis of tailored nanomaterials to test and establish two critical hypotheses for predictive hazard assessment has been initiated. In WP6 the specific measurement data connected to the value-

chain case studies are currently being collected for subsequent gap-analysis. A number of candidates for new case-studies are investigated for the moment to start the first exposure and environmental release field measurement campaign in the second half of 2017.

In WP7, the work has been initiated as planned with model parameter analysis and selection of the procedure of the sensitive analysis. Sensitivity analysis and model user testing will be conducted in the next phase.

WP8 has started with stakeholder alignment, which will continue into the second year of the project. A draft risk governance framework has been launched for further development. WP8 will be fully active during the remaining part of the project.

WP9 has primarily focussed on establishment of the projects public relations and stakeholder consultations, stakeholder libraries and the project dissemination, including establishment of the project templates. WP9 also played a major role in establishment of an industrial safety-by-design workshop in Bilbao on April 24-25, 2017 and preparations for our participation at the EuroNanoForum in Malta on June 21-23, 2017. Finally caLIBRAte was present with a booth at the NANOEH 2017 Conference in Elsinore, Denmark, May 29 to June 1, 2017. For specific project information on deliverables and project dissemination, please find pamphlets, fact sheets and presentations at our website www.nanocalibrate.eu.

## 8 Directory

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

| First Name | Last Name | Affiliation | e-mail |
|---|---|---|---|
| Adrienne | Sips | RIVM | adrienne.sips@rivm.nl |
| Agnes | Lampke | DIA | geschaeftsleitung@dialogik-expert.de |
| Agnes | Oomen | RIVM | agnes.oomen@rivm.nl |
| Alejandro | Vilchez | LEITAT | avilchez@leitat.org |
| Aleksander | Jovanovic | R-Tech | jovanovic@risk-technologies.com |
| Alex | Rinkus | NIA | alex.rinkus@nanotechia.org |
| Alex | Zabeo | GD | alex.zabeo@greendecision.eu |
| Anders | Baun | DTU | abau@env.dtu.dk |
| Andrea | Porcari | AIRI | porcari@nanotec.it |
| Anja | Verschoor | RIVM | anja.verschoor@rivm.nl |
| Anna-Kaisa | Viitanen | FIOH | anna-kaisa.viitanen@ttl.fi |
| Anne Thostrup | Saber | NRCWE | ats@nrcwe.dk |
| Antti Joonas | Koivisto | NRCWE | jok@nrcwe.dk |
| Antonio | Marcomini | UNIVE | marcom@unive.it |
| Anze | Zupanic | EAWAG | Anze.Zupanic@eawag.ch |
| Arto | Säämänen | FIOH | arto.saamanen@ttl.fi |
| Bernd | Nowack | EMPA | bernd.nowack@empa.ch |
| Blanca | Suarez | GAIKER | suarezb@gaiker.es |
| Camilla | Delpivo | LEITAT | cdelpivo@leitat.org |
| Christiaan | Delmaar | RIVM | christiaan.delmaar@rivm.nl |
| Cindy | Bekker | RIVM | cindy.bekker@rivm.nl |
| Claus | Svendsen | NERC | csv@ceh.ac.uk |
| Cornelle | Noorlander | RIVM | cornelle.noorlander@rivm.nl |
| Danail | Hristozov | GD | danail.hristozov@greendecision.eu |
| Dario | Greco | UH | dario.greco@helsinki.fi |
| David | Rees | NIOH | david.rees@nioh.nhls.ac.za |
| David | Spurgeon | NERC | dasp@ceh.ac.uk |
| Elisabetta | Borsella | AIRA | borsella@nanotec.it |
| Elma | Lahive | NERC | elmhiv@ceh.ac.uk |
| Elvio | Mantovani | AIRI | mantovani@nanotec.it |

| First Name | Last Name | Affiliation | e-mail |
|---|---|---|---|
| Emeric | Frejafon | INERIS | Emeric.FREJAFON@ineris.fr |
| Flor Angela | Quintero | R-Tech | fq@risk-technologies.com |
| Francois | Bourgeois | RIVM | francois.bourgeois@rivm.nl |
| Håkan | Wallin | NRCWE | hwa@nrcwe.dk |
| Hannu | Norppa | FIOH | hannu.norppa@ttl.fi |
| Harri | Alenius | FIOH / UH | Harri.Alenius@ttl.fi |
| Helene | Stockmann-Juvala | FIOH | Helene.Stockmann-Juvala@ttl.fi |
| Henrik | Wolff | FIOH | henrik.wolff@ttl.fi |
| Howard | Jacobs | UH | howard.jacobs@helsinki.fi |
| Isabel | Rodriguez | GAIKER | rodriguez@gaiker.es |
| Ismo Kalevi | Koponen | NRCWE | ikk@nrcwe.dk |
| Jean-Marc | Willems | NIA | jean-marc.willems@nanotechia.org |
| Jessica | Adams | NERC | jesams@ceh.ac.uk |
| Jitka Stilund | Hansen | NRCWE | jsh@nrcwe.dk |
| Joris | Quik | RIVM | joris.quik@rivm.nl |
| Julia | Catalan | FIOH | julia.catalan@ttl.fi |
| Kai | Savolainen | FIOH | Kai.Savolainen@ttl.fi |
| Kailen | Boodhia | NIOH | Kailen.Boodhia@nioh.nhls.ac.za |
| Katharina Christiane | Jeschke | NRCWE | kcj@nrcwe.dk |
| Keld Alstrup | Jensen | NRCWE | kaj@nrcwe.dk |
| Khara D. | Grieger | RTI-HS | kgrieger@rti.org |
| Kirsten I. | Kling | NRCWE | kil@nrcwe.dk |
| Hanna | Kivisaari | FIOH | hanna.kivisaari@tut.fi |
| Kristin | Schirmer | EAWAG | kristin.schirmer@eawag.ch |
| Lee | Walker | NERC | leew@ceh.ac.uk |
| Lisa | Pizzol | GD | lisa.pizzol@greendecision.eu |
| Marianne | Matzke | NERC | martzk@ceh.ac.uk |
| Marina G. | Moser-Johansen | NRCWE | mgm@nrcwe.dk |
| Mark | Wiesner | DUKE | wiesner@duke.edu |
| Martine | Bakker | RIVM | martine.bakker@rivm.nl |
| Mary Elizabeth | Reiss | RTI-HS | mreiss@rti.org |
| Mary | Gulumian | NIOH | Mary.Gulumian@nioh.nhls.ac.za |
| Mehmood | Ahmad | R-Tech | mahmad@risk-technologies.com |
| Michael | Burkard | EAWAG | Michael.Burkard@eawag.ch |
| Miikka | dal Maso | TUT | miikka.dalmaso@tut.fi |
| Mikko | Poikkimaki | TUT | mikko.poikkimaki@tut.fi |
| Minne | Heringa | RIVM | minne.heringa@rivm.nl |
| Ninell | Mortensen | RTI-HS | nmortensen@rti.org |
| Olivier | Aguerre-Chariol | INERIS | olivier.aguerre-chariol@ineris.fr |
| Pekka | Kohonen | KI | pekka.kohonen@ki.se |
| Penny | Nymark | KI | penny.nymark@ki.se |
| Pete | Kines | NRCWE | pki@nrcwe.dk |
| Piet | Sellke | DIA | sellke@dialogik-expert.de |
| Rambabu | Atluri | NRCWE | rba@nrcwe.dk |
| Rebecca | Dupre | DUKE | rld3@duke.edu |
| Rob | Stierum | TNO | rob.stierum@tno.nl |
| Roland | Grafström | KI | Roland.Grafstrom@ki.se |

| First Name | Last Name | Affiliation | e-mail |
|---|---|---|---|
| Sandra | Dissel | RIVM | sandra.dissel@rivm.nl |
| Sara Nørgaard | Sørensen | DTU | sans@env.dtu.dk |
| Sean | Kelly | NIA | sean.kelly@nanotechia.org |
| Serenella | Rizzieri | UNIVE | s.rizzieri@unive.it |
| Sesto | Viticoli | AIRI | viticoli@airi.it |
| Socorro | Vásquez-Campos | LEITAT | svazquez@leitat.org |
| Steffen Foss | Hansen | DTU | sfha@env.dtu.dk |
| Stephen | Lofts | NERC | stlo@ceh.ac.uk |
| Thies | Oosterwijk | TNO | thies.oosterwijk@tno.nl |
| Tiina | Berg | UH | tiina.berg@helsinki.fi |
| Tomi | Kanerva | FIOH | tomi.kanerva@ttl.fi |
| Ulla | Vogel | NRCWE | ubv@nrcwe.dk |
| Vesa | Hongisto | MISVIK | vesa.hongisto@gmail.com |
| Walter | Brand | RIVM | walter.brand@rivm.nl |
| Well | Utembe | NIOH | Wells.Utembe@nioh.nhls.ac.za |
| Willie | Peijnenburg | RIVM | willie.peijnenburg@rivm.nl |
| Wim | de Jong | RIVM | wim.de.jong@rivm.nl |
| Wouter | Fransman | TNO | wouter.fransman@tno.nl |

# 9 Copyright

# EC4SafeNano

## European Centre for Risk Management and Safe Innovation in Nanomaterials & Nanotechnologies

Contract Agreement: Website: www.EC4SafeNano.eu

Coordinator: Emeric FREJAFON, INERIS – Institut National de l'Environnement Industriel et des Risques.
emeric.frejafon@ineris.fr

*Table 1 Consortium List.*

| No. | Beneficiary name | Short name | Country |
|---|---|---|---|
| 1 | Institut National de l'Environnement Industriel et des Risques (Coordinator) | INERIS | FR |
| 2 | European Virtual Institute for Integrated Risk Management | EUvRI | DE |
| 3 | Nederlandse Organisatie voor Toegepast Natuurwetenschappelijk Onderzoek | TNO | NL |
| 4 | Bundesanstalt für Materialforschung und -prüfung | BAM | DE |
| 5 | Tyoeterveyslaitos | FIOH | FI |
| 6 | Vlaamse Instelling voor Technologisch Onderzoek | VITO | BE |
| 7 | SP Sveriges Tekniska Forskningsinstitut | SP | SE |
| 8 | National Centre for Scientific Research "DEMOKRITOS" | Dem | GR |
| 9 | Tecnalia Research & Innovation Foundation | Technalia | ES |
| 10 | Health and Safety Executive | HSE | UK |
| 11 | National Research Centre for the Working Environment | NRCWE | DK |
| 12 | Paris Lodron University Salzburg | PLUS | AT |
| 13 | Université Libre de Bruxelles | ULB | BE |
| 14 | University of Birmingham | UoB | UK |
| 15 | Agenzia Nazionale per le nuove tecnologie, l'energia e lo sviluppo sostenibile | ENEA | IT |

## Contents



## 1 Summary

Project Duration: 36 months

Project Funding: €2M

A central challenge to ensure the sustainable production and use of **nanotechnologies** is to understand the risks for environment, health and safety associated with this technology and the resulting materials and products (engineered nanomaterials), and how to identify and implement practical **strategies to minimise these risks**. Knowledge about nanotechnology-enabled processes and products is growing rapidly, achieved through numerous European or national programs launched over the last decade, but effective use of this knowledge for risk management by market actors is lagging behind.

Therefore, an initiative has been created to bridge the gap between scientific knowledge and the market, linking the nanosafety scientific community, including expert institutes/organizations active in translational research, with the wider stakeholder community. These are experienced partners working to assess and manage risks who already provide knowledge and technical services to public and private organizations, to industry and to public authorities and regulatory bodies. The proposed partnership will set up a structure to

integrate activities across the member states, and provide the **interface** between the scientific community and these other parties to develop and supply knowledge and technical services appropriate to each community.

For this, the project will gather together partner national institutes and agencies. The EC4SafeNano core group has also invited any interested organization to take part in the initiative as an Associated Partner helping to design the future European Centre and establish harmonized approaches for the proposed solutions and services. The **Associated Partners** will be active at the European level through their participation in the Focus Networks and will act in an 'ambassador' role for the member state where they are based. EC4SafeNano seeks to establish a record of accomplishment in developing **'fit-for-purpose' solutions** and providing access to **reliable data and experience** to help solve the range of environment, health and safety challenges that will be required to develop **safe and sustainable innovation for** nanotechnology.

EC4SafeNano also seeks to establish principles for safe management of nanotechnology based on the experience of its core group and associate members, and to assist public and private organizations and industry in the application of these principles. The core group and associates are experienced in providing expert advice to industry and other private actors, to public authorities and regulatory bodies and in communicating evidence based expertise to these different target audiences.

## 2 Scientific and technological challenges

A central challenge to ensure the sustainable production and use of nanotechnologies is to understand the risks for environment, health and safety associated with this technology and resulting materials and products (engineered nanomaterials), and how to identify and implement practical strategies to minimise these risks. Knowledge about nanotechnology processes is growing rapidly, achieved through numerous European or national programs launched over the last decade, but effective use of this knowledge for risk management by market actors is lagging behind.

Indeed, more than 50 projects on hazard characterization, exposure evaluation, and risk assessment of nanomaterials were conducted over the last decade1, generating substantial knowledge. However, a lot of scientific publications are not relevant or not useful2. Furthermore, the current education content in the field of nanosafety does not meet industry and job market needs3. The scientific community per se is not always well connected to industry and regulators. In order to bridge the gap between scientific knowledge and the market, the nanosafety scientific community should be consolidated by expert institutes/organizations, defined as being active in research and expertise, showing a high level of confidence in the results that they deliver and being able to assess and manage risks for themselves, for other public or private organizations, for industry and as support to public authorities and regulatory bodies. Acting as an interface between the scientific community, the industry, the regulators and the public society, these expert institutes/organizations can help to provide and communicate operational approaches and tools adapted to each community.

There is the need for the scientific and expert community to encourage, to integrate and harmonize (in terms of technical approaches, of services offered, of information delivered) and to further develop these initiatives so as to set up and make available to industry and other stakeholders concerned a European-wide, up-to-date, science-based, organisational structure to act at the interface between research organisations, industries, regulatory bodies, and the civil society (Figure 1), identifying, gathering and benchmarking all technologies, skills, tools, approaches and processes relevant for a robust, reliable and impartial expertise in safe innovation.


Figure 1: EC4SafeNano positioning at the interface between stakeholders

This need is urgent: there is evidence of serious current health and safety concerns related to nanoparticles. For instance in the field of occupational safety, there is a scientific consensus that inhalation of low toxicity nanomaterials is more hazardous compared to larger particles with the same chemical composition when exposure is quantified as mass, and there is evidence of European work place exposures that exceed the proposed nano-specific OELs.

The partnership have a track record in developing 'fit-for-purpose' solutions as well as access to reliable data and experience to help solve the range of environment, health and safety challenges that industry is facing. The core members have an established record in conducting safe operations and managing risks, for themselves, and for public and private organizations as well as for industry. They are sought after to give expert opinions to industry and other private actors (NGOs, citizens...), to public authorities.

## 3 Objectives

The overall objective of the EC4SafeNano project is to develop a distributed **Centre of European organisations for Risk Management and Safe Innovation for Nanomaterials & Nanotechnologies.** This will be independent and science-based and will support industry, safety service providers, regulators and public stakeholders. To do so the project will define and validate appropriate operating principles, as well as the necessary governance strategy to develop a sustainable self-funding structure. The structure of the Centre will be a hub-based network

of organizations operated by a core group of public-oriented bodies providing risk management and safe innovation support to all stakeholders. It will attract 'Associate Partners' to expand the capabilities, resources and services available, and it will interact with 'mirror' national hubs.

A secondary objective of the EC4SafeNano project is to **produce and promote guidance documents** on available tools, Standard Operational Procedures (SOPs), best practices, and an inventory of infrastructures etc. These actions will support market actors in implementing safe management of nanotechnology and enhance the overall capabilities and expertise in risk management and safe innovation for Nanomaterials and Nanotechnologies.

The overall resources and capabilities available within the EC4SafeNano Centre will make it possible to provide expert knowledge and technical solutions to enable the **safe production and use of nanotechnologies**. These solutions will address the needs of industry and governments to enhance European industrial innovation and competitiveness, and will evolve to reflect changing stakeholder needs for suitable tools and knowledge. The Centre will seek financial support from these stakeholders and service users to sustain the services in the longer term.

The general objectives of the EC4SafeNano project are to apply appropriate governance to the described structure, to develop operating procedures and to evaluate the Centres operational capabilities based on several case studies. These case studies will be defined during the project, and exemplify how the Centre for Risk Management and Safe Innovation for Nanomaterials & Nanotechnologies will operate on behalf of its stakeholders.

The operational objectives of EC4SafeNano are therefore:

- To **understand the needs of the various stakeholders** (private and public) and achieve a mapping of needs, both current and likely in the near future on a 5 year-horizon;
- To **identify the resources and capabilities available inside/outside the consortium to address the identified stakeholder needs**. This will be conducted inside & outside the consortium, including emerging / associated countries, with both a geographical and domain mapping of all resources available;
- **To provide solutions and build a range of services**, based on selected resources that answer stakeholder needs across the innovation value chain. Examples are: conducting routine tests, hazards and risks assessment, training, support in standardization or certification, sharing knowledge, offering access to communicative platforms and informatics tools;
- To **develop mechanisms and operating procedures** to facilitate periodic updating of "needs and resources" mapping and of the services proposed, to always propose the best available practices to meet the emerging needs expressed by the various stakeholders;
- To **test and benchmark the services** in order to check their relevance to address the needs but also to evaluate the governance of the structure delivering the proposed services;
- To **develop a sound exploitation plan and business plan** to prepare the self-sufficient operation of such a hub of expertise and services beyond the project lifetime.

# 4 Organisation

EC4SafeNano will act as an independent science-based European Centre to promote a harmonized vision of services for risk assessment and management for nanotechnologies and nano-enabled products across all industry and regulatory sectors. It will do this by working with existing EU and international networks or platforms. This will be achieved by sharing knowledge, tools and expertise as well as working collaboratively both at national and EU levels. The EC4SafeNano core group (see Table 1) has invited any interested organization to take part in the initiative as an Associated Partner helping to design the future European Centre and establish harmonized approaches for the solutions and services to be provided.

EC4SafeNano will deliver expertise for the public and private sectors based on the highest level of collective knowledge and operational tools and methods, as illustrated in Figure 2. It will also provide services to industry and regulators to increase EU competitiveness by enabling the safe development and commercialization of nanotechnology, as well as developing strategies to ensure the sustainability of these services.

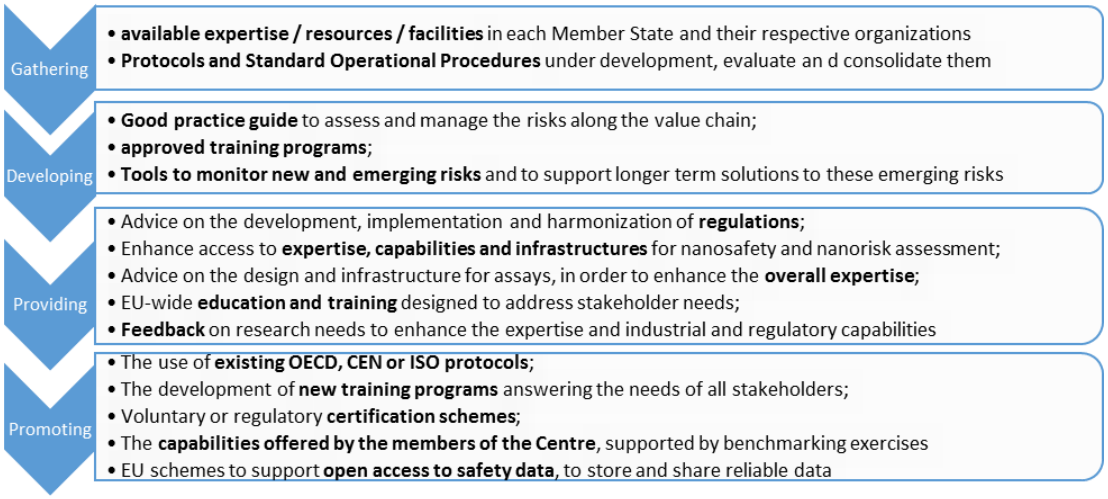



*Figure 2: Services to be provided by EU4SafeNano Centre*

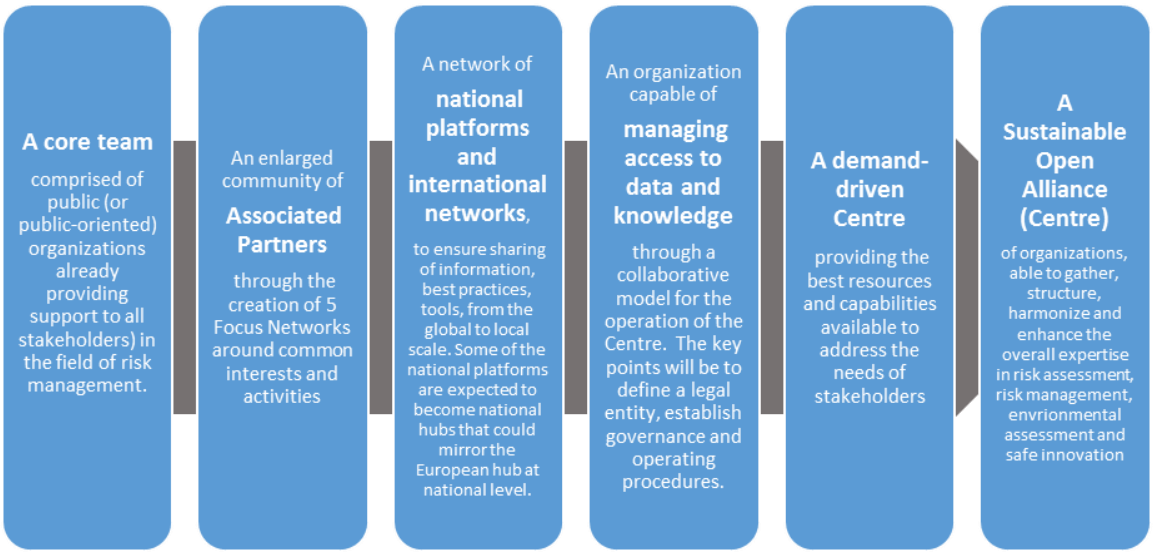



Figure 3: Pillars of the EC4SafeNano project

## 5 Implementation

A cornerstone of EC4SafeNano is to build a community to improve risk management and safe innovation for nanotechnology. The consortium has invited numerous companies/ institutions to join as **Associated Partners**. Partnership opportunities are still available.

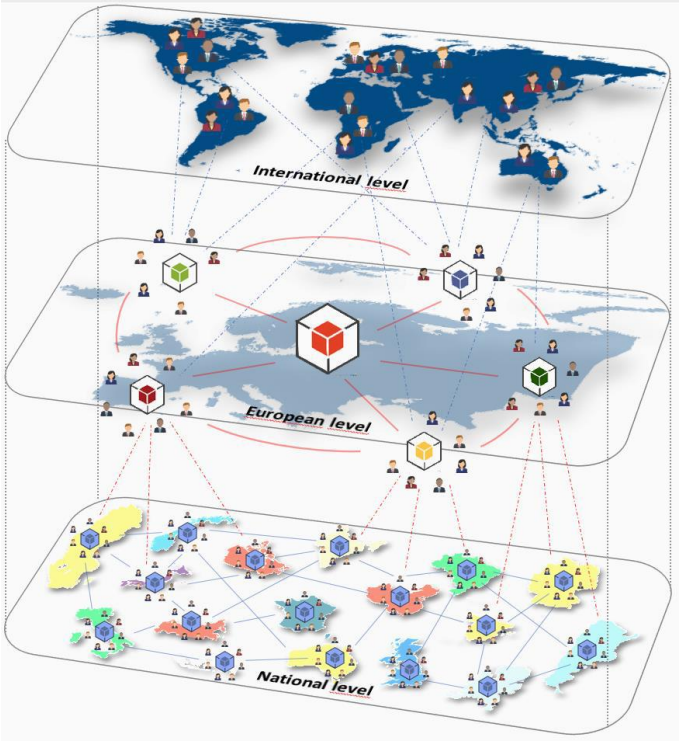



*Figure 5: The 3 levels of cooperation enable a quick exchange of knowledge and implementation of best available techniques at all scales for safety management.*

To deliver this project, seven work packages (WPs) were designed, lead by Core members with a partner for each separate task in each WP. The interrelationship between WPs is illustrated below.

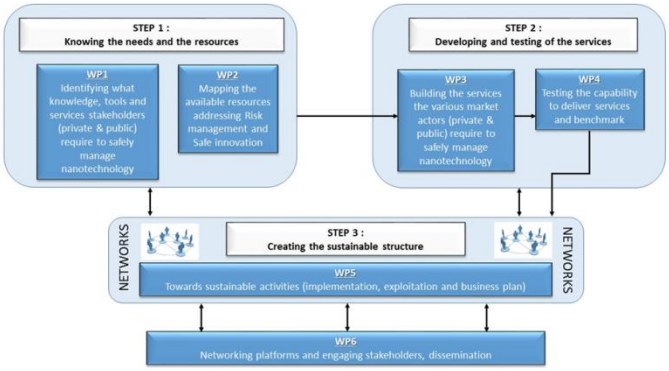



*Figure 4 : Flow chart of the work programme*

## 6 Progress to date

Partners in WPs 1-3 have prepared a survey, divided into 5 stakeholder groups to assess needs of all stakeholders, existing resources available throughout the nanosafety community, and the services available and required. Based on this survey, EC4SafeNano partners will construct the offer to the stakeholder community in terms of:

- Research
- Education and training
- Regulation and policy
- Standardization / certification
- Industrial innovation.

The Survey "Demand and supply of services in nanosafety" is currently open for respondents until 15.09.2017 via the project website: www.EC4SafeNano.eu

Events including a stakeholder workshop and contribution to the 2nd Open workshop on standardisation were held as satellite events to the EuroNanoForum meeting in Malta on June 19 and 20, 2017.

Progress towards outlining the different potential modalities of service provision is underway, including looking into the legalities of each mode, and the optimal way to develop and build the services.

A workshop on barriers to data sharing is being organised in May 2018 in conjunction with the Nanosafety Cluster and potentially the NanoObservatory.

The various stakeholder networks (WP6) will have their official launches in autumn 2017.

## 7 Expected Impact

**Five different types of impacts are expected.**

First, the project will be organised to allow information to be collated from the different partners and experts in the field of risk analysis and nanotechnologies. The goal is to address barriers to the ready exchange of knowledge and evidence.

Therefore, the project will map and analyse the requirements of its stakeholders whether industrial, research or public organizations. Based on the proposed catalogue of services, it will provide solutions such as methods, practices, standards, training or certification.

The main deliverables of EU4SafeNano are expected to be:

1. A robust collaborative open structure, gathering and sharing the best available resources and knowledge from across Europe and globally to promote safe innovation in nanotechnologies;
2. A set of operating procedures to operate the Centre and offer services to stakeholders along the innovation value chain, focused on removing barriers currently limiting knowledge sharing and distribution, and reducing uncertainty regarding environmental protection, safety and risk;
3. A dynamic cooperation through integration of networks, platforms and hubs connecting the nanosafety community and stakeholders to identify and solve issues related to nanotechnologies, including knowledge transfer to emerging economies and accession states;
4. 'Pathway' documents that identify the needs of the stakeholders and summarise the services, infrastructure and tools that the EU4SafeNano hub will provide for each stakeholder group.
5. Guidance documents setting out good practice standards relevant to market actors supporting safe innovation in nanotechnology.

## 8 Directory

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

| First Name | Last Name | Affiliation | Address | e-mail |
|---|---|---|---|---|
| Wolfgang | UNGER | BAM | Berlin, Germany | wolfgang.unger@bam.de |
| Effie | MARKOULAKI | DEMIKRITOS | Athens, Greece | emarcoulaki@ipta.demokritos.gr |
| Simona | SCALBI | ENEA | Rome, Italy | simona.scalbi@enea.it |
| Benoit | HAZEBROUCK | EU-VRi | Stuttgart, Germany | bh@eu-vri.eu |
| Kai | SAVOLAINEN | FIOH | Helsinki, Finland | kai.savolainen@ttl.fi |
| Gareth | EVANS | HSL | Buxton, UK | gareth.hsl.evans@hsl.gsi.gov.uk |
| Emeric | FREJAFON | INERIS | Ile de France, France | emeric.frejafon@ineris.fr |
| Ulla | VOGEL | NRCWE | Copenhagen, Denmark | ubv@nrcwe.dk |
| Albert | DUSCHL | PLUS | Salzburg, Austria | albert.duschl@sbg.ac.at |
| Andrea | FORNARA | SP | Borås, Sweden | andrea.fornara@sp.se |
| Jesus | LOPEZ DE IPINA | TECNALIA | Bilbao, Spain | jesus.lopezdeipina@tecnalia.com |
| Maaike | LE FEBER | TNO | Leiden, Netherlands | maaike.lefeber@tno.nl |
| Anthony | BOCHON | ULB | Brussels, Belgium | abochon@ulb.ac.be; |
| Iseult | LYNCH | UoB | Birmingham, UK | i.lynch@bham.ac.uk |
| Hilda | WITTERS | VITO | Mol, Belgium | hilda.witters@vito.be |

# 9 Copyright

# HISENTS

## High level Integrated SEnsor for NanoToxicity Screening

Grant Agreement: 685817 - H2020-NMP-2015-two-stage Website: http://www.hisents.eu

Coordinator: Professor Andrew Nelson, School of Chemistry, University of Leeds, UK

Deputy Coordinator: Dr Karen Steenson, Faculty of Engineering, University of Leeds, UK

*Table 1 Consortium List.*

| No. | Beneficiary name | Short name | Country |
|---|---|---|---|
| 1 | University of Leeds (Coordinator) | UNIVLEEDS | UK |
| 2 | Fraunhofer-Gesellschaft zur Foerderung der Angewandten Forschung e. V. - Institut fuer Biomedizinische Technik | Fraunhofer-IBMT | DE |
| 3 | Tel-Aviv University | TAU | IL |
| 4 | Blueprint Product Design Ltd | BPDES | UK |
| 5 | Slovak University of Technology in Bratislava | STUBA | SK |
| 6 | Technische Universitaet Wien | TUW | AU |
| 7 | Ustav Experimentalnej Onkologie Biomedicinska Centrum SAV | UEOSAV | SK |
| 8 | Fundacio Institut Catalá de Nanociencia I Nanotecnologia | ICN | ES |
| 9 | Universitaet des Saarlandes | USAAR | DE |
| 10 | Tyndall National Institute – University College Cork | TNI-UCC | IRL |
| 11 | Norsk Institut For Luftforskning (Norwegian Institute for Air Pollution Research) | NILU | NO |

## Contents



## 1 Summary

Project Duration: 36 months (April 2016-March 2019)

Project Funding: €6,332,825.00

The HISENTS vision is to address the problem of the dearth of high-quality tools for nanosafety assessment by introducing an innovative multimodular high throughput screening (HTP) platform including a set of individual modules each representing a critical physiological function connected and integrated in a hierarchical vectorial manner by a microfluidic network. The increase of the capacity to perform nanosafety assessment will be realised by innovative instrumentation developments for HTP and high content analysis (HCA) approaches. Toxicogenomics on chip is also one embedded objective. Our interdisciplinary approach focuses on tools to maximise the read-across and to assess applicable endpoints for advanced risk assessment of nanomaterials (NM). The main goal is thus to establish individual chip-based microfluidic tools as devices for (nano)toxicity screening which can be combined as an on-line HTP platform. Seven different chip-based sensor elements will be developed and hierarchically combined via a flow system to characterise toxicity pathways of NM. The HISENTS platform allows the grouping and identifying of NM. Parallel to the screening, the pathway and interaction of NM in biological organisms will be simulated using the physiologically based pharmacokinetic (PBPK) model. Using the different sensor modules from the molecular to cell to organ level, HISENTS can input quantitative parameters into the PBPK model resulting in an effective pathway analysis for NM and other critical

compounds. The developed platform is crucial for realistic nano-safety assessment and will also find extensive application in pharmaceutical screening due to the flexible modifications of the HTP platform. The specific objective is the development of a multimodular HTP platform as new a screening tool for enhancing the efficiency of hazard profiling. Currently, no such flexible, easy-to-use screening platform with flexibly combinable chip-based sensors is available on the market.

## 2 Background

State-of-art toxicology has been an experimental subject whereby the effects of toxicants on living organisms were assessed through *in vivo* experiments leading to dose-response curves which delivered parameters of median effective concentration (EC50) and/or median lethal concentration (LD50) which are effective and/or lethal respectively to 50 % of a population of animals. These parameters are the standard output of toxicologists. Parallel to the experimental routines, a theoretical predictive enterprise of organism toxicity has been applied for a long time. This is very popular since it avoids the expense and time associated with animal testing and allows for the screening out of putative toxicants. Originally these toxicity predictions were based on partition coefficient data and latterly comparisons with experiment where the hydrophobicity of the toxicant was correlated with its toxicity and/or bioavailability. These so called structure-activity predictive models have become more refined relating the electronic structure of the molecules to their biological activity. Nonetheless toxicology has always been basically a phenomenon-based science and there has been a dearth of a mechanistic understanding in toxicant biological activity. In addition the in vitro testing has been slow and not robust enough. This together with the emergence of many newly synthesised NM with uncertain biological activity has led to a drive for more rapid toxicity testing using *on-line* methodology and high throughput testing. Commonly these tests involve in vitro models but of increasing sophistication than earlier. Procedures are being developed employing artificial tissue on chip-based systems embedded in microfluidic platforms but to date there has been no attempt to integrate these into a global assay system.

This project therefore attempts to advance TWO arms of toxicity testing. The first is to develop these toxicity tests at successive levels of biological organisation and miniaturise them onto a fabricated chip-based platform embedded in a microfluidic network and the second is to integrate them into a global network so a novel systems biological and pathway analysis PBPK type approach can be taken in assessing the biological activity of the NM. The main goal of HISENTS is to establish a comprehensive multimodular screening platform for manufactured NM. An innovative miniaturised HTS tool will be developed with the capability of reliably analysing nano-bio-interactions within a platform of increasing complexity of biological organisation. The smart screening platform will be developed side by side with a PBPK model for predicting the ADME of NMs.

## 3 Scientific and technological challenges

The project HISENTS focuses on the development and application of a novel technology for nanosafety assessment. The novelty of this technology is that it allows the development of a multimodular HTS platform where each module represents one particular physiological target/barrier and each target/barrier is monitored separately (see Figure 1). Any kind of monitoring method is possible. All devices will be integrated into one screening platform through innovative instrumentation developments. The design of the multimodular platform will follow the AOP model which accounts for the critical pathway NMs in an organism. At the same time we will simulate the pathway and interaction with targets of a number of NMs in a model biological organism.

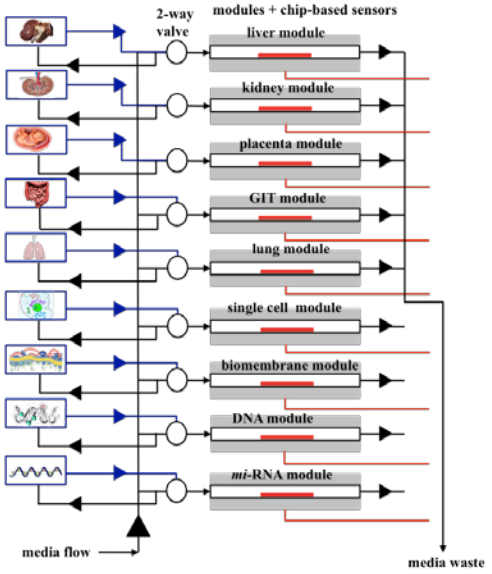



*Figure 1:* The HISENTS experimental/modelling platform.

The modelling procedure we will use is the PBPK model which employs ordinary differential equations to describe transport of compounds/materials from compartment to compartment. A reaction-diffusion approach as well as a MM and QC simulation to describe interaction with targets will be applied to develop the model which qualitatively at first deals with all modules in our proposed sensing platform. The modules will be developed in a hierarchy of organisation. Thus the first module will be the biomembrane which is the primary environment/organism interface, then the lung and intestine which are the routes of intake of NMs, then cells, genetic material and the liver as a site of metabolism of toxicants. Accordingly as a result of the experiments with the different modules, quantitative parameters will be inputted into the model and an effective pathway analysis for the NM as well as for their interaction with critical targets will be extracted. At the same time the simulation development will aid the design of the screening modules. During the project all of the modules will be integrated together according to the PBPK model to realise the concept of a multimodular screening system. The stress throughout the project is to miniaturise modules so that all screening will be chip-based and integrated within a microfluidic network. The project aim is to show by PoC that the multimodular design works which will act as a guiding light or

goal throughout the project. The screening program will be carried out systematically. Initially the screens will be tested and calibrated with the water soluble toxicant tricyclic phenothiazine, chlorpromazine. Once a consensus result has been obtained throughout the consortium, the devices will be tested with related tricyclic pharmaceuticals which have very well characterised ranked biomembrane activity and toxicology to achieve PoC for the toxicity sensing technologies. Subsequently standard and then novel NMs will be screened.

## 4 *Objectives*

The objectives as described below will be followed, and implemented as shown in Figure 2:

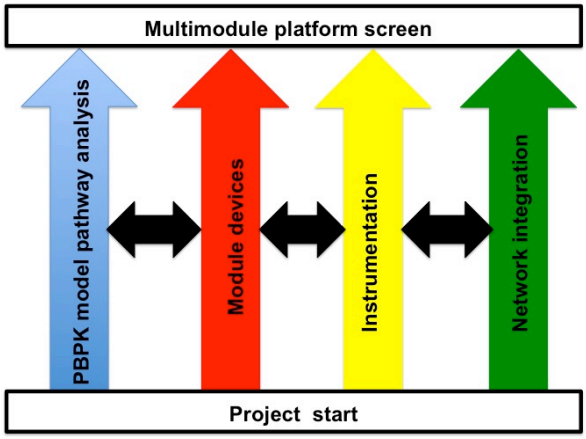



*Figure 2:* Overall strategy of the project HISENTS. Horizontal and vertical arrows indicate activity alignment and programme direction respectively.

**Objectives:**

1) Synthesise and characterise NM for use as standard test analytes for demonstrating individual device and platform performance.

2) Design and test effective screening devices, each representing a particular physiological function which can be incorporated in a multimodular platform as novel, relevant but realistic devices for sensing nanotoxicity. Individual chip-based tools will be set up as individual devices for (nano)toxicity screening and can be combined as an on-line High Throughput Screening (HTS) and/or High Content Analysis (HCA), as appropriate, multimodular platform. Nine different chip-based sensing modules will be developed each carrying a separate interrogable function/process associated with a biochemical/physiological function. All modules are integrated within a hierarchical flow system to characterise toxicity pathways for the individual toxicants and materials.

3) Configure robust electrochemical and optical techniques to interrogate the individual devices. Develop and innovate instrumentation for interfacing to sensor chips.

4) Incorporate and holistically integrate the screening devices into miniaturised and effective flow systems for HTS of NM along a directional pathway.

5) Develop smart automated signal processing data recognition techniques for qualitative and quantitative analysis of results.

6) Carry out comprehensive performance evaluation of platform initially with respect to each individual device. Calibration of results with corresponding *in vivo* data using standard invertebrate and mammalian indicator organisms.

7) Employ the concept of adverse outcome pathway (AOP) for deepening knowledge about the progression of toxicity events across scales of biological organisation which lead to adverse outcome (AO) relevant for risk assessment by performing an independent systems biological simulation of mechanistic processes derived from the PBPK model. This will involve the transport and bioactivity of individual NM, mechanistic pathway analysis and, correlation with experimental results.

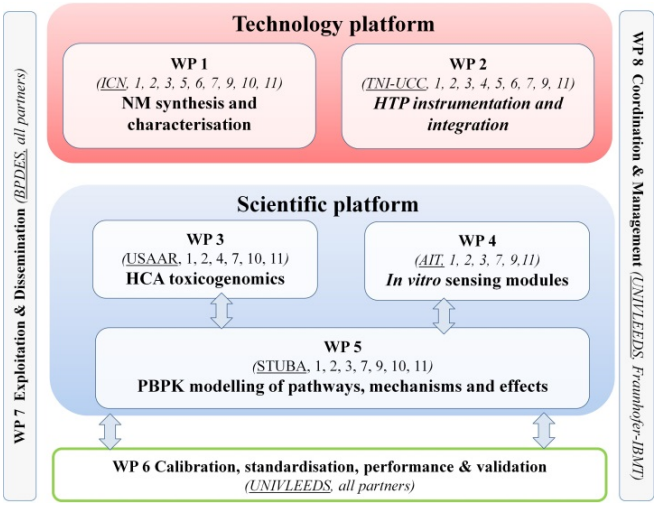



*Figure 3:* Detailed WP inter-relationship of HISENTS.

## 5 *Organisation*

The scientific (RTD) activities are conducted within six workpackages (WP1-6), with one other work package being specifically concerned with dissemination and exploitation (WP7). Each workpackage is managed by a workpackage manager who is responsible for the timely delivery of deliverables, to budget, to the Coordinator who in turn represents the Consortium to the Commission. WP8 concerns project management, which cuts across all work packages: WP1: NM synthesis and characterisation (Victor Puntes, ICN); WP2: HTS smart instrumentation and integration (Vladimir Ogourtsov, TNI-UCC); WP3: HCA toxicogenomics (Eckart Meese, USAAR); WP4: in vitro sensing modules (Peter Ertl, TUW); WP5: Modelling mechanisms, pathways and effects (Peter Simon, STUBA); WP6: Calibration, standardisation, performance and validation (Andrew Nelson, UNIVLEEDS); WP7: Dissemination and exploitation (Mick Karol, BPDES); WP8: Management (Andrew Nelson, Coordinator/Scientific Coordinator, UNIVLEEDS). Figure 3 above shows how these WPs are inter-related.

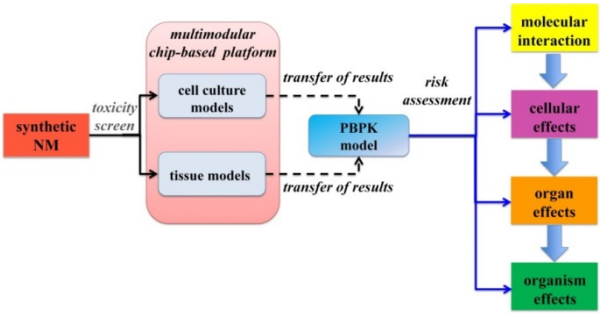



*Figure 4:* Adverse Outcome Pathway represents the scientific organisation of HISENTS.

## 6 Scientific achievements 2016-2017.

**Microfluidoc flow system prototype**: The University of Leeds has progressed well with the construction of a flow system prototype with the membrane sensor element module. The existing peristaltic pumps have been replaced with syringe pumps which are more easily controlled by automatic system. Computer software is being developed for automatic control of flow systems and the membrane module has been further miniaturised (*Figure 5*). The next steps are to introduce an oxygen removal system and to move from single module to multimodular.

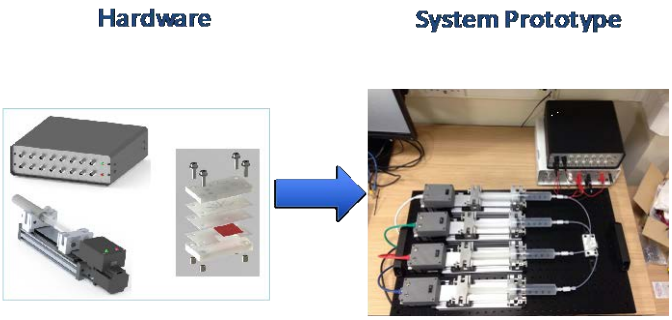



*Figure 4*: Photograph of automated membrane module prototype with streamlined flow system and flow cell.

**Modules for cell cultivation and imaging**: Modules have been developed for cell cultivation, imaging and electrical impedance measurement. These modules will be incorporated into a microfluidic system for the initial individual tissue prototype screeners (*Figure 6*).

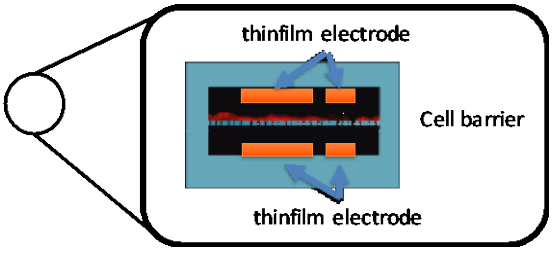



*Figure 5*: Diagram of first designed tissue screening module.

**Incorporation of DNA and *mi*RNA module into the membrane sensor element modular system:** Double-stranded 100 base pair long DNA conjugated to lipid tails .have been incorporated into phospholipid vesicles. The vesicles require an incubation time of up to an hour for successful DNA-lipid incorporation. The DNA-phopholipid doped vesicles as sensor element are then spread on to a phospholipid coated microelectrode. Proof of concept experiments show that the DNA-lipid conjugates sensitise the sensor element to methyl methanesulphonate (MMS) damage by a factor of 100 showing that the methylation of DNA by mms upsets the self-organisation of the phospholipid sensor element which is detected electronically.

**Reference NM interaction with biomembranes in the microfluidic module:** A series of home synthesised Au and Ag NM of varying shapes and sizes have been screened by the biomembrane sensor element module. Results show that the assay is rapid and indicates a response inversely proportional to particle size and directly proportional to aspect ratio. Ag particles indicate a very clear interaction with the biomembrane sensor element in contrast to soluble silver which shows no significant interaction. A series of JRC standard NM and NM sent out to all partners by ICN Barcelona have also been routinely screened in the microfluidic modular system as part of initial intercalibration experiments within the consortium.

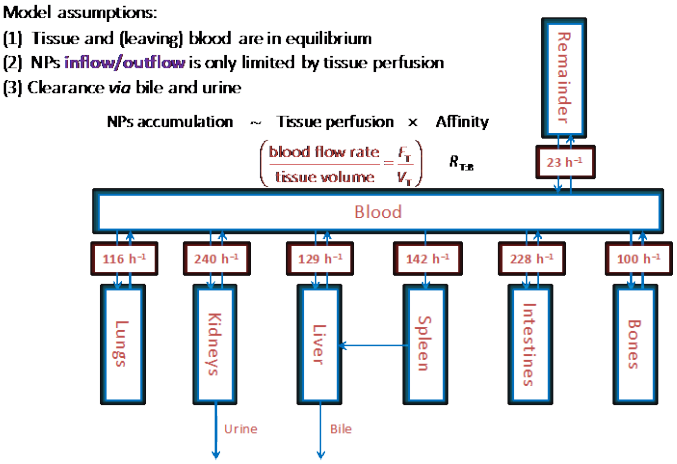



*Figure 7*: PBPK model for NM biodistribution

**Computer program for PBPK model**: The PBPK software is being developed. A model system has been built comprising seven separate tissue compartment modules including blood (*Figure 6*). Results for tissue NM concentrations versus time have been plotted and compared to published data. A good comparison was obtained. These results were extremely encouraging. Subsequent work will focus on comparing the output data from the program with experimental data obtained within the consortium from the membrane and tissue modules

**Device performance with standard calibrated toxicants**: An intercalibration exercise was carried out within the consortium with the aim of aligning and relating together of all the toxicity assays used by each partner. It was planned to use three water soluble toxicant compounds whose physical and toxicological properties were well established. The three toxicants chosen were chlorpromazine, colchicine and methyl

methanesulphonate (MMS). Chlorpromazine is biomembrane active, colchicine is a microtubule inhibitor affecting cell division and MMS methylates the DNA molecule. Results showed for the biomembrane module and cell culture assays a ranking order of toxicity of the toxicant compounds of chlorpromazine > MMS > colchicine. This indicates that toxicity to cell cultures is of a non-specific biomembrane damage nature. Comet assay showed only MMS to damage the DNA of A549 and HepG2 cell lines which is commensurate with its methylating properties. Colony forming efficiency chronic exposure tests showed the toxicant ranking order as colchicine > MMS > chlorpromazine. Colchicine is a microtubule inhibitor and therefore will inhibit cell division to form the cell colony.

## 7 Expected Impact

The emphasis of this programme is on high level interdisciplinarity which is indispensable for developing a platform technology. This programme brings together the most outstanding workers in nanotechnology, surface and colloid chemistry, toxicology, instrumentation and mathematical modelling to attack the innovative and ambitious problem of developing a global toxicological simulation model and aligning it to a comprehensive and integrated screening platform. The study has a wide impact since it delivers a third generation toxicity screening platform which has a mechanistic and pathway analysis functionality and possesses apparent commercial attractiveness as provides a cheap solution for many valuable practical application such as drug delivery and drug discovery.

The HISENTS project develops a new and comprehensive toxicity testing protocol combining the most up-to-date simulation procedures aligned with a multimodule screening platform where each module carries a specific (bio)barrier and/or (bio)target represented in the simulation. The close reinforcement of dual feedback of simulation with experiment and experiment with simulation is a disruptive technology and a step change in innovation. This innovation fills an at present unoccupied niche in the toxicity screening area. The new technology improves innovation capacity through its broad interdisciplinarity. The developments in fabrication technology, microfluidics, instrumentation and the interrogation of novel endpoints on a wafer-based platform are each at the cutting edge of 21st century science and can be developed independently for other applications e.g. drug delivery, drug discovery and chemical analysis. The rapid and comprehensive screening ability associated with the simulation model will enable many NM batches to be screened which enables the development of a close understanding of the detailed mechanism of the (bio)nanointeractions. Indeed it was shown in the previous FP7 project ENNSATOX that a close understanding of the toxicity mechanism could only be attained by a rapid screening. This is because NMs change their structure and conformation over time sometimes rapidly.

The HISENTS concept which is the combination of the multimodular screen with the PBPK model is a novel idea. It contains the distinctive features and possesses the positive effects which discriminate the platform from the prior art solutions thereby confirming its high patentability. This screening platform will attract much interest from pharmaceutical and personal care companies and health and safety, water, defence and security industries. There are also several international screening companies such as Cyprotex Ltd who would benefit enormously from adopting this new regime of toxicity testing thus improving their competitiveness. Personal care companies such as Unilever who are not able to use in vivo testing for the environmental and health screening of their products would find the technology developed during this project highly applicable since its multimodular facility incorporates all tests in one platform and is underwritten by a simulation programme. Pharmaceutical and cosmetic companies will be especially concerned with the structure-activity relationships and also the protocols developed to determine these.

## 8 Directory

*Table 2: Directory of people involved in this project – main contact highlighted in bold.*

| First Name | Last Name | Affiliation | Address | e-mail |
|---|---|---|---|---|
| Eva Beate | Andersen | Norsk Institutt For Luftforskning | Health Effects Laboratory, Environmental Chemistry Dept, Instituttveien 18, Kjeller 2027, Norway | eba@nilu.no |
| Neus | Bastus | Fundacio Institut Catala De Nanociencia I Nanotecnologia | Inorganic Nanoparticles Group, Campus De La Uab Edifici Q ICN2, Bellaterra, Barcelona 08193, Spain | neus.bastus@icn.cat |
| Paul | Beales | University of Leeds | School of Chemistry, Leeds LS2 9JT, UK | p.a.beales@leeds.ac.uk |
| Rik | Brydson | University of Leeds | School of Chemical & Process Engineering, Leeds LS2 9JT, UK | r.m.drummond-brydson@leeds.ac.uk |

| First Name | Last Name | Affiliation | Address | e-mail |
|---|---|---|---|---|
| Tibor | Dubaj | Slovenska Technicka Univerzita V Bratislave | Dept Physical Chemistry, Faculty of Chemical and Food Technology, Vazovova 5, Bratislava 81243, Slovakia | tibor.dubaj@stuba.sk |
| **Maria** | **Dusinska** | Norsk Institutt For Luftforskning | Health Effects Laboratory, Environmental Chemistry Dept, Instituttveien 18, Kjeller 2027, Norway | maria.dusinska@nilu.no |
| **Peter** | **Ertl** | Technische Universitaet Wien | Karlsplatz 13, 1040 Vienna, Austria | peter.ertl@tuwien.ac.at |
| **Alena** | **Gabelova** | Ustav experimentalnej onkologie, Biomedicinska Centrum SAV | Dubravska Cesta 9 845 05 Bratislava, Slovakia | alena.gabelova@savba.sk |
| Martin | Hanson | Blueprint Product Design Ltd | Main Office, 38 Market Street, Keighley BD21 5AD, UK | martin@blueprintproductdesign.com |
| Nicole | Hondow | University of Leeds | School of Chemical & Process Engineering, Leeds LS2 9JT, UK | n.hondow@leeds.ac.uk |
| Nik | Kapur | University of Leeds | School of Mechanical Engineering, Leeds LS2 9JT, UK | n.kapur@leeds.ac.uk |
| **Mick** | **Karol** | Blueprint Product Design Ltd | Main Office, 38 Market Street, Keighley BD21 5AD, UK | mick@blueprintproductdesign.com |
| Thorsten | Knoll | Fraunhofer-Insitut für Biomedizinsiche Technik IBMT | Ensheimer Str. 48, St. Ingbert 66386, Germany | thorsten.knoll@ibmt.fraunhofer.de |
| **Yvonne** | **Kohl** | Fraunhofer-Insitut für Biomedizinsiche Technik IBMT | Ensheimer Str. 48, St. Ingbert 66386, Germany | yvonne.kohl@ibmt.fraunhofer.de |
| **Rafi** | **Korenstein** | Tel Aviv University | Marian Gertner Institute for Medical Nanosystems, Dept of Physiology & Pharmacology, Ramat Aviv 69979, Israel | korens@post.tau.ac.il |
| Katarina | Kozics | Ustav experimentalnej onkologie, Biomedicinska Centrum SAV | Dubravska Cesta 9, 845 05 Bratislava, Slovakia | katarina.kozics@savba.sk |
| Nicole | Hondow | University of Leeds | School of Chemical & Process Engineering, Leeds LS2 9JT, UK | N.Hondow@leeds.ac.uk |
| Nicole | Ludwig | Universitaet Des Saarlandes | Dept of Human Molecular Genetics, building 60, Homburg 66421, Germany | n.ludwig@mx.uni-saarland.de |
| Lea | Madi | Tel Aviv University | Marian Gertner Institute for Medical Nanosystems, Dept of Physiology & Pharmacology, Ramat Aviv 69979, Israel | levana.madi@gmail.com |
| **Eckart** | **Meese** | Universitaet Des Saarlandes | Dept of Human Molecular Genetics, building 60, Homburg 66421, Germany | eckart.meese@uks.eu |
| Steve | Milne | University of Leeds | School of Chemical & Process Engineering, Leeds LS2 9JT, UK | s.j.milne@leeds.ac.uk |
| **Andrew** | **Nelson (Coordinator)** | University of Leeds | School of Chemistry, Leeds, LS2 9JT, UK | a.l.nelson@leeds.ac.uk |

| First Name | Last Name | Affiliation | Address | e-mail |
|---|---|---|---|---|
| **Vladimir** | **Ogurtsov** | Tyndall National Institute | Dyke Parade, Cork, Ireland | vladimir.ogurtsov@tyndall.ie |
| **Victor** | **Puntes** | Fundacio Institut Catala De Nanociencia I Nanotecnologia | Inorganic Nanoparticles Group, Campus De La Uab Edifici Q ICN2, Bellaterra, Barcelona 08193, Spain | victor.puntes@icn.cat |
| Mario | Rothbauer | Technische Universitaet Wien | Karlsplatz 13, 1040 Vienna, Austria | mario.rothbauer@tuwien.ac.at |
| Elise | Rundén Pran | Norsk Institutt For Luftforskning | Health Effects Laboratory, Environmental Chemistry Dept, Instituttveien 18, Kjeller 2027, Norway | elise.runden.pran@nilu.no |
| **Peter** | **Simon** | Slovenska Technicka Univerzita V Bratislave | Dept Physical Chemistry, Faculty of Chemical and Food Technology, Vazovova 5, Bratislava 81243, Slovakia | peter.simon@stuba.sk |
| **Karen** | **Steenson (Deputy Coordinator)** | University of Leeds | Engineering Research & Innovation Service, Faculty of Engineering, Leeds LS2 9JT, UK | k.a.steenson@leeds.ac.uk |
| Bob | Wadey | Blueprint Product Design Ltd | Main Office, 38 Market Street, Keighley BD21 5AD, UK | bobw@blueprintproductdesign.com |

# 9 Copyright

# LORCENIS

## Long Lasting Reinforced Concrete for Energy Infrastructure under Severe Operating Conditions


Contract Agreement: 685445 Website: https://www.sintef.no/projectweb/lorcenis/
Coordinator: SINTEF

*Table 1 Consortium List.*

| No. | Beneficiary name | Short name | Country |
|---|---|---|---|
| 1 | STIFTELSEN SINTEF | SINTEF | Norway |
| 2 | NATIONAL TECHNICAL UNIVERSITY OF ATHENS | NTUA | Greece |
| 3 | HELMHOLTZ-ZENTRUM, GEESTHACHT ZENTRUM FUR MATERIAL- UND KUSTENFORSCHUNG GMBH | HZG | Germany |
| 4 | UNIVERSIDADE DE AVEIRO | UAVR | Portugal |
| 5 | ACCIONA INFRAESTRUCTURAS S.A. | ACCIONA | Spain |
| 6 | CBI Betonginstitutet AB | CBI | Sweden |
| 7 | AGENCIA ESTATAL CONSEJO SUPERIOR DE INVESTIGACIONES CIENTIFICAS | CSIC | Spain |
| 8 | UNIVERSITEIT GENT | UGent | Belgium |
| 9 | FUNDACION AGUSTIN DE BETANCOURT | FUNAB | Spain |
| 10 | FUNDACION CIDETEC | CIDET | Spain |
| 11 | KVAERNER CONCRETE SOLUTIONS AS | KVAER | Norway |
| 12 | SMALLMATEK – SMALL MATERIALS AND TECHNOLOGIES LDA | SMT | Portugal |
| 13 | SIKA TECHNOLOGY AG | SIKA | Switzerland |
| 14 | DYCKERHOFF GMBH | DYCK | Germany |
| 15 | VATTENFALL AB | VATT | Sweden |
| 16 | CHEMSTREAM BVBA | CHEMS | Belgium |

## Contents



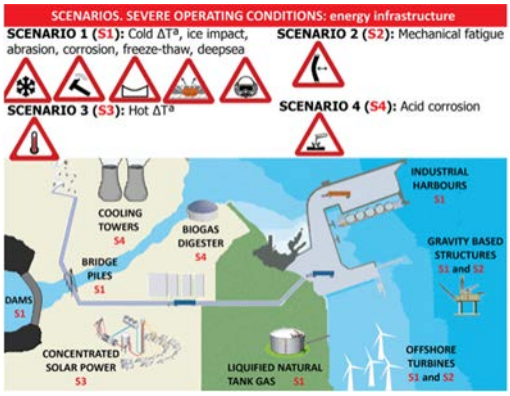
## 1 Summary

Project Duration: 48 months

Project Funding: 7,970,130.00 €

There will be an increasing demand for energy worldwide in the coming 30 years as population is steadily growing. New infrastructure projects for energy or transport require long service life spans, which often exceed those formulated in standards. In particular in aggressive environments, long-term durability of conventional concretes is not always in tune with service life expectations and limited funding or possibilities for maintenance.
The service life of engineering structures depends on their use, the environment they are exposed to, the materials they are built with and how they are built. Durable materials are paramount for safety and functionality of structures. Existing and emerging energy technologies require materials that perform in more and more extreme operating conditions as they are installed in sub-

arctic/arctic areas (low temperatures, ice-abrasion), desert areas (high temperatures), along coast lines (high chloride contents), deep-sea or underground (large temperature gradients and high pressure). At the same time, our society has to face environmental aspects caused by increased $CO_2$ emissions. New energy technologies are necessary to meet the energy request from carbon-based sources more efficiently at short term and to move to renewable energy sources at a longer term.




## 2 Background

The Consortium consists of partners with long experience from European research cooperation. Some of the partners have already established well-accomplished research collaboration and the partners complement each other very well within the Consortium. Each partner is given a clearly defined role in the work packages it is involved in and the WPs are established to give a well-defined outcome and contribution to the final project objective. Each one of the partners is highly experienced in the field of multi-functionalized nanomaterials and/or advanced concrete mix designs for energy infrastructures, as well as modelling actions and predictive simulations with engineering relevance.

## 3 Scientific and technological challenges

The need to develop materials which can perform well in severe operating environments is increasing with advances in technology and requirements for higher efficiency in all areas such as manufacturing, energy, transport and communications, deep-sea technologies etc. The general aim is to develop new products or components with a step change in efficiency or performance compared to existing ones, for operation in e.g. high radiation environments, highly corrosive environments, under high friction conditions, low temperature environments, deep sea or space environments, or other extreme climate conditions. Another important driver for advanced functionalities, e.g. self-diagnosis and self-healing, comes from the incorporation of nanoscale and molecular materials components. This poses a major challenge for materials science, and requires a fundamental understanding of how the processing, microstructure, nanostructure and properties of such material interact in order to enhance their response under more severe conditions.

## 4 Objectives

i) Concrete infrastructure in deep sea, arctic and subarctic zones: Offshore windmills, gravity based structures, bridge piles and harbours

ii) Concrete and mortar under mechanical fatigue in offshore windmills and sea structures

iii) Concrete structures in concentrated solar power plants exposed to high temperature thermal fatigueConcrete cooling towers subjected to acid attack

The goal will be realized through the development of multifunctional strategies integrated in concrete formulations and advanced stable bulk concretes from optimized binder technologies. A multi-scale show case will be realized towards service-life prediction of reinforced concretes in extreme environments to link several model approaches and launch innovation for new software tools.

## 5 Progress and Outcomes to date

During the first 12M of LORCENIS project, a variety of different multi-functional admixtures have been developed with increased compatibility with the cementitious matrices. Additionally, based on the deterioration mechanism of cement, different sub-scenarios related to the exposure conditions have been identified. For each scenario the best performing State-of-the-Art mix design has been defined with respect to the requirements on workability and strength development. Based on optimal combination of novel technologies including optimized binder technologies and multifunctional additives. These concrete mix designs represent the benchmarks for the further investigations and improvements in the LORCENIS project.

## 6 Expected Impact

The durability of sustainable advanced reinforced concrete structures developed will be proven and validated within LORCENIS under severe operating conditions based on the TRL (Technology Readiness Level) scale, starting from a proof of concept (TRL 3) to technology validation (TRL 5).

# 7 Directory

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

| First Name | Last Name | Title | Address | e-mail |
|---|---|---|---|---|
| Christian | Simon | Research Manager | Strindveien 4, Trondheim | Christian.Simon@sintef.no |
| Monika | Pilz | Dr. | | Monika.pilz@sintef.no |
| Costas | Charitidis | Prof. | Heroon Polytechneiou 9 Str. | charitidis@chemeng.ntua.gr |
| Elias | Koumoulos | Dr. | Heroon Polytechneiou 9 Str. | elikoum@chemeng.ntua.gr |
| Daniel | Höche | Dr. | Max-Planck-Straße 1 | Daniel.Hoeche@hzg.de |
| Mário | Ferreira | Prof. | | mgferreira@ua.pt |
| Jose Vera | Agullo | Dr. | Avda. de Europa, 18 | jose.vera.agullo@acciona.com |
| Urs | Mueller | Dr. | Brinellgatan 4 | Urs.Mueller@cbi.se |
| Maria Cruz | Alonso | Dr. | Serrano, 117 | mcalonso@ietcc.csic.es |
| Nele | De Belie | Prof | Sint-Pietersnieuwstraat 33 | Nele.DeBelie@UGent.be |
| Antonio L. Lara | Galera | Dr. | | alargal@fundacionabetancourt.org |
| German | Cabañero | Dr. | P° Miramón, 196 | gcabanero@cidetec.es |
| Kjell | Tore Fossa | Prof. | Oksenøyveien 10 | Kjell.Tore.Fossa@kvaerner.com |
| João | Tedim | Dr. | Rua dos Canhas | joao.tedim@smallmatek.pt |
| Emmanuel | Gallucci | Dr. | Zugerstrasse 50 | gallucci.emmanuel@ch.sika.com |
| Dirk | Qvaeschning | Dr. | Biebricher Straße 68 | Dirk.Qvaeschning@dyckerhoff.com |
| Tiina | Tarvis | Dr. | Evenemangsgatan 13 | Tiina.Tarvis@vattenfall.com |
| Geert | Deroover | Dr. | Drie Eikenstraat 661 | geert.deroover@chemstream.be |

# 8 Copyright

# NanoFASE

## Nanomaterial FAte and Speciation in the Environment

Contract Agreement: 646002 Website: http://www.nanofase.eu/

Coordinator: Claus Svendsen, Natural Environment Research Council, Centre for Ecology & Hydrology, Wallingford, UK

*Table 1 Consortium List.*

| No. | Beneficiary name | Short name | Country |
|---|---|---|---|
| 1 | Natural Environment Research Council | NERC | UK |
| 2 | University of Birmingham | UoB | UK |
| 3 | Acondicionamiento Tarrasense | LEITAT | ES |
| 4 | Eidgenössische Anstalt für Wasserversorgung, Abwasserreinigung und Gewässerschutz | EAWAG | CH |
| 5 | Institut für Energie- und Umwelttechnik e.V. | IUTA | D |
| 6 | Gothenburg University | UGOT | S |
| 7 | University of Vienna | UNIVIE | A |
| 8 | Wargeningen University | WU | NL |
| 9 | Oxford University | UOXF.DJ | UK |
| 10 | European Virtual Institute For Integrated Risk Management | EU-VRi | D |
| 11 | HEMPEL | HEMPEL | ES |
| 12 | Promethean Particles | PP | UK |
| 13 | Technical University of Liberec | TUL | CZ |
| 14 | FCC Construcción S.A. | FCCCO | ES |
| 15 | AMEPOX | AXME | PL |
| 16 | Inotex | ITEX | CZ |
| 17 | Applied Nanoparticles | AppNano | ES |
| 18 | Eidgenössische Materialprüfungs- und Forschungsanstalt | EMPA | CH |
| 19 | Institut National de l'Environment Industriel et des Risques | INERIS | F |
| 20 | Environmental, technical and scientific services | ETSS | CH |
| 21 | TNO Netherlands Organisation for Applied Scientific Research | TNO | NL |
| 22 | Rijksinstituut voor Volksgezondheid en Milieu, Ministerie van Volksgezondheid, Welzijn en Sport | RIVM | NL |
| 23 | University of Geneva | UoGEN | CH |
| 24 | Malvern Instruments Limited | MIL | UK |
| 25 | University of Plymouth | UoP | UK |
| 26 | University of Ljubljana | UNI-Lj | SLV |
| 27 | VU University Amsterdam | VU-Vumc | NL |
| 28 | University of Aveiro | UAVR | P |
| 29 | Stichting Dienst Landbouwkundig Onderzoek | RIKILT | NL |
| 30 | Universita Ca' Foscari di Venezia | UniVE | IT |
| 31 | PENSOFT | PENSOFT | BG |
| 32 | SYMLOG France | SYMLOG | F |
| 33 | GBP consulting | GBP | UK |
| 34 | Perkin Elmer | PE | S |
| 35 | Swedish University of Agricultural Sciences | SLU | S |

## Contents

## 1 Summary

Project Duration: 48 months (Sep 2015-Aug 2019)

Project Funding: €11,296,701.25

The overarching objective of NanoFASE is to deliver an integrated Exposure Assessment Framework of models and characterisation protocols that will allow all stakeholders to assess the full diversity of industrial nano-enabled products to a standard that; i) is acceptable in regulatory registrations, ii) allows industry a cost-effective product-to-market process, and iii) delivers the understanding at all levels to underpin public and consumer confidence.

NanoFASE will ensure the Framework is delivered in a form that supports both the regulatory and technical guidance developments needed, via direct and continuous industrial and regulator stakeholder engagement and dialogue, throughout the project lifetime. Through this emphasis on stakeholder engagement and input to the Framework development process, NanoFASE will develop models and methods that are ideally positioned for incorporation into current mainstream chemical assessment tools, policy and regulation (e.g. EUSES & REACH). We will provide the underpinning science to enable the state of the art in ENM Fate and exposure assessment to move towards a level at least comparable with that for conventional chemicals.

## 2 Background

To make tangible progress on nano exposure assessment in the environment and its inclusion in nano regulation, a pragmatic and realistic approach is needed to reduce complexity. This was evident from the 3 day OECD expert meeting on categorisation of manufactured nanomaterials (Washington DC, Sept 2014). At this key event, attended by NanoFASE experts, the need to move focus away from the physical/chemical properties of pristine ENMs and understand the functional and behaviour patterns of ENM in exposure relevant environments was identified as a priority. In addressing fate and transport in real environments, NanoFASE meets this challenge.

## 3 Scientific and technological challenges

NanoFASE will develop a set of novel concepts and approaches to underpin the Framework, developed as common themes linking the research, exploitation and dissemination across the different WPs.

1) "Reactors": NanoFASE will consider different release processes (e.g. weathering or direct release), waste-streams (e.g. incinerators, wastewater treatment, landfill) and environmental compartments (e.g. air, soil, water/sediment, biota) as a set of "reactors" that are able to transform ENMs from the highly engineered high energy states achieved during fabrication to environmentally transformed lower energy forms (Fig. 1). The concept of "reactors" aligns the different environmental compartments in the order that released ENMs will encounter them. This approach is consistent with the structure of multimedia fate models, where each "reactor" can be defined as a physicochemical or biological environment encountered by ENMs, in which transformations occur that govern further fate pathways. Each "reactor" model can be developed independently, and networks of reactors can be linked through a spatial transport framework to efficiently construct spatially–realistic simulations of real product value chains and environments.

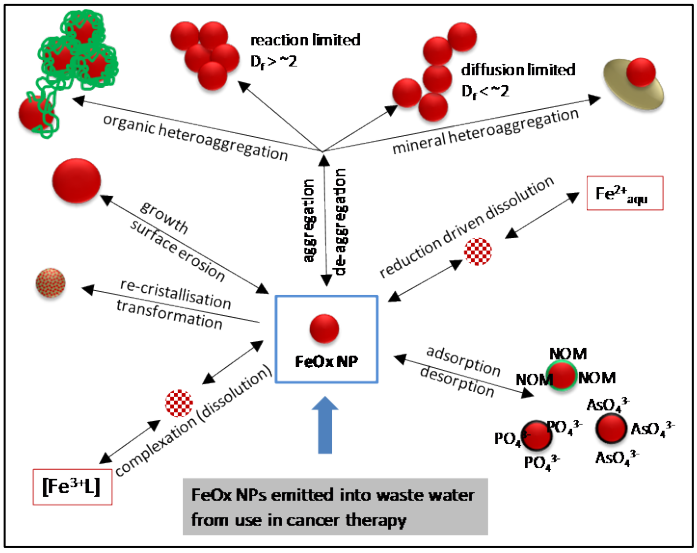



Figure 1: Likely transformations of FeOX NPs in water reactors.

2) Functional fate groups: a tool to understand and reduce complexity: Each ENM form, whether transformed or not, has properties that determine its subsequent transformations. The interplay between particle properties and conditions in the environmental "reactor" in which the particle resides will determine which reactions will occur, and how fast. From the vast diversity of future ENMs and possible "reactor" characteristics, a myriad of possible outcomes could occur (see Fig. 2), especially when the potential for ENMs to pass through different reactors is taken into account. However, dominant pathways of transformation can be identified when ENM properties, hydrochemistry and environmental colloid chemistry parameters for each "reactor" type are taken into account and once environmentally realistic observation timescales of days and weeks are considered instead of minutes. The dominance of some processes at these longer temporal scales will in many cases act to reduce complexity, e.g. through conversion of several different applied coating forms into the same effective environmentally derived coating (eco-coronas) or converging core transformations (see Fig. 3).

   Information on such dominant transformation and transport processes will allow ENMs to be grouped into Functional Fate Groups according to their "most probable" fate pathways. The Functional Fate Groups (FFG) concept can thus condense the richness and variability of existing and future ENMs into behavioural categories that summarise likely environmental fate and behaviour. This offers the potential to use read

across to similar material as a common approach to understanding post release behaviour.

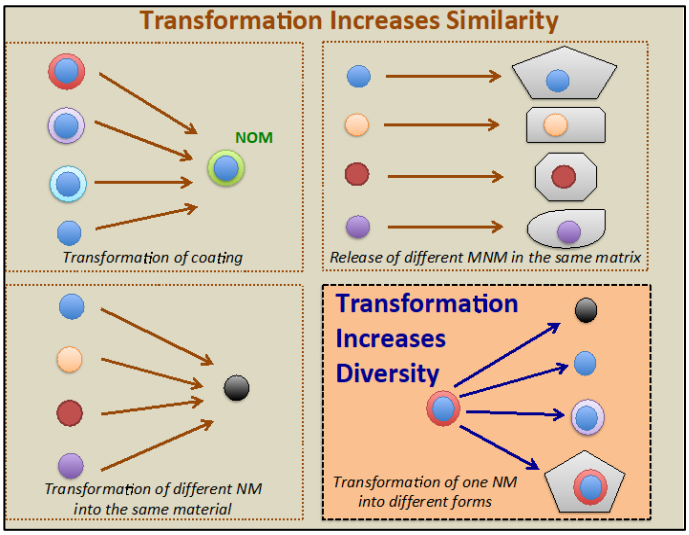



Figure 2: Mitrano & Nowack (EMPA), et al. "Report on environmental transformation reactions" NanoMILE D3.1

3) Multimedia fate modelling: The major tools for fate prediction within the overall Framework will be the two multimedia fate models, SimpleBox4Nano and the NanoFASE model system. SimpleBox4Nano simulates at regional to continental scale for screening level fate assessment. The NanoFASE model system will perform more complex, spatially-explicit simulations at smaller scales. It will simulate geographical area(s) as a network of cells. Within each cell, "reactors" (as described above) will be linked by transport functions (e.g. sedimentation, deposition, effluent release, soil runoff, biota uptake). Implementation of material flow among cells (e.g. water flow, air movement) will enable multimedia transport modelling and fate prediction. The model system can be made geo-specific using various GIS overlays (see Fig.1). Understanding the dynamic rates of the transformation processes in each "reactor" and how the properties of the resulting transformed ENMs affect their within- and between-compartment transport then becomes central to predicting their fate and ultimately environmental exposure

4) Use of real industrial and bespoke aged ENM test sets: To develop understanding of specific processes, NanoFASE will use a carefully selected "research and training set" of "as manufactured" and "bespoke transformed ENMs", to represent the relevant product, release, and process or environmentally aged ENM forms, in the development of the specific "reactor" process studies. A set of case study products developed by NanoFASE Industry partners will be used to provide a "validation set" including current Industrial on market, and novel near- or future-market ENMs to provide a series of cross cutting case studies for validation of the overall fate and exposure framework developed within NanoFASE.

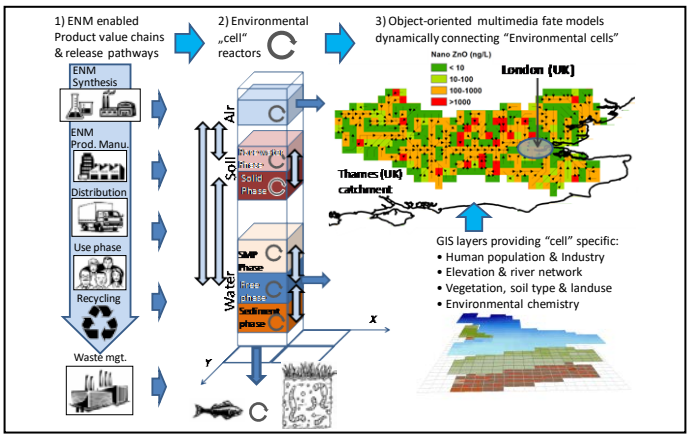



Figure 3: NanoFASE conceptual workflow for delivering dynamic multimedia fate prediction both in a generalised model environment and GIS enabled mode.

5) Method, parameter and model catalogues: The reactor–based process understanding of ENM transformation and transport gained in NanoFASE will underpin the derivation of parameterised submodels for specific fate processes in air, soils, waters, sediments and biouptake (e.g. dissolution, (hetero)agglomeration etc.). These submodels will be incorporated into the NanoFASE model system, which will couple transformation and transport processes into a spatial framework and link them to exposure and bioaccumulation. However, to provide greater flexibility and usability of the submodels, rather than designing only a single "closed code" modelling tool, NanoFASE will also collate a catalogue of parameterised submodel algorithms, describing transformation processes, that can be incorporated by stakeholders into existing exposure and risk assessment frameworks in order to "nano-enable" them. As a demonstration of this flexibility, NanoFASE will incorporate suitable, parameterised process algorithms into the SimpleBox4Nano screening fate assessment model. SimpleBox4Nano is a development of SimpleBox, which underpins the widely used EUSES tool. As a product of model parameterisation and the Functional Fate Group categorisation, a "method catalogue" will be compiled of functional assays, including standardised ENM characterisation analysis methods that are operationalised (with the instrumentation manufacturers) for real environmental analysis. The combination of the model catalogue, and identification of the Functional Fate Groups, will also aid Safe by Design and Benign by Design Concepts, as it will inform on how basic ENM properties will affect their final environmental form(s) and distribution following environmental release, allowing this to be a relevant consideration in the design phase.

## 4 Objectives

To deliver a streamlined approach to regulation that supports sustainable innovation in nanotechnology, NanoFASE aims to produce a new state of the art framework for evaluating environmental release, fate and exposure for ENMs. Our vision is

to move from the current mainly mass-based lifecycle and release flow approaches towards systems that can account for spatial and temporal variability of ENM release, environmental transport and fate. The framework, supported by standard operating procedures (SOPs), parameter values, models and guidance, will incorporate (i) the behaviour of the actual relevant ENM forms released from ENM products (a distribution of composite bound and free particles); (ii) how reactions in waste management and environmental compartments transform such release-relevant ENMs (integrating environmental speciation with ENM properties); and (iii) the consequences of these transformations for transport and fate and among the different environmental compartments including organism uptake.

The detailed objectives to be delivered by NanoFASE to achieve this shift in the state of the art are:

1. Enabling "form-specific" release modelling, by development of detailed understanding of i) product-type and product-use based release forms; and ii) release pathways of ENMs across ENM–enabled product chains (i.e. manufacturing, accidents, weathering, use, recycling and waste management) (WPs 4,5).

2. Optimising current, routine "clean media" methods for ENM characterisation to deliver repeatable and reproducible results in environmentally relevant "complex" matrices. These protocols will provide practical methods to support future ENM exposure prediction and monitoring (WPs 3,6,7,8,9). We will continue to impart such state-of-the-art knowledge on metrology and standardisation issues into the international harmonisation and regulation efforts through exploiting consortium links with the OECD, ISO and CEN, via already engaged partners and our Advisory Board (WP11,12).

3. Developing a catalogue of process-informed compartment models, to describe how the distributions/populations of ENM forms entering all key waste management or environmental compartments transform in time (WPs 5,6,7,8,9).

4. Working closely with stakeholders to develop a fate and exposure assessment framework (WP1) comprising validated SOPs, product value chain and waste management release modules (WP4,5), parameterised transformation algorithms (WP4-9) and multimedia fate/exposure models (SimpleBox4Nano and the NanoFASE model system) (WP2), along with guidance for stakeholder use. The framework will be validated and road–tested using real–world case studies to ensure completeness and quality of the methods, data and models included (WP3, 10, 11).

5. Ensuring that the method and model developments have the widest and highest possible impact, by working with stakeholders to enable incorporation of developed methods and standards into existing exposure prediction tools to increase the nano-capability of regimes for general chemicals (e.g. REACH), product, waste and environmental assessment, facilitated by direct contact with ECHA and the OECD WPMN via the Advisory Board.

The achievement of the above objectives is critical to ensure the framework will;

• Be applicable to the full diversity of ENM–based product types and uses within the current industrial nano-market and can take account of other near-market and future market products and requirements

• Assess processes across the full product value chain to determine release of ENMs including their form

• Employ analytical methods that can quantify ENMs along the product value chain, in managed waste-stream and the environment and track their transformations and fate behaviour, while being tractable to operationalise.

• Develop methods and standards that are applicable for contract-laboratories working to Good Laboratory Practice (GLP) criteria.

## 5 Progress and Outcomes to date

To facilitate delivery, NanoFASE will be coordinated (WP11) through a set of WPs linked through the common aim, objectives and concepts of the project. In short the project will be guided from the stakeholder focused WP1, which will initially draw up the outline specification for the Exposure Assessment Framework. WP1 will, with the other two crosscutting WPs (WPs 2 and 3), enable and drive overall scientific coordination and delivery around the common goal of framework development.

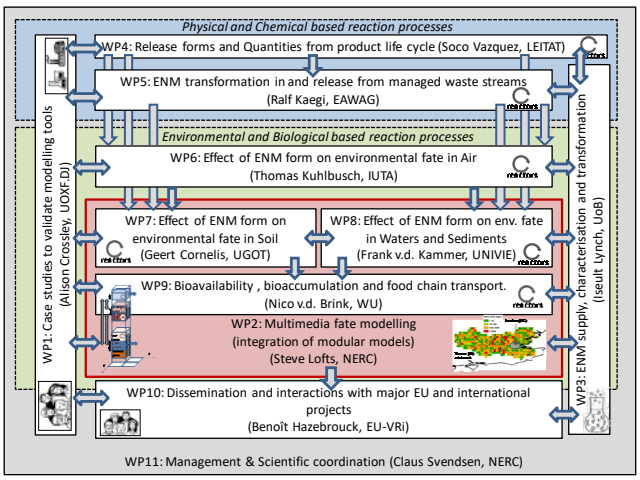



Figure 4: NanoFASE PERT chart shows the interrelationship of work packages and flow of materials, information and between WPs.

To date WP1 has produced an outline of the exposure assessment framework (EAF). Development of the EAF will continue throughout the course of the project until it launches in June 2019 (Deliverable D1.3). In the last year, a range of particle producers, regulators and academic experts have been consulted with regard to the format, scope and stakeholder/user community likely to utilize this framework.

A second major deliverable (D1.2) from WP1 (in collaboration with WP4 and the industrial partners in the project) was to produce a report on a pathway analysis along and beyond the product value chain to establish for our case study particles (i) potential release points to the environment (with WP4, 5) and (ii) potential pathways through the environment following disposal/release (with WP2, 6–9) to develop real case study profiles of production, use and disposal of ENMs. We have included the source, volume and type of starting material (nanoparticles and coatings), how this is incorporated into products and transported, market details and disposal options where known. This will inform WP2 on model development and integration.

In the coming months WP1 will report on feedback from stakeholders on development of our case studies (D1.5; due September 2018). WP1 tried to include current uses as well as near–market developments and have identified products in the pipeline which will be improved by reducing the particle size of the active ingredients to the nano scale. These include both improvement in properties and conservation of resources. WP2 is thus guiding the experimental work packages to focus on the issues that industry and regulators prioritise and to ensure as much as possible consistency across WPs 5-9 in terms of choice of nanomaterials to use in experiments. It is hoped any ENM transformations can be identified in all compartments leading to improved and tested models. The experimental work packages have challenging objectives so when required will use related material provided via WP 3 to develop experimental protocols and modelling tools to be then tested on the industrial case studies where possible. Throughout the project WP1 will continue to have a pivotal role and will work closely with the other partners in the project and external stakeholders including industry, regulators and government departments to ensure the quality, effectiveness and ease of use of the outputs.

WP2 is responsible for developing the spatially-explicit modelling frameworks, for predicting nanomaterial fate and biouptake in the environment, that form core outputs of the NanoFASE project. To do this, we are developing two complementary modelling systems. For screening level assessment we will further develop the SimpleBox4Nano model. SimpleBox4Nano is a nanomaterial-specific development of the SimpleBox model, which underpins the EU's chemical risk and safety decision-support tool EUSES. For the more complex, spatially explicit fate and biouptake assessment, we will develop a coupled, gridded, soil-water-sediment transport framework with descriptions of nanomaterial transport, transformation and biouptake – the NanoFASE model.

The aim of developing a new, nanomaterial-specific model is to take advantage of state-of-the-art approaches to model structuring, specifically the concept of object–orientation. This allows the physical structure of the environment (including nanoparticle populations) to be represented in code by a structured collection of discrete 'objects' (Fig. 5). It permits an efficient separation of transport and transformation algorithms for nanoparticles. It also allows the definition of a nanoparticle 'type', i.e. the set of algorithms that describe the behaviour of a specific group of nanoparticles having common transformation behaviours. New types will be readily added to the model system, and existing types extended, making the NanoFASE model able to be extended to consider types of nanoparticles not yet subject to environmental release.

The development of the NanoFASE model follows a clear set of stages and milestones:

• Development of a written specification for the model (D2.1; completed November 2016). This document is the blueprint for the actual coding work, including connecting the model to the atmospheric compartment (WP6) and the product value chain and managed waste sources (WP4, WP5).

• Coding of the gridded spatial transport model for the soil and water compartments; (D2.2; Due to complete November 2018)

• Initial incorporation and parameterisation of algorithms for the transformation and biouptake of nanoparticles within soils, waters and sediments;

• Initial testing of the model against specific case studies (WP1);

• Final updating and parameterisation of algorithms, final testing against case studies.

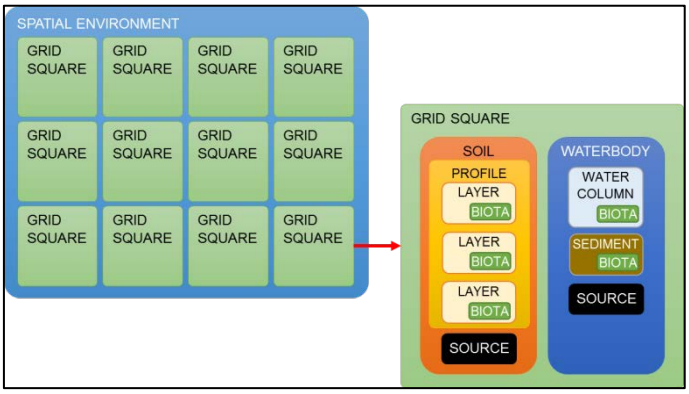



Figure 5: Proposed object structure for the soil and water components of the NanoFASE model.

WP3 contributes centrally to the project through its overarching aim to ensure timely supply of ENMs and their environmentally aged (functional fate form) variants to each of the environmental receptors workpackages (WP5-WP9) and the characterisation of all NanoFASE ENM under the relevant exposure conditions. WP3 is also integrating the Standard Operational Procedures (SOPs) for characterisation of ENM including so-called Functional Fate Assays, which aim to quickly and simply understand the environmental transformations and final forms of ENMs in each of the different environmental compartments, including their associated biomolecule coronas. WP3 in responsible for managing and integrating the NanoFASE datasets to facilitate linking of the ENM physico-chemical characteristics under different environmental conditions to their fate and behaviour data, including speciation and bioaccumulation. All NanoFASE data will be incorporated into the NanoFASE Knowledge Base, thereby ensuring harmonisation of fate and (previously generated) hazard data on the common ENMs (see below for more details).

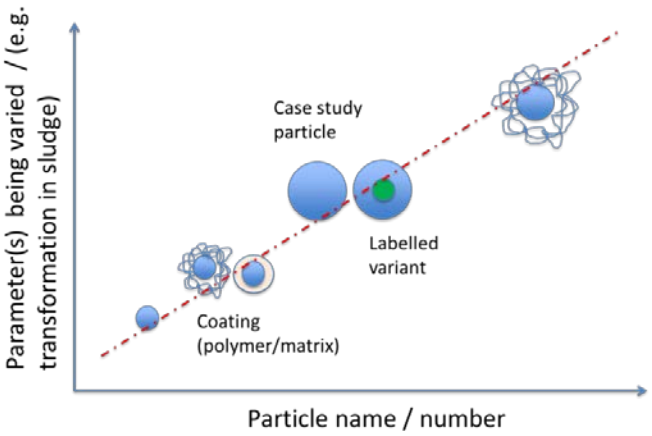



Figure 6. Schematic representation of the NanoFASE approach to integrating real-world industry case study materials and the bespoke or commercial particles required to facilitate determination of key model parameters (e.g. labelled for traceability and discrimination from natural background). Thus, the case study particles will be central and specific parameters will be varied around this core, such as particle size, coating, surface

ageing etc.) to facilitate integration of case studies throughout the models developed.

Based on the key datasets needed for the integrated spatially-explicit modelling frameworks for predicting nanomaterial fate and biouptake in the environment, WPs 5-9 have provided a list of ENM families and key characteristics that should be systematically varied (coating composition, size, doping with tracer elements etc.) for WP3 partners to produce / provide. Initial selections included Ag ENM from partner Amepox (8 and 6nm), a range of TiO2 ENM with different coatings from PROM, and a set of bespoke core-shell particles of holmium cores (to allow distinction from natural background) surrounded by titania or ceria shells. Other particles developed for NanoFASE include dyspium-doped Cu(OH)2 nanowires for use in the soil mesocosm studies (WP9) in order to enable detection against background and map to the nano-pesticide case study, and Ag2S particles to mimic the form of Ag ENMs that results from waste water treatment plants.

Central to WP3 is the need to develop innovative approaches for producing gram-scale quantities of ENM artificially aged to produce materials representing the identified functional fate groups (FFGs) for further fate testing – this will be achieved in collaboration with our industry partners PROM and AppNano, based on transformations identified in the environmental receptors via WPs 5-9. PROM recently completed a pilot project (FP7 SHYMAN) in which they developed a 1,000 tonne/annum facility and are currently producing large batches of numerous coated and uncoated metal oxide ENMs for NanoFASE.

NanoFASE has also agreed with FP7 project NanoMILE and its data management partner Biomax to extend the KnowledgeBase to cover the additional aspects of relevance to NanoFASE on fate characterization of ENMs in the environment. While initially each project will have its own entry to the portal, and will work with its own set of ENM (although with overlaps as far as scientifically possible), the data are organized in a manner that allows progressively open sharing of the data, for example, as datasets get published. Fate is the key parameter for exposure, and exposure and hazard combined determine the risk. The agreement with Biomax allows the possibility to easily combine hazard and fate data for risk assessment and safety-by-design. This first construction of sister databases could show the way to a meta-database on nanosafety that would allow to capitalize and better exploit the huge amount of high -value data obtained in years and tens of millions of Euros of European nanosafety research, as currently actively discussed within the NanoSafety Cluster. Other projects are welcome to join this initiative.

WP4 contributes to the transversal NanoFASE modelling and experimental work aiming to predict the quantities and forms of ENM released to each environmental compartment at all the life cycle stages of nano-enabled products. This work package focuses on the identification and quantification of ENM releases from nano-enabled products. Sectors and categories with higher potential of ENMs release to the environment will be identified and its life cycle stages analyzed. Furthermore, an integrative model for predicting environmental releases, including those stemming from accidental situations, will be developed.

Since project start in October 2016, WP4 has established communication with the NanoFASE industrial partners and obtained information about the case studies developed in WP1. This information has been used to perform pathway analyses of different ENM, detecting the environmental compartments in which most of the released ENM will end up as well as the transformation that the ENM could experience. More detailed and quantitative information will be obtained through organizing field campaigns in ENM production plants and performing lab scale simulations of the case studies for manufacturing, use phase and recycling processes including accidental release scenarios in all of them. The data collected will be shared with other WPs to design any additional studies needed to understand the transformation of these released ENM in the different environmental compartments (i.e. air, water, soil, and biota).

In deliverable D4.1 an inventory of estimates of ENMs and nano-enabled products value chain along with a first country-specific ENM release model for Europe has been produced (for example see Fig. 6). Releases are quantified for the different stages (production, manufacturing, consumption of ENMs), and also the ENM flows into waste management (e.g. landfilling, recycling, incineration) are improved. Data from WP1 case studies considering production, manufacturing, consumption, recycling and end-of-life in the EU countries were gathered and structured to generate a release database with all the parameters needed by the new design of the release model. Moreover, a second model will be developed to predict accidental emissions of ENMs to the environment, focusing on major accidents (i.e. factory explosion). The model will enable assessment of not only the extent of potential environmental exposure but also the probability of occurrence.

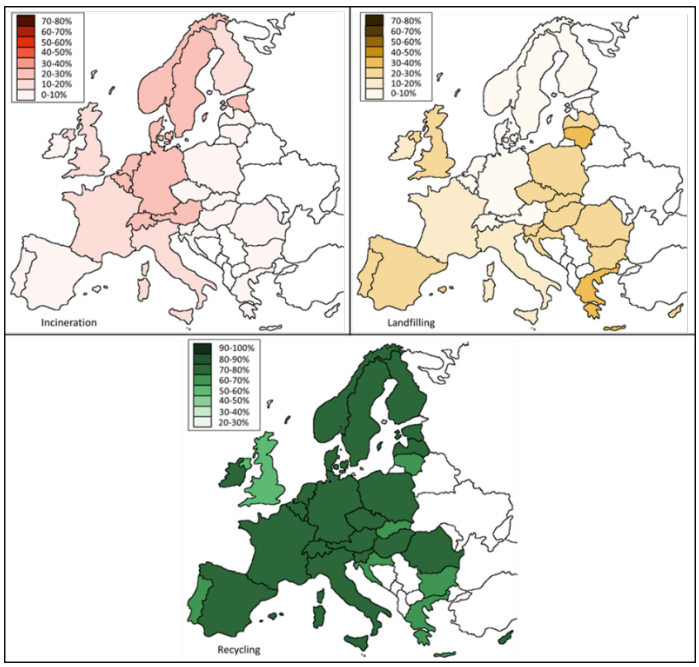



Figure 6. Carbon black ENM waste stream shares of landfilling, incineration and recycling in European countries

WP5 will establish transformation and release rates of ENM during their passage through different reactors. We are focusing on wastewater treatment plants (WWTPs), solid waste and dedicated sewage sludge incinerators as well as landfills (Fig. 7). Additionally, lab-scale experiments using pristine and well characterized materials, representing the realistic fate relevant forms at each stage, will allow us to obtain a mechanistic understanding of the transformation processes in waste treatment reactors. Our experimental results will feed directly into the development of a

mathematical model describing the transformation and transfer of ENMs through the investigated reactors.

WWTPs are major hubs for ENMs released from consumer products during their use phase. We are moving to fill in the knowledge gaps. Right now WP5 is spiking a pilot WWTP with selected ENMs over a period of several months. We'll monitor the transfer of the ENMs through the WWTP, using a combination of bulk analyses and single particle based methods. This will reveal to which extent ENMs are transformed in the WWTP and whether particles are discharged as individual nanoparticles or as (homo-/hetero-) agglomerates. Following these initial experiments, ENM enriched sewage sludge will be incinerated and/or pyrolyzed and the resulting ashes and biochars will be investigated for the presence of (transformed) ENMs.

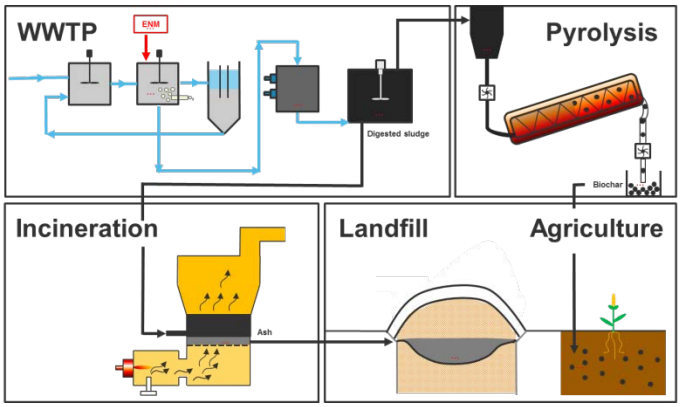



Figure 7. "Reactor" processes related to waste water treatment plants studied in WP5 of the NanoFASE project.

In the coming year WP5 will report on completion of studies on ENM fate in wastewater treatment plants and degradation and exchange of particle coating. The main results will included fate process information and likely particle characteristics that will be used to produce bespoke particles to be produced in WP3 for use in environmental compartment focussed work packages.

The overall goal of WP6 is the investigation and description of ENMs in ambient air: their emission, transformation as well as their direct atmospheric impact. We will study the photochemical effects of $TiO_2$, coated $TiO_2$ and $CeO_2$ in a reaction chamber to enable the modelling of atmospheric chemical reactions and the degradation of coatings under different conditions. The concentrations to be used in the local and regional model will be derived from an extended literature study but more importantly from dedicated laboratory and field measurements e.g. around a production plant. A general measurement strategy was developed and first tests showed promising results. In addition, WP6 partners have produced a review paper on emissions and possible environmental implication of engineered nanomaterials in the atmosphere and an international workshop of ENM fate in air has been organised for November 2017.

WP7 develops a modelling framework for engineered nanomaterials in soils. Nanomaterials could reach soils mainly after the deposition of wastewater treatment sludge. This sludge contains much valuable organic carbon and nutrients for agricultural soils. Soils therefore are a sink for nanomaterials, where concentrations develop over time.

Much of the model development for soils revolves around developing fate descriptors, i.e. the physical parameters required for a model to calculate, as well as the methods of how to determine them. The most important fate descriptor for soils is the partitioning coefficient in the case of conventional chemicals. However, such a coefficient assumes some sort of equilibrium develops where the chemical's concentration reaches a steady state both in the liquid part of the soil, the soil pore water, and the solid part of the soil. Such an equilibrium does not develop for nanomaterials and the transport of nanomaterials has to be looked at with different eyes and from a kinetic perspective.

A major task of WP 7 is therefore the quest for such a descriptor and the method to determine it. Column tests are possibly a suitable alternative, but these are lengthy and expensive tests that are thus not fit to provide the large amount of data we need. We wish to shed light on how different nanomaterials with different properties behave in different soil types. This requires a faster method to provide a lot of data.

We also seek to model how the speciation of nanomaterials changes in soil as a function of time. Many nanomaterials are highly reactive and change to less reactive counterparts. If such is the case or many different nanomaterials so that they all evolve to the same form, risk assessment becomes less complex to describe, because the original diversity of nanomaterials matters much less in that case.

The model itself then combines both speciation and fate descriptors to predict the fate of a wide range of nanomaterials in different soils. This allows us to predict and classify the soil types that are most vulnerable should they be exposed to nanomaterials. At the same time, it also provides us with invaluable knowledge for those cases where nanomaterials are intentionally added to soils. Two cases are studied: zerovalent iron and pesticides. Zerovalent iron is being developed to remediate certain organic and inorganic contaminants such as halogens, chromium and arsenic in soils. Nanopesticides are developed as a more efficient and safe alternative to conventional pesticides. The efficient and safe application of both these nanomaterials requires knowledge of how they are transported in different soils, thus extending the impact of NanoFASE to both current nanomaterials and future applications.

WP7 has recently produced a research report on speciation and transformation of nanomaterials in soil (D7.1). This report grouped method developments to monitor and quantify speciation in conditions representative of a soil environment and it outlines model approaches for how these speciation changes will be modelled in the overall NanoFASE framework.

WP8 has developed a multidimensional parameter testing matrix after identifying the major driving parameters affecting the behaviour of ENMs in water (Fig. 8). The matrix consists of three dimensions: pH, natural organic matter (NOM) concentration and electrolyte concentration (represented by $CaCl_2/MgSO_4$ in a fixed 4:1 ratio). The parameters will be varied within ranges representative of water chemistries of European surface and coastal waters.

WP8 has selected a first set of ENMs for testing, chosen to represent different types of ENM reaction/behaviour types, while covering several ENMs relevant for the NanoFASE case studies. A common dispersion protocol for these ENMs was developed within WP8. WP8 has produced an overview of the main driving forces with respect to the behaviour of ENMs in surface waters (D8.2) which will be published as papers later in the project.

A small scale two-component cluster-cluster heteroaggregation model for studying interactions between ENMs and NOM has been developed. This model will be expanded to a three-component system, capable of modelling interactions between ENMs, NOM and inorganic colloids.

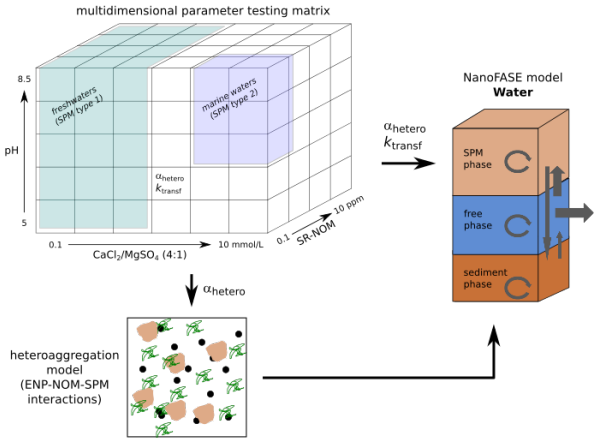



Figure 8. Design of multi-dimensional experiments to parameterize the NanoFASE water model, including a component heteroaggregation model.

Our next steps are to decide on suitable analytical methods to study heteroaggregation, dissolution and other transformation processes. To this aim, a thorough literature review on existing heteroaggregation methods has been performed and first approaches are being tested by different WP8 partners. Furthermore, a novel, infinite-sink approach to study CuO ENM dissolution is being developed within WP8 and compared with batch experiments.

The main objective of WP9 is to quantify the uptake of NMs in selected species (aquatic and terrestrial), and to assess the effect of ageing of NMs under environmentally relevant conditions on the uptake and bioaccumulation. In this WP exposure experiments will be conducted with relevant species of both aquatic and terrestrial ecosystems, e.g. earthworm, isopods, wheat and snails for terrestrial systems and algae, daphnids and fish for aquatic systems. The modelling will be based on so-called physiological based kinetic models which assess bioaccumulation as a result from uptake and elimination processes (Figure 9).

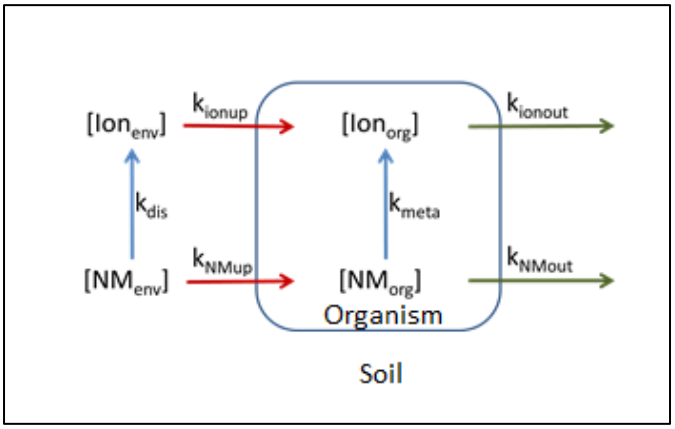



Figure 9. The conceptual model for accumulation modelling in organisms. [Ion$_{env}$]: ionic concentration of NP-material in environment.

In these models, uptake of different forms of NPs can be calculated. For this, the different uptake and elimination rate parameters need to be derived for different species, particles and potentially also environmental conditions. Furthermore, effects that organisms have on the NPs will also be studied, e.g. changes in surface functionalisation/chemistry. The models will specifically include local conditions (e.g. gut/intestinal tract for animals, rhizosphere for plants) that may affect accumulation rates.

In the 18 months of the project we have established an array of species that are relevant for the different compartments that will be included in the experimental work. These species have different routes of uptake/elimination and potentially different (gut) conditions. The array of species include some vertebrate species as well (fish and rat). It is chosen to perform the first experiments with Ag-NPs because 1) they are relevant for some case studies, 2) they show relevant environmental fate processes (e.g. dissolution, ageing), 3) environmental background levels are low, so there are problems with background levels and 4) methods are available to quantify and characterise Ag-NPs in difficult matrices like soil and tissues. Besides the pristine particles, also sulfidised Ag-NPs will be included, reflecting aged Ag-NPs. All species will be exposed to the same particle type, enabling the exchange of information on for instance characterisation of NPs as much as possible.

In addition, WP8 partners have completed a study i) to derive the dynamic ranges of parameters that play a role in accumulation efficiency, uptake, and translocation of nanomaterials in the gut across a range of (eco)toxicological species, and ii) to identify critical parameters for prediction of the bioavailability and bioaccumulation potential of NPs and how to apply them to improve in vitro and in silico testing procedures.

In the coming period *in vivo* single species experiments will continue to be conducted in order to assess uptake and elimination rate constants for pristine particles. Furthermore, a review will be compiled on the gut-conditions in different species, which will be the base for the in vitro experimental exposure studies. Mesocosm studies will be conducted to validate the in vitro and in vivo single species tests. The characterisation will be performed in close cooperation with WP3, the mesocosms studies with WP7 and 8, while the uptake rate constants will feed into WP2 (model development).

At Month 6, the plans and the infrastructure for the integration, communication and dissemination of information are established. On the project website relayed on the NanoFASE social media accounts (Twitter: @NanoFASE_EU, LinkedIn, YouTube, Facebook), news and video interview inform about the project, its progress and its actors from the work package leader to the "young Scientist". Effective internal exchange of documents takes place through the Internal Communication Platform.

Information of and integration from the targeted stakeholders (industry, OECD, ECHA, scientists, consultants… etc.) has taken place from the very start of the project, among others at conferences in and beyond Europe (ICEEN, Pacifichem, EuroNanoForum 2017), at the Advisory Board meeting and at a specific meeting with industry. These activities are planned and recorded in a dedicated tool.

The next step, beside the continuous communication in journals, at events and in our newsletter, is the development of a clickable framework that will and explain on the website the work

performed at the different levels of the project (from the work package level to the individual assay or model module).

## 6 Expected Impact

NanoFASE aims to specifically address the need for regulatory development for nanotechnology by reducing uncertainties in the "fate" related aspects of risk assessment as a key link in the delivery of objectives for nanosafety (Fig. 10). The project will support the development of innovative solutions to support better standardisation of risk assessment approaches, thereby helping to bring sustainable innovative nanotechnology products and services into the market. Combined with research initiatives on the other component of "hazard" assessment for health and the environment (e.g. OCED Sponsorship Program), risk assessment (e.g. NANoREG); and safe-by-design (e.g. future NANoREG II), NanoFASE will bring a significant improvement in exposure assessment and reduction of risks related to ENMs over their life cycle (production, transport, use and disposal). NanoFASE will address all of the expected impacts of the work program as well as more generally:

i. improve innovation capacity and integrate new knowledge to provide significant economic and commercial;
ii. inform policy by enhanced EMN risk assessment in REACH and other regimes;
iii. input to future cultural and social impacts for nanotechnology/nanosafety.


Figure 10: The nanosafety puzzle showing how fate and exposure assessment link hazard, risk and safety by design in an integrated system – NanofASE will deliver the fate understanding.

## NanoFASE publications to date (May 2017)

Clavier, A., F. Carnal and S. Stoll (2016). "Effect of Surface and Salt Properties on the Ion Distribution around Spherical Nanoparticles: Monte Carlo Simulations." The Journal of Physical Chemistry B 120(32): 7988-7997.

Cornelis, G. and S. Rauch (2016). "Drift correction of the dissolved signal in single particle ICPMS." Analytical and Bioanalytical Chemistry 408(19): 5075-5087.

John, A., M. Küpper, A. Manders-Groot, B. Debray, J.-M. Lacome and T. Kuhlbusch (2017). "Emissions and Possible Environmental Implication of Engineered Nanomaterials (ENMs) in the Atmosphere." Atmosphere 8(5): 84.

Lahive, E., M. Matzke, M. Durenkamp, A. J. Lawlor, S. A. Thacker, M. G. Pereira, D. J. Spurgeon, J. M. Unrine, C. Svendsen and S. Lofts (2017). "Sewage sludge treated with metal nanomaterials inhibits earthworm reproduction more strongly than sludge treated with metal metals in bulk/salt forms." Environmental Science: Nano 4(1): 78-88.

Li, C. C., Y. J. Wang, H. Du, P. Cai, W. J. Peijnenburg and D. M. Zhou (2017). "Influence of bacterial extracellular polymeric substances on the sorption of Zn on gamma-alumina: A combination of FTIR and EXAFS studies." Environ Pollut 220(Pt B): 997-1004.

## 7 Directory

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

| First Name | Last Name | Affiliation | Address | e-mail |
|---|---|---|---|---|
| Claus | Svendsen | NERC | United Kingdom | csv@ceh.ac.uk |
| Stephen | Lofts | NERC | United Kingdom | stlo@ceh.ac.uk |
| Dave | Spurgeon | NERC | United Kingdom | dasp@ceh.ac.uk |
| Lee | Walker | NERC | United Kingdom | leew@ceh.ac.uk |
| Iseult | Lynch | UB | United Kingdom | i.lynch@bham.ac.uk |
| Socoro | Vazquez | LEITAT | Spain | svazquez@leitat.org |
| Ralf | Kaegi | EAWAG | Switzerland | ralf.kaegi@eawag.ch |
| Carmen | Nickel | IUTA | Germany | nickel@iuta.de |
| Martin | Hassellöv | UGOT | Sweden | martin.hassellov@marine.gu.se |
| Frank | von der Kammer | UNIVIE | Austria | frank.kammer@univie.ac.at |

| First Name | Last Name | Affiliation | Address | e-mail |
|---|---|---|---|---|
| Antonia | Praetorius | UNIVIE | Austria | antonia.praetorius@univie.ac.at |
| Nico | van den Brink | WU | Netherlands | nico.vandenbrink@wur.nl |
| Alison | Crossley | UOXF.DJ | United Kingdom | alison.crossley@materials.ox.ac.uk |
| Kerstin | Jurkschat | UOXF.DJ | United Kingdom | Kerstin.jurkschat@materials.ox.ac.uk |
| Benoît | Hazebrouck | EU-VRi | Germany | bh@eu-vri.eu |
| Eduardo | Andres | HEMPEL | Spain | edan@hempel.com |
| Marta | Santiago | HEMPEL | Spain | masr@hempel.com |
| Selina | Tang | PP | United Kingdom | selina.tang@proparticles.co.uk |
| Miroslav | Cernik | TUL | Czech Republic | miroslav.cernik@tul.cz |
| Christian | Cortés | FCCO | Spain | ccortesfu@fcc.es |
| Andrzej | Moscicki | AMEPOX | Poland | amepox@amepox.com.pl |
| Olga | Chybová | ITEX | Czech Republic | chybova@inotex.cz |
| Jan | Marek | ITEX | Czech Republic | marek@inotex.cz |
| Victor | Puntes | AppNano | Spain | victor.puntes@gmail.com |
| Bernd | Nowack | EMPA | Switzerland | bernd.nowack@empa.ch |
| Bruno | Debray | INERIS | France | bruno.debray@ineris.fr |
| Fadri | Gottschalk | ETSS | Switzerland | fadri.gottschalk@etss.ch |
| Ingeborg | Kooter | TNO | Netherlands | ingeborg.kooter@tno.nl |
| Willie | Peijnenburg, | RIVM | Netherlands | willie.peijnenburg@rivm.nl |
| Serge | Stoll | UNIGE | Switzerland | serge.stoll@unige.ch |
| Patrick | Hole | MIL | United Kingdom | patrick.hole@malvern.com |
| Richard | Handy | UoP | United Kingdom | r.handy@plymouth.ac.uk |
| Damjana | Drobne | Uni-Lj | Slovenia | damjana.drobne@bf.uni-lj.si |
| Kees | van gestel | VU-Vumc | Netherlands | kees.van.gestel@vu.nl |
| Susana | Loureiro | UAVR | Portugal | sloureiro@ua.pt |
| Antonio | Marcomini | UNIVE | Italy | marcom@unive.it |
| Lyubomir | Penev | PENSOFT | Bulgaria | penev@pensoft.net |
| Pavel | Stoev | PENSOFT | Bulgaria | pavel.e.stoev@gmail.com |
| Claire | Mays | SYMLOG | France | clairemaysnanofase@gmail.com |
| Barry | Park | GBP | United Kingdom | barry.park@gbpconsulting.co.uk |
| Chady | Stephan | PE | Sweden | chady.stephan@perkinelmer.com |
| Geert | Cornelis | SLU | Sweden | geert.cornelis@slu.se |

# 8 Copyright

# NANOGENTOOLS

## Developing and implementation of a new generation of nanosafety assessment tools

Contract Agreement: 691095 Website: http://www.ubu.es/iccram
Coordinator: Universidad de Burgos-ICCRAM (Dr. Santiago Cuesta-López)

*Table 1 Consortium List.*

| No. | Beneficiary name | Short name | Country |
|---|---|---|---|
| 1 | UNIVERSIDAD DE BURGOS | UBU | Spain |
| 2 | UNIVERSITA DEGLI STUDI DEL PIEMONTE ORIENTALE AMEDEO AVOGADRO | UPO | Italy |
| 3 | THE UNIVERSITY OF BIRMINGHAM | UoB | United Kingdom |
| 4 | BIONANONET FORSCHUNGSGESELLSCHAFT MBH | BNN | Austria |
| 5 | Research and Development of Carbon Nanotubes S.A. | NTHINX | Greece |
| 6 | SITEX 45 SRL | S45 | Romania |
| 7 | NOVAMECHANICS LIMITED | NovaM | Cyprus |
| 8 | NANOTECHNOLOGY INDUSTRIES ASSOCIATION AISBL | NIA | Belgium |

# Contents



## 1 Summary

Project Duration: 48

Project Funding: 706.500€

NanoMaterials (NMs) safety is of great societal concern and raises many questions for the general public, governments, industry, scientists and regulators. Identifying and controlling the hazards associated with NMs is required to ensure the safety in parallel to exploiting the technological benefits. NANOGENTOOLS answers this challenge by creating a collaborative excellence-based knowledge exchange network that will: i) push forward knowledge via method development and pre-validation, ii) train scientists in new methodologies to assess long term nanosafety, and iii) support their inclusion in standardization and EU regulations. NANOGENTOOLS combines toxicogenomics, proteomics, biophysics, molecular modeling, chemistry, bio/chemoinformatics to develop fast in vitro high through put (HTS) assays, with molecular based computational models for nanotoxicity. Its objectives are to:· Provide solutions for faster, more reliable assessment of NM toxicity and propose HTS and omics tools for predicting toxicological properties of NMs.· Develop new bioinformatics methodologies for analyzing -omics data and create an open database in collaboration with the EU Nanosafety Cluster.· Conduct research and training on biophysical techniques and mathematical models for accurate and fast nanotoxicity prediction.· Build/improve the safe by design concept, demonstrated using carbon-NMs and nanosensors.· Place our new knowledge in the context of regulations and EU roadmaps. NANOGENTOOLS brings together cutting edge research, innovative knowledge-transfer and codevelopment, and cross-sectoral and cross-disciplinary secondments linking EU academic institutes/networks with industry and policy makers across 8 countries.

## 2 Background

Nanotechnology will be one of the key technological drivers in building an innovation European Union (EU) based on smart, sustainable and inclusive growth, and has been identified as a key-enabling technology (KET) for the EU.

The value of Nanotechnology relies in its potential to bring new materials with new or enhanced physico- chemical properties for industrial applications that are different from their micron-sized counterparts. As a result, the engineered nanomaterials (NMs) market is growing rapidly worldwide, and fostering the development of new consumer products that greatly impact almost every industrial and manufacturing sector worldwide. In the last decade, advances in NMs and nanotechnology have impacted the cosmetics, food and packaging industries and more recently biomedical applications, such as drug delivery, gene therapy or medical imaging have emerged.

By the end of 2015, the Nanotechnology World Market Size was predicted to hit €3trillion across a broad range of sectors (chemical manufacturing, pharmaceuticals, aerospace, electronics, materials etc.)[1-2], while by 2025, nanotechnology is expected to be a mature yet still growing industry, with countless mainstream products in all industrial sectors. In this context, Europe aims to play a market leader position, increasing its competitiveness in all sectors where nanotechnology may have a strong added value. However, growth and commercialization of nanotechnology must be guided and fostered by responsible innovation taking account of social and sustainability aspects.

Without doubt one of the most difficult challenges faced in the exploitation of nanotechnology for the benefit of European society (and beyond) has been the uncertainty surrounding the potential associated risks. Moreover, 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. Consequently, NM safety is of great societal concern and raises many questions for the general public, governments, industry, scientists and regulators. Identifying and controlling the hazards associated with NMs is required to ensure the safety of the general public, workers and the environment in parallel to exploiting the technological benefits associated with nanotechnologies.

While current data available on environmental and human toxicity of numerous NMs is starting to reach satisfactory levels, the time taken to produce safety dossiers is high and joint efforts between research scientists from academia and industry are needed to enhance the pace and reduce the cost of assessing the safety of NMs and nano-enabled products, to cope with the vertiginous growth of NMs expected for the next decade.

To meet this challenge, toxicological testing methods suitable for the detection of different/specific health effects are needed urgently, in addition to identification and/or development of suitable (new) toxicological models (in vivo & in vitro), and fast screening options for investigation of toxicity during the product development phase.

## 3 Scientific and technological challenges

NANOGENTOOLS answers this challenge by fostering a timely key action joining industry and academia to create a collaborative excellence-based knowledge exchange network pushing forward and training scientists in new methodologies to assess long term nanosafety, testing their applicability for NMs and pre-validating them, and finally discussing their relevance and suitability for standardization and inclusion in present and future EU regulations.

NANOGENTOOLS will answer this need and request, by combining genomics (toxicogenomics), proteomics and multidisciplinary science (biophysics, molecular modeling, chemistry, bioinformatics, chemoinformatics) to develop fast in vitro high throughput (HTS) assays, combined with molecular based computational models that will lead to better understanding of the molecular fundamentals behind nanotoxicity, and plant the seed for development of long term valuable predictive nanosafety tools for industry, including online assays during product development.

## 4 Objectives

NANOGENTOOLS pursues the main objective of generating a common solid knowledge basis arising from the Associated with fruitful cross-sectorial synergy between forefront research centers in nanosafety and industry, in a cross-fertilization multidisciplinary approach that will provide new tests and methodologies (or improve existing ones) to assess the long term risks of nanomaterials (NMs) in a rapid and cost effective manner suitable for regulatory inclusion. Its objectives are to:

· Provide solutions for faster, more reliable assessment of NM toxicity and propose HTS and omics tools for predicting toxicological properties of NMs.

· Develop new bioinformatics methodologies for analyzing -omics data and create an open database in collaboration with the EU Nanosafety Cluster.

· Conduct research and training on biophysical techniques and mathematical models for accurate and fast nanotoxicity prediction.
·Build/improve the safe by design concept, demonstrated using carbon-NMs and nanosensors.

· Place our new knowledge in the context of regulations and EU roadmaps.

The objectives converge to the main concept of NANOGENTOOLS, summarized in Figure 1.

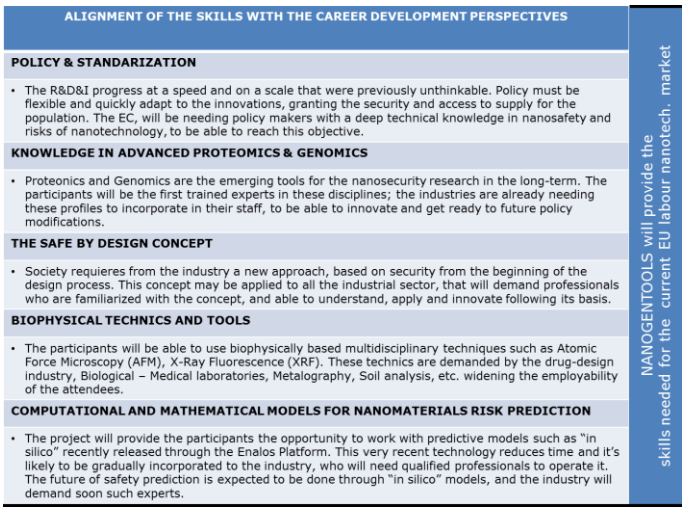



*Figure 1: NANOGENTOOLS will provide technical skills to the individuals involved in the project, of relevant value for their career development and aligned with the present EU nanotechnology labour market needs.*

## 5 Organisation

The MSCA-RISE (Research and Innovation Staff Exchange) project, NANOGENTOOLS, will ensure the delivery and alignment of key functionalities, which will allow exploitation of the exchange services in order to answer key questions important to both academic researchers and firm interests in the field of nanotoxicity and in the production of *safe by design* nanomaterials.

NANOGENTOOLS will focus on the following innovation activities & research methodologies:

The first action targeted by NANOGENTOOLS will foster research on new methodologies and tests based on -omics approaches.

A new approach to understand the molecular mechanisms of toxicity induced by NMs and its Mode of Action is toxicogenomics. This approach provides knowledge of the complex interaction between the structure and activity of the genome and adverse biological effects caused by exogenous agents such as NMs. The genomic approach provides information about specific mechanisms at the molecular level (e.g. oxidative stress); toxicogenomics has proved to be a powerful tool for the direct monitoring of patterns of cellular perturbations in specific pathways, through identification and quantification of global shifts in gene expression resulting within treated cells [5].

Gene expression profiles by RNA-sequencing is the first molecular apparatus that NANOGENTOOLS plans to use and validate with carbon-NMs as a HTS tool for long term prediction of their associated risks. Quantitative RNA-sequencing by Illumina Hi-Seq will provide the required profundity to identify robust gene changes. A typical experimental design for RNASeq will encompass 20 million 100 bp paired-end sequence reads for each analyzed condition with biological triplication (60M PE sequences per condition). Such analyses will provide the applicability and ability of QSAR models for read-across within & across NM families.

The second action on which NANOGENTOOLS will focus is the **development and adaptation of a new collection of approaches based on multidisciplinary science and biophysics**, as a new tool to complement the -omics methods in an effort to provide efficient fast assessment of nano safety to industry. This activity will facilitate the combination of biophysical methods (Microscopy, Raman spectroscopy, AFM, DSC, ITC, molecular and elemental imaging) with -omics to enhance understanding of NMs toxicity mechanisms and identify novel predictive endpoints based on elemental/molecular signatures of induced toxicological processes.

The third line of action carried out within NANOGENTOOLS will be development of better understanding of the concept of 'safe by design' (WP4). As a conclusion of the research and learning activity, the safe-by-design concept will be applied to the construction of a nanosensor based on a carbon-NM. The whole know-how and data arising from WP2 and WP3, applied to carbon-NMs converge to improve the safe-by-design approach and support a safe design of a nanosensor (WP4).

Finally, NANOGENTOOLS, will conduct research into understanding the recent policy efforts and regulation mechanisms, as well as the priority roadmap for nano safety research 2015-2025 set up by the Nanosafety cluster and the EU Commission (WP5). According to such priority roadmaps, there is a clear urgency to regulate occupational and environmental exposure to NMs, as well as to develop new fast screening methods for the risk and heath issues prediction of engineered NMs, paying attention to: suitability for implementation in industry (particularly SMEs); cost reduction; and reduced use of animals, thus putting emphasis on *in vitro* or modeling methodologies.

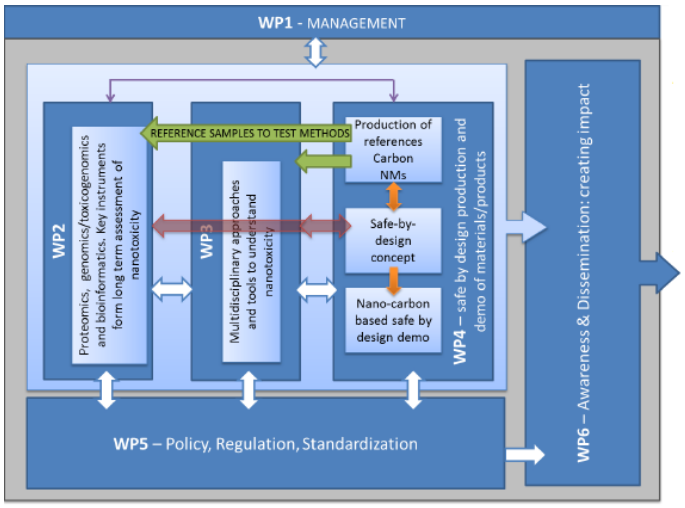



*Figure 2. NANOGENTOOLS RISE action work package structure.* The overall work package structure of NANOGENTOOLS RISE action and their dependencies. Interactions between WPS and activities as well as the multidisciplinary aspect are depicted here.

The following approach and methodology for knowledge sharing will be used: NANOGENTOOLS will use a two-fold approach for ensuring a clear and efficient sharing of knowledge across the partnership.

1. Dedicated trans-national and trans-sectoral multidisciplinary secondments designed to have a high degree of complementarity and sharing of knowledge and ideas from research to market (and vice-versa). With respect to the training activities, early stage

researchers will be advised throughout all their secondment periods by host-organization staff (see Figure 3). Joint on-line meetings will also be organized, to ensure continuity between the ESRs in secondments and the senior staff.

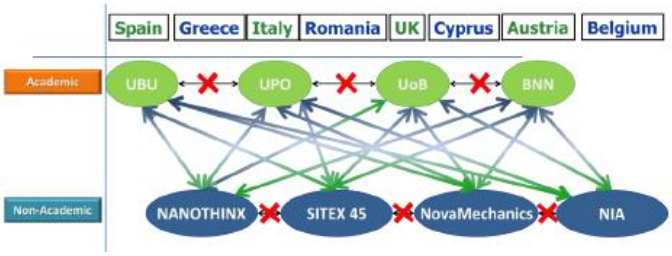



*Figure 3: NANOGENTOOLS network of eligible secondments*

2. Dedicated training, learning and dissemination activities to enhance the continuous knowledge flow between Academia experts in different techniques to predict materials nanosecurity and enterprises related to nanotechnology working on the concept of "Safe by Design" and interested in developing know-how about new technologies that ensure long-term absence of the hazards of nanotech based products.

## 6 Progress and Outcomes to date

The main progress up to now is summarised below, related to the project milestones:

MS1 progress:

A collection of methods has been set for genotoxicity and safety assessment of nanomaterials. Efficient exchange of this knowledge has been started through secondments, and analysis of carbon based nanomaterials (CNTs and graphene family products) started.

MS2 progress:

Several Biophysically based techniques have been set up in UBU-ICCRAM (i.e. AFM of NPs+cells, AFM-RAMAN, RAMAN). Preliminary validation tests are being performed in TiO2 NPs and monolayer graphene oxide. Methods and knowledge are ready to be transferred through hosted secondments during 2017 and 2018.

MS4 progress:

Safe by Design (SbD) methodology has been trained through different secondments and consortium discussions. A preliminary prototype for a SbD product based on nanoelectronics and graphene-based inks has been drafted. Work to progress in this line is specifically planned through secondments in 2017 and 2018.

Consortium is improving and setting up new updates within the ENALOS platform (secondments at NovaM)

An international industrial workshop on SbD concept merging results of different EU projects dealing with nanosafety and the SbD concept has been designed and organized, and was hold in Bilbao on April 2017, helping to develop new relationships between the H2020 projects in SbD and industrial stakeholders

MS5 progress:

Preliminary discussions on regulation and standardization have started. This topic was part of the industrial workshop organized in Bilbao on April 2017.

Also a Nanotechnology Round table was organized in Brussels on May 2017 with the objective of discussing current thinking and regional interest in nanotechnologies and examining specific areas of interest such as nano-safety

MS6 progress:

Activities within the workshop organized (SAFE BY DESIGN INDUSTRIAL WORKSHOP - safety critical issues and their impact on EU society and industry-) had as target industrial dissemination and communication.

Moreover, a specific campaign of visiting nanotechnology based companies to review their products under the SbD concept was carried out during 2017.

MS7 progress:

A communication and dissemination plan has been designed and implemented. Project web site was designed, open made public and it is periodically updated.

Several communications activities and measures have already been done. Project objectives has been communicated to the EU Nanosafety cluster, and a couple of publications in newsletters delivered (EU-Nanosafety Cluster e-newsletter (03/2016 and 07/2016 - Compendium)).

Specific training targeting new generation of researchers is scheduled and planned for Autumn 2017, through the EU NANOGENTOOLS AUTUMN SCHOOL "Advanced Training in understanding the Safety of Nanomaterials".The event will be held three days between 27th-29th of September 2017 at the University of Burgos facilities in Burgos, awaking the curiosity of young researchers in the related subject.

## 7 Expected Impact

NANOGENETOOLS will contribute to foster regulation, discuss standards and validate new fast tests suitable to be incorporated in the assessment of the risks of nanotechnology, being in this regard aligned with the strategy research agenda "*Nanosafety in Europe 2015-2025: Towards Safe and Sustainable Nanomaterials and Nanotechnology Innovations*". As the labour market grows, the need for new specific protocols and specialized personnel will be demanded directly by the industry to protect their workers.

NANOGENTOOLS targeted breakthroughs will likely merit publication in high-impact journals. Opting for open access publication, and accompanying such with press releases and media interview, will ensure wide dissemination of NANOGENTOOLS outcome to the scientific community and the general public, opening the field to further scientific inquiry and technological innovations.

The NANOGENTOOLS meetings, workshop and Summer School scheduled will further disseminate NANOGENTOOLS outcome to relevant stakeholders. All these will prepare the ground for the participating SMEs, well established in their respective fields, and any other entity sprouting from NANOGENTOOLS that will promote nanosafety needs. The initiatives carried out by the

academic institutions, and the consolidated role held by the participant PIs as "opinion makers" by the major national and international media (newspapers, TV/Radio channels, Nature News, etc.) will ensure proper dissemination of NANOGENTOOLS objectives, to the general public. Thus, these actions will provide a sound proof for support provided by the EU policy to excellent research and international collaborations.

Both NIA and BioNanoNet (BNN) network structures will assure a wide impact at all their interactions levels that include a huge amount of specialized companies and national networks, in a unique multidisciplinary perspective.

## 8 Directory

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

| First Name | Last Name | Affiliation | e-mail |
|---|---|---|---|
| Santiago | Cuesta-López | ICCRAM Director & Project Coordinator | scuesta@ubu.es |
| Kalliopi | Legaki | NTHINX | kalliopi.legaki@nanothinx.com |
| Francesco | Dondero | Università del Piemonte Orientale "Amedeo Avogadro" | francesco.dondero@uniupo.it |
| Dumitru | Ulieru | SITEX45 R&D Director | sitex45@gmail.com |
| Andreas | Falk | BIONANONET Manager | andreas.falk@bionanonet.at |
| Iseult | Lynch | University of Birmingham | I.Lynch@bham.ac.uk |
| Sean | Kelly | Nanotechnology Industries Association | sean.kelly@nanotechia.org |

## 9 Copyright

# NANoREG II

## Development and implementation of Grouping and Safe-by-Design approaches within regulatory frameworks

NanoReg2

Contract Agreement: 646221 Website: http://www.nanoreg2.eu/
Coordinator: Dr. Hab. Emeric FREJAFON / INERIS / FRANCE

*Table 1 Consortium List.*

| Nr | Name of partner | Acronym | Country | type |
|---|---|---|---|---|
| 1 (co) | Institut National de l'Environnement Industriel et des Risques | INERIS | FR | RES |
| 2 | European Virtual Institute for Integrated Risk Management | EU-VRi | DE | SME |
| 3 | Nanotechnology Industries Association AISBL | NIA | BE | IND |
| 4 | TEMAS AG Technology and Management Services | TEMAS | CH | SME |
| 5 | Bundesinstitut fuer Risikobewertung | BfR | DE | GOV |
| 6 | Rijksinsituut voor Volksgezondheid en Milieu | RIVM | NL | RES |
| 7 | Det Nationale Forskningscenter for Arbejdsmiljo | NRCWE | DK | GOV |
| 8 | Fundacion Gaiker | Gaiker | ES | RES |
| 9 | Instituto Superiore di Sanità | ISS | IT | GOV |
| 10 | University Utrecht | UU | NL | RES |
| 11 | University of Gdansk | UG | PL | RES |
| 12 | Institute of Occupational Medicine | IOM | UK | RES |
| 13 | Leibniz Institut für neue Materialien | INM | DE | RES |
| 14 | Joint Research Centre, Ispra | JRC | BE | GOV |
| 15 | Nordic Quantum Computing Group AS | NQCG | NO | SME |
| 16 | Norsk Institutt for Luftforskning | NILU | NO | RES |
| 17 | Veneto Nanotech S.C.p.A. | VN | IT | RES |
| 18 | Centre National de la Recherche Scientifique, CEREGE | CNRS | FR | RES |
| 19 | Institute of Physical Chemistry "Ilie Murgulescu" of the Romanian Academy | IPC | RO | RES |
| 20 | Roumen Tsanev Institute of Molecular Biology - Bulgarian Academy Sci. | IMB-BAS | BG | RES |
| 21 | Aristotle University of Thessaloniki | AUTh | GR | RES |
| 22 | Avanzare Innovación Tecnológica S.L. | Avanzare | ES | SME |
| 23 | Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria | INIA | ES | RES |
| 24 | Karolinska Institut | KI | SE | RES |
| 25 | Fondazione Istituto Italiano di Tecnologia | IIT | IT | RES |
| 26 | Nederlandse Organisatie voor Toegepaste Natuurwetenschappelijk Onderzoek | TNO | NL | RES |
| 27 | Commissariat à l'Energie Atomique et aux Energies Alternatives | CEA | FR | RES |
| 28 | Association Francaise de Normalisation | AFNOR | FR | RES |
| 29 | HiQ-nano | HiQ-nano | IT | SME |
| 30 | Grupo Antolin | Antolin | ES | IND |

| | | | | |
|---|---|---|---|---|
| 31 | l'Association Saint Yves, Université Catholique de l'Ouest | UCO | FR | RES |
| 32 | NANOGAP SUB-NM-POWDER, S.A | NanoCap | ES | SME |
| 33 | IdeaConsult Ltd. | IDEA | BG | SME |
| 34 | PINEXT BV | Pub-Imp | NL | SME |
| 35 | DSM | DSM | NL | IND |
| 36 | New Media Design s.r.o. | NANOCOMO | CZ | SME |
| 37 | NANOMAKERS (EU Validation under progress to become a partner) | Nanomakers | FR | SME |

# Contents



## 1 Summary

Project Duration: 36 months, starting on September 2015

Project Funding: 10 M€

One of the greatest challenges facing regulators in the ever changing landscape of novel nano-materials is how to design and implement a regulatory process which is robust enough to deal with a rapidly diversifying system of manufactured nanomaterials (MNM) over time. The challenge is to build a regulatory system which is flexible enough to be able to deal with new targets and requirements in the future, and this can be helped by the development and introduction of Safe by Design (SbD) principles. The credibility of such a regulatory system, underpinned by the implementation of SbD, is essential for industry, who while accepting the need for regulation demand it is done in a cost effective and rapid manner. The NANoREG II project, built around the challenge of coupling SbD to the regulatory process, will demonstrate and establish new principles and ideas based on data from value chain implementation studies to establish SbD as a fundamental pillar in the validation of a novel MNM.

## 2 Background

As stated by the European Economic Forum in their reporting on the Innovation Principle . Innovation is the single most important driver of societal prosperity and is indispensable for sustainable development and economic growth. Without innovation European industry will lose competitive advantage and attractiveness for investment and steadily fall behind other economies".

Despite efforts of innovators and despite regulations that require safe products at market, innovations with high economic potential are faced with questions about their safety for man and its environment. Too often these questions pop up after or around market penetration. The questions are merely related to the innovative aspects of the product or technology and appear not easy to be answered. At that time investments have been done by innovators and interests are thereby established. On the other hand, regulators act in a reactive way which implies that regulations are tuned to innovations rather than giving clear guidance to innovations. At a more abstract level regulations are of course in place by stating that products should be safe when entering market, but for innovations the devil is in the details. For innovative products it is sometimes not clear which regulations are appropriate for the product, sometimes the validity of the testing methods might be absent whereas in others regulations do not cover the required test methods to prove safety. On the other hand, innovators tend to approach the safety issues of their innovations in a conservative way. In the end, when products near market, regulatory dossier requirements appear not to cover the information needed to address the question whether a product can be regarded safe or not. This makes the discussion about safety of innovative products a discussion about uncertainty whether there is a risk or not, rather than about the exact height of the risk.

The situation as described above makes clear that the pace of innovations reaching market is much higher than the pace of developing regulatory requirements tailored to innovative nanomaterials. This leads to conclusions that taking risks is unavoidable to come to innovations, as stated by CEO's of large European companies in their letter to the European Commission and European Parliament last year. On the other hand, there is awareness among the regulatory community that the regulatory system should evolve to a system that is improved to absorb new safety aspects in a more proactive way and thereby facilitating innovation processes better.

## 3 Scientific and technological challenges

Nanosafety is expected to improve significantly following acceptance of SbD as a tool to guarantee safety at work, for

consumers and in the environment. In this sense SbD is not an aim in itself. SbD is an appealing concept, although it currently lacks agreed procedures, data collection along the R&D and innovations processes. Also acceptance of e.g. registration dossiers by ECHA to simplify the registration processes for SbD MNMs has not been discussed or agreed, and therefore, currently the SbD processes are not integrated in any legal framework and associated procedures such as REACH.

The challenge is therefore to devise a system which will channel the process of new nanomaterial acceptance through a number of interlinked regulatory steps in a rapid and effective manner. Currently industry needs to generate data to demonstrate safe use of their products; either by performing risk assessment prior to product release (e.g. for drug dossiers or for REACH non-phase-in chemicals) or after materials have been placed on the market (REACH, for phase-in chemicals). We have to progress from rules to processes: now regulators check that the rules are followed, while in the future a procedure focusing on how to confirm the safety of new products and materials along their value chains could be envisaged.

A large consortium of partners has been formed to address this call, comprising regulators, researchers, and industry. Since it is industry who must take up this (SbD) challenge in future, it is essential to have industrial involvement, so that industrial concerns can be identified and addressed. Industry is placing nano-enabled products on the market in exponentially increasing numbers, with more than 500% growth from 2006 to 2011 registered in the USA. There is a strong industrial participation, and clear commitment with an estimated 6.5 Million Euro own contribution by the consortium partners, in addition to the H2020 funding.

To achieve the above, the NANoREG II approach combines experts from different disciplines such as material scientists, toxicologists, engineers, market experts and regulators.

## 4 Objectives

OB-1 Identify and define regulatory requirements, build safe-orientated grouping approaches linked with Intelligent Testing and non-testing strategies (ITS). "Grouping approaches" are well established for chemicals, for example through the identification of chemical categories ("category approach") or analogues ("analogue approach"). It can largely reduce the amount of tests and furthermore should strongly facilitate Regulatory Risk Assessment and Management. For MNMs, as reported recently, grouping concepts are mostly unavailable and their development pose additional (i.e. nano-specific) challenges as MNMs are defined according to their physical size and synthesized in a huge variety of different chemistries, sizes, shapes and surfaces which might change during their life cycle, influencing their behaviour and toxicological effects. Thus, identifying grouping needs and possibilities for current and future regulations, defining criteria addressing physical and chemical descriptors and toxicological end-points and evaluating results from a socio-economic point of view should be done. Such approach will be place in a global Intelligent non-Testing (eg. grouping and prediction tools) and Testing (eg. high throughputs screening, in-vitro models, short term in-vivo) Strategies (ITS) designed for toxicological, eco-toxicological and physical hazards.

OB-2 Identify and select materials as candidates for value chain demonstrators in collaboration with industry, then develop life cycle maps and identify existing and potential exposure scenarios (based on work carried out in other projects). A structured approach will be developed and applied that allows for grouping of similar release and exposure scenarios, taking into account the nanomaterial, the product, the process and environmental conditions. This will help to define the framework for evaluating changes in environmental and human health risk and to identify "hot spots" where exposure and/or risks appear relatively high.

OB-3 Evaluate the relative change in environmental and human health risk, following implementation of the SbD process (for occupational and product (consumers and environment) safety), estimate any residual risk following SbD and recommend additional risk mitigation/management measures as appropriate.

OB-4 Develop and adapt supportive technical and organizational tools for Safe by Design, based on regulatory orientated grouping approaches. Based on grouping approaches placed in a general ITS, and based on a defined value chain, develop a comprehensive model supporting SbD principles to reduce uncertainties regarding safety along the value chain and therefore promoting safer innovations. This will be relevant when marketing should be balanced with competitiveness and social constraints.

Develop supportive tools for identifying and integrating efficient hazard and functionality testing during the innovation process that aims to minimize uncertainty on health risks for workers, consumers and the environment, analyze present SbD approaches applied in the processes extending from basic research to the market and illustrate added value of experimentally obtained safety data during the innovation process; Adapt currently applied stage-gate innovation models for use between organizations/actors wanting to facilitate the introduction of safety aspects for nanomaterials, nanoproducts at an early stage thereby supporting responsible innovation, and at the same time evaluate options to share information between players; Develop an action plan to achieve dedicated multi-stakeholder interactions (in any form) and define required actions aiming to facilitate responsible innovation.

OB-5 Identify and overcome barriers to the application of SbD concepts, including development of approaches to adequately address such barriers, taking into consideration grouping approaches and Risk Management (RM) requirements, with a view to ultimately eliminate the barriers and provide examples of guidance towards achieving SbD.

OB-6 Disseminate Safe by Design tools and SOPs, promoting regulatory orientated guidelines. The final objective is to establish viable grouping and SbD approaches, through the provision of industrial SbD-tools and Standard Operation Procedures (SOPs) that can become standards, the development of specific tools to help sharing knowledge between stakeholders (e.g. Newsletters, training support, web platform, helpdesk and public-private-partnership based platforms), and the provision of Grouping approaches that can become regulatory tools or guidelines for regulators. More precisely, standardization can support the regulatory process by providing the necessary measurement procedures and appropriate guides and specifications for products and processes. Public-private partnerships (PPP) such as helpdesk on SbD or such as pre-validation platform on non-testing and testing methods are techniques promoting innovation by developing instruments to anticipate regulations.

## 5 Organisation

WP 1: Regulatory orientated activities establishing a framework of grouping approaches will: 1- identify, define and harmonize current and prospective regulatory requirements and needs for MNM risk assessment, in order to implement grouping approaches and SbD. Information requirements in different regulatory frameworks such as REACH, biocides and pesticides regulations, cosmetics directive and SCCS, and feed and food regulations will be reviewed. 2- develop and apply grouping concepts for MNM and ultimately provide tools applicable for regulatory purposes. First grouping concepts from different sources will be compiled and reviewed. Through co-operations and partner's contributions, information on data management, testing protocols and MNM characterization will be shared and grouping criteria and concepts will be defined, taking also into account regulatory needs including Analyses of Alternatives and Socio-Economic Analysis. A minimum number of tests will be performed only if important data gaps need to be addressed. Grouping criteria will be verified using various datasets, including those collected and made available to the project by industry. 3- combine grouping concepts with other non-testing concepts such as Read-across and QSARs or QSPRs under development in several on-going projects or European initiatives. Then to associate these non-testing concepts with already existing testing concepts like High Throughputs screening, In-vitro models and in-vivo short term assays. All these testing and non-testing concepts will be associated in a general principle named as an Intelligent non-Testing and testing Strategy (ITS). 4 - build on a data management on well-established expertise developed in NANoREG and the ISA-TAB-Nano template approach.

WP 2: Nanomaterials for industrial markets and their corresponding value chains will 1 - Identify and select existing and new materials as candidates for value chain demonstrators in collaboration with WP1, WP3 and WP4. For each of the selected existing and new nanomaterials, develop life cycle maps, identifying existing and potential exposure scenarios. 2 - develop where possible a structured approach allowing grouping approaches of similar release and exposure scenarios, taking into account the nanomaterial, the product, and the process and environmental conditions. 3 - evaluate any relative change in environmental and human health risk following process of Safe-by-design (for production process safety and product safety) and carry out risk profiles of selected materials and/or products before and after application of SbD approaches in the case studies, to evaluate the impact of the SbD applied on the product and/or production process in terms of (potential) health and environmental risk and estimate any residual risk following SbD and recommend additional risk mitigation/management measures.

WP3: Safe by Design (SbD) aims of define a system of tools, guidance and checklists – which in its coherence is referred to as the safe innovation approach - to be used by various actors along innovation chains, supporting improved dealing with new safety issues of innovative nanomaterials and products on one hand but also supporting improved regulatory preparedness on the other hand. The safe innovation approach envisages a future proof approach, able to deal with upcoming generations of nanomaterials. Ultimately, safe innovation should also benefit to the public by improved warranting of safety of new products.

WP4: Demonstration and verification of Safe by Design concepts, will 1- -identify barriers to the application of Safe-by-Design (SbD) concepts and adequate address such barriers under consideration of grouping principles and Risk Management (RM) requirements, with a view to ultimately eliminate/reduce the barriers and provide examples of guidance towards achieving SbD, 2 - demonstrate the integration of the SbD approaches to the innovation process of the industrial partners. 3 - establish commercially viable grouping and Safe-by-Design concepts, through the provision of industrial SbD-Tools and SOPs.

WP 5: Liaisons and network activities, will establish and strengthen the strategic and operational liaisons with: a) Member States and Associate Countries, inclusive regulation authorities; b) International organisations; c) EU and related organisations, d) FP7 and H2020 project consortia; e) Industries and industry associations. f) Public laboratories. These liaisons will be formalized by agreements and memorandums. The objective is to exchange actual information and future needs with regulation authorities, industry and the society as well as the research community with respect to actual and future needs about SbD. The close collaboration with experts of the ongoing NANoREG project, CSA ProSafe, and future H2020 projects, will guarantee complementary activities and prevent focusing on the same topics.

WP 6: Knowledge management, dissemination and exploitation, is addressing the management of the knowledge and the dissemination of new knowledge from external sources into the project and from the project to its relevant stakeholder groups. Partners participating in WP6 have been selected due to their strong background in knowledge management and dissemination, their experience in the strategic planning and conduct of dissemination activities, and their existing liaisons and partnerships with external stakeholder that are central to the furthering of knowledge dissemination in the nanotechnology community and the provision of training to the community, where the impact of this workpackage will be evaluated. While the general public is not amongst the immediate target groups of the NANoREG II project, the public face of NANoREG II will be created and maintained with issues of societal awareness in mind: true to its name, NANoREG II will highlight the safety-aspect of nanotechnology and thus strengthen the societal awareness of nanotechnology as a 'key enabling solution' to grand societal challenges.

WP 7: Project Management and Scientific coordination, will provide the project coordination as defined in the Grant Agreement, the relations with EC, the project administration, regular reporting, financial management, quality assurance and impact monitoring. An Executive Board will ensure an effective information flow and knowledge exchange within the consortium and with coordinators of other relevant FP7 or H2020 research projects, a high quality and timely reporting, and management of risks and resolution of problems.

## 6 Progress to date

The progress within NanoReg II to date is summarised according to the main themes of the project, as follows:

**Grouping strategies:**

- An International Advisory Panel (IAP) was established. Regulatory Needs for implementing grouping were identified.
- A Report on existing Grouping Strategies (NANoREG, OECD, ECHA or others) and available datasets from other projects

(e.g. NANoREG, NanoTEST, MARINA) State of the Art Analysis has been made.
- A Report on MNM selection, criteria for grouping was also finalized.
- An overview on available methods expertise was collected as a basis for the validation of the grouping approach.
- A solution team of database experts has been established.

**Nanomaterials for industrial markets and their corresponding value chains**
- The selection of a set of materials for case studies was realised. More than 450 nano-enabled products have been identified.
- The definition of a group of relevant risk assessment (RA) tools for preliminary RA to suit each case study has been accomplished.
- LCA activities have thereby been split into two distinct elements within the Nanoreg2 project: the development of necessary interfaces in order to be able to integrate LCA into the SbD approach and its related tools, and the development of various, currently missing elements for a comprehensive application of the LCA framework in the area of NM applications (development of adequate characterisation factors, CFs, for releases of NMs and the establishment of comprehensive and transparent inventory data covering the production of ENMs).

**Safe by Design:**
- A first description of a conceptual and operational framework of the Safe Innovation Approach (SIA).
- A literature search was also performed of the current available tools, impact assessments and socio-economic analysis.
- An inventory on Regulatory Preparedness in other domains was performed and the usability for SIA will be elaborated.
- SbD training modules were developed.
- A first version of a safety dossier was used as input for the SbD concept and implementation trainings.

**Case studies demonstration:**
6 case studies are underway. We have also identified data and knowledge gaps. Case studies should be completed within the next 9-12 months.
A workshop was organised during which a number of focus groups with industrial and technical partners were held.

**Liaisons and dissemination activities:**
- The project website (www.nanoreg2.eu) has been established and continues to be maintained. Further dissemination materials have been made available to project partners (including the project flyer and newsletter).
- Participation to meetings such as of the NanoSafety Cluster (NSC), OECD, ECHA, Nanomaterials Working Group (NMWG), CASG Nano.
- Organisation of the workshop on Regulatory Preparedness as part of the NR2 Safe Innovation Approach (SIA) with key regulatory authorities such as ECHA, etc. During this event, finally the open question of the acceptance of the Regulatory Preparedness (prepare regulators for innovations) or "not-acceptance" by important organisations can hopefully been answered.
- Important connections have been continued - following ProSafe activities - with potential partners in South Africa and Australia and with the CEINT data base at Duke University (US).

### 7 Expected Impact

The expected potential impact (including the socio-economic impact and the wider societal implications of the project so far) include:

***For industry:***
Safer products, less uncertainties, overall saving of time and money us well us faster time to market thanks to timely identification of uncertainties and risks to manage them, finding alternative solutions as early as possible

***For regulators:***
Be prepared for up-coming innovations & respective products thanks to insight into the innovation processes, dossier compatible data along the development process, regulation oriented tools and SOPs, etc.

**For the R&D community:**
Strengthening R&D and innovation process through the Safe-by-design concept thanks to being well connected to the development and implementation of regulatory driven tools, SOPs, data bases, etc.

***For society:***

Transparent and traceable information on the safety of nanomaterialss and nano inspired products but also the capability to understand and compare products on the market.

## 8 Directory

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

| No. | First Name | Last Name | Affiliation | Address | e-mail |
|---|---|---|---|---|---|
| 1 | Emeric | FREJAFON | INERIS | France | Emeric.frejafon@ineris.fr |
| 2 | Benoit | HAZEBROUCK | EU-VRi | Germany | bh@eu-vri.eu |
| 3 | David | Carlander | NIA | Belgium | David.carlander@nanotechia.org |
| 4 | Karl | HOEHENER | TEMAS | Switzerland | karl.hoehener@temas.ch |
| 5 | Andrea | HAASE | BFR | Germany | andrea.haase@bfr.de |

| No. | First Name | Last Name | Affiliation | Address | e-mail |
|---|---|---|---|---|---|
| 6 | Cornelle | NOORLANDER | RIVM | Netherlands | cornelle.noorlander@rivm.nl |
| 7 | Håkan | WALLIN | NRCWE | Denmark | hwa@arbejdsmiljoforskning.dk |
| 8 | Blanca | SUAREZ | GAIKER-IK4 | spain | suarezb@gaiker.es |
| 9 | Paola | DIPROSPERO | ISS | Italy | paola.diprospero@iss.it |
| 10 | Gerhard | BLAB | Utrecht University | Netherlands | g.a.blab@uu.nl |
| 11 | Tomasz | PUZYN | Gdansk University | Poland | t.puzyn@qsar.eu.org |
| 12 | Martie | VAN TONGEREN | IOM | United Kingdom | martie.van.tongeren@iom-world.org |
| 13 | Annette | KRAEGELOH | INM | Germany | Annette.Kraegeloh@inm-gmbh.de |
| 14 | Hugues | CRUTZEN | JRC | European Commission | Hugues.CRUTZEN@ec.europa.eu |
| 15 | Axel | MUSTAD | NQCG | Norway | axel@nqcg.com |
| 16 | Maria | DUSINSKA | NILU | Norway | Maria.Dusinska@nilu.no |
| 17 | Federico | BENETTI | VENETO NANOTECH | Italy | federico.benetti@venetonanotech.it |
| 18 | Jerome | ROSE | CEREGE | France | rose@cerege.fr |
| 19 | Speranta | TANASESCU | Inst. Of Phys. Chem. | Romania | stanasescu@chimfiz.icf.ro |
| 20 | Margarita | APOSTOLOVA | IM-BAS | Bulgaria | margo@obzor.bio21.bas.bg |
| 21 | Christoforos | GRAVALIDIS | AUTH University | Greece | cgrava@physics.auth.gr |
| 22 | Marta | PEREZ | AVANZARE | Spain | marta@avanzare.es |
| 23 | José María | NAVAS | INIA | Spain | jmnavas@inia.es |
| 24 | Eva | BJÖRNDAL | Karolinska Institutet | Sweden | eva.bjorndal@ki.se |
| 25 | Stefania | SABELLA | ITT | Italy | Stefania.sabella@iit.it |
| 26 | Ingeborg | KOOTER | TNO | Netherlands | Ingeborg.kooter@tno.nl |
| 27 | Sylvie | CHEVILLARD | CEA | France | Sylvie.chevillard@cea.fr |
| 28 | Emilie | LANGLOIS BERTRAND | AFNOR | France | emilie.langloisbertrand@afnor.org |
| 29 | Mariada | MALVINDI | HIQ-NANO | Italy | mariada.malvindi@hiqnano.com |
| 30 | Cesar | MERINO | Grupo Antolin Ingenieria | Spain | cesar.merino@grupoantolin.com |
| 31 | Catherine | MOUNEYRAC | UCO – Asso St Yves | France | catherine.mouneyrac@uco.fr |
| 32 | Allen | REID | NANOGAP | Spain | a.reid@nanogap.es |
| 33 | Nina | JELIAZKOVA | IDEA Consult | Netherlands | jeliazkova.nina@gmail.com |
| 34 | Teun | SLUIJTERS | PUBLIC Impact | Netherlands | t.sluijters@publicimpact.nl |
| 35 | Germ | VISSER | DSM Chemical Tech. | Netherlands | Germ.Visser@dsm.com |
| 36 | David | HOFMAN | NANOCOMO | Czech Republic | david.hofman@nanocomposix.eu |
| 37 | Jean-François | PERRIN | NANOMAKERS | France | jfperrin@nanomakers.fr |

# 9 Copyright

# PANDORA

## Probing safety of nano-objects by defining immune responses of environmental organisms

Contract Agreement: 671881 Website: www.pandora-h2020.eu
Coordinator: Consiglio Nazionale delle Ricerche – Istituto di Biochimica delle Proteine – Italy – Dr. Diana Boraschi

*Table 1 Consortium List.*

| No. | Beneficiary name | Short name | Country |
|---|---|---|---|
| 1 | Consiglio Nazionale delle Ricerche | CNR | Italy |
| 2 | Paris-Lodron Universität Salzburg | PLUS | Austria |
| 3 | Università di Genova | UNIGE | Italy |
| 4 | Academy of Sciences of the Czech Republic | IMIC | Czech Republic |
| 5 | Eberhard-Karls-Universität Tübingen | EKUT | Germany |
| 6 | University of Ljubljana | UNILJ | Slovenia |
| 7 | Cardiff University | CU | UK |
| 8 | Natural Environment Research Council | NERC | UK |
| 9 | AvantiCell Science Ltd. | ACS | UK |
| 10 | Fundaciò Institut Català de Nanociencia i Nanotecnologia | ICN2 | Spain |

## Contents



## 1 Summary

Project Duration: 48 months

Project Funding: EUR 2,814,491.16

PANDORA (Probing safety of nano-objects by defining immune responses of environmental organisms) shall assess the global impact of engineered nanoparticles (NP) on the immune responses of representative organisms covering all evolutionary stages and hierarchical levels from plants to invertebrates and vertebrates. Immunity is a major determinant of the survival and fitness of all living organisms, and therefore immunosafety of engineered NP is a key element of environmental nanosafety. PANDORA will tackle the issue of global immunological nanosafety by comparing the impact of widely used NP (*e.g.*, iron, titanium and cerium oxide) on the human immune response with their effects in representative terrestrial and marine organisms. This comparison will focus on the conserved system of innate immunity / stress response / inflammation, aiming to identify common mechanisms and markers across immune defence evolution shared by plants (Arabidopsis), invertebrate (bivalves, echinoderms, earthworms), and vertebrate (human) species. PANDORA's objectives are: 1. To identify immunological mechanisms triggered by nano-objects, and predictive markers of risk *vs.* safety; 2. To do so by a collaborative cross-species comparison, from plants to human, of innate immune defence capacity, using selected, industrially relevant NP; 3. To design predictive *in vitro* assays to measure the immuno-risk of NP to the environment and human health, as new approaches to industrial and environmental nanosafety testing. PANDORA will train 11 PhD students in an overarching training programme involving training-by-research, joint courses of technical, scientific and transferrable skills, participation to public scientific events, and an intense intersectoral networking exchange plan. The PANDORA consortium encompasses academic institutions, research centres, and SMEs, all with proven experience in higher

education and training, and state-of-the art scientific and technical expertise and infrastructures.

## 2 Background

PANDORA aims at studying the effects of engineered NP on the immune and defensive responses of environmental organisms and at identifying common mechanisms/markers across species (from plants to humans) that could be used for novel assays for assessing immuno-nanosafety.

The particulate nature of NP dictates a preferential interaction with cells of the immune system deputed to recognition and elimination of foreign particulate matters. It is therefore of key importance that, even for NP that are non-toxic (*i.e.*, unable to kill cells or organisms) according to regulatory approved standard assays, additional evaluation of their interaction with the immune system is performed. PANDORA will focus on the interaction of NP with cells and molecules of the innate immune system. Innate immunity is the rapid and non-specific defence system that reacts to and eliminates foreign materials (infectious microorganisms, dusts and particles) that enter the body. Innate immunity is the only mechanism of immune defence in plants, invertebrates and lower vertebrates, while in higher vertebrates adaptive immunity is also present, adaptive immunity (more specific but much slower). The full functioning of innate immunity is of central importance for the survival and health of environmental organisms.

Whether NP may induce an anomalous innate reaction or interfere with a protective reaction (*e.g.*, against an infectious agent) is an issue of high relevance for predicting a nanorisk.

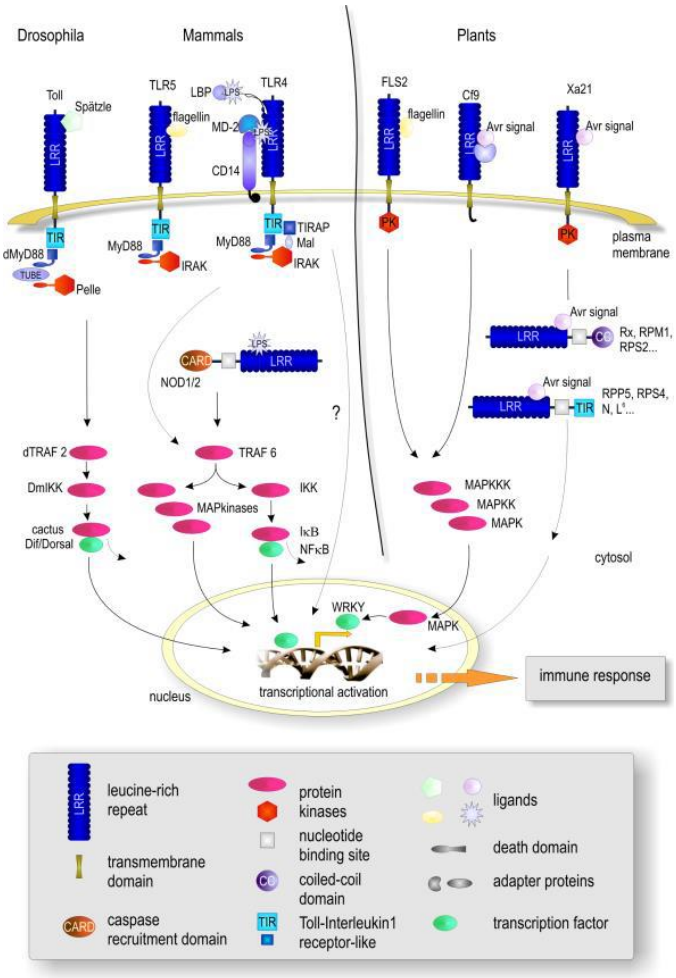
## 3 Scientific and technological challenges

PANDORA aims at identifying the effects of NP on the immune responses of environmental organisms.

The rapid development of nanotechnologies worldwide has fostered intense studies regarding the safety of engineered NP, which enter in an increasingly larger number of consumers' products and industrial applications and are released into the environment. The effects of NP on human health are actively studied also in relation to the development of nanomedicine. The immunosafety of NP is a major issue for human health, because of the possibility that NP, even if not directly toxic, may alter the functionality of immune cells thereby posing significant health risks.

Environmental nanotoxicology, on the other hand, has dedicated little/no attention to the effects of NP on the immune responses of environmental species. Defensive immune responses are present in practically all living organisms and some of the basic mechanisms are remarkably conserved throughout evolution, in particular those of the so-called "innate" immune system. Pathogen-sensing receptors such as the human Toll-like receptors (TLR) are practically identical to invertebrate Toll receptors, and to the pathogen receptors found in plant cells. Innate effector molecules (antimicrobial peptides, degrading enzymes, complement) and innate effector cells (phagocytes) are practically identical in most living organisms.

The study of the immune response, integrated with ecological, evolutionary and population biology information, has led to the development of one of the most rapidly expanding fields of biology, Ecological Immunology, which examines the impact of environmental stressors on the immune response and how these stresses maintain the variation in the immune function in the context of evolution and ecology. The importance of defensive mechanisms (that ensure survival but also physical fitness and consequently reproductive capacity) underlines the need of assessing the effects of NP on the immune response of environmental organisms.




## 4 Objectives

PANDORA aims at training eleven PhD students into the issue of immuno-nanosafety. The focus on the effects of NP on the functionality of the immune system, and consequently on the overall health protection of the target organism, will be broadened from the attention to human health to encompass a variety of environmental organisms (plants, invertebrates, marine, earth). The programme will therefore lay the basis for an integrated approach to environmental nanosafety that includes immunosafety as a key element. All the selected students will be involved in a highly stimulating training programme, both at the local and at the network-wide level. The training programme comprises:

1. The implementation of the individual research project at the host institution. The research project will involve collaborations with other PANDORA institutions, to be implemented through secondments.

2. Each researcher will be involved in local training sessions.

3. Joint scientific courses and meetings will be organised by the PANDORA consortium, together with short courses for transferable skills training, including a course on ethics.

4. Organisation of a final joint workshop on "Immuno-nanosafety: innate immune mechanisms in environmental species".

The research activities implemented in PANDORA have the following objectives:

1. To identify immunological mechanisms triggered by nano-objects, and predictive markers of risk vs. safety;

2. To do so by a collaborative cross-species comparison, from plants to human, of innate immune defence capacity, using selected, industrially relevant NP;

3. To design predictive *in vitro* assays to measure the immuno-risk of NP to the environment and human health, as new approaches to industrial and environmental nanosafety testing.

PANDORA will address the challenge through the collaborative efforts of eleven partners with different expertise that complement each other towards the overall goals of the project. One partner (10) will produce the NP, examine their interaction with relevant organic/biological matrices (blood, soil, marine water, *etc.*), and correlate NP characteristics with effects raised in different species. Four partners (4, 6, 7 and 8) will focus on the toxicology and innate reactivity of different terrestrial invertebrates, two will study the innate immune reactivity of marine organisms (3 and 1.b), and one (5) will address plant immunity. Two projects (1 and 2) will study vertebrate (human) innate immunity, aiming at comparing cells and mechanisms involved in reaction to NP across species, in order to identify common pathways and markers. One project (9) will exploit the consortium expertise and accumulated knowledge for developing predictive assays. Partners will work in strict collaboration, with frequent secondments of the fellows to other partners' labs.

## 5 Organisation

The Consortium will act under a strong and well-organised management structure, encompassing three bodies: the Project Coordinator, the External Advisory Board, the Supervisory Board and the Thesis Committees.

## 6 Progress to date

The overall project kick-off meeting took place at the CNR, Napoli, Italy on January 26$^{th}$-27$^{th}$, 2016, where the procuedures for recruitment of fellows and other key consortium-wide issues were agreed. Following the recruitment phase, this was followed by a **Fellow's Kick-Off Meeting** on February 22$^{nd}$ – 23$^{rd}$, 2017 Residència d'Investigadors, Barcelona, Spain. The photos below show the Pandora Fellows and the entire Pandora consortium.

The exposure of the Pandora fellows to different disciplines (from nanotechnology to evolution of immune responses to human immunity to safety/toxicology) and the intense exchange programme will allow them to expand the range of their scientific and technical knowledge and to learn how to work in multidisciplinary collaborations.

Likewise, the industrial interest of the participating SMEs in the field of nanosafety assessment and screening will provide the fellows with a technical capability that is both specialised and marketable and, with their additional experience of working at the interface between academia and industry, should enhance their career prospects in both sectors.

Moreover, the transferable skill courses foreseen in PANDORA will provide the Early Stage Researchers with the necessary tools to become project managers, to find funds and to communicate effectively to different stakeholders or non-scientific media.

The courses will guarantee that all ESRs will receive enough knowledge on various complementary topics to foster their entrepreneurial mind-set. The intersectoriality and interdisciplinary aspects of the partnership will create a generation of scientists able to adapt to changes in global technology and that will represent an investment into the research livelihood of the European Union.

## 7 Expected Impact

PANDORA presents a good mixture of scientific objectives coupled to technical objectives, with the technological developments being

weighted equally in significance with the research, as each will foster the other during the course of the project. This mixture is considered likely to equip the individual trainees immediately with eminently marketable skill-sets, which should be attractive to a range of potential employers. The exposure of fellows to different disciplines (from nanotechnology to evolution of immune responses to human immunity to safety/toxicology) and the intense exchange programme will allow them to expand the range of their scientific and technical knowledge and to learn how to work in multidisciplinary collaborations.

Regarding the industrial interest, one of the drivers of ACS participation is the attraction the novel cell-based systems of immune detection will have in the field of nanosafety assessment and across multiple sectors of the life science industry.

In the longer-term, the trainees' career progression is expected to benefit from the deliberate combination of academic / commercial research experience, wherein the existing, close working relationship between the partners will create a dynamic working environment spanning the public and private sectors.

Another longer-term benefit arising from successful realisation of a truly interactive programme of R&D will be the imbuing of an entrepreneurial spirit amongst project trainees. This will be encouraged by trainees' secondments to the industrial partners of the consortium and by exposing them to the industrial environment and commercial considerations that shape industrial research.

The proposed research training programme wants to be considered a step towards the establishment of Europe-wide PhD School of Immuno-nanosafety, a scheme PANDORA will start implementing as soon as the project begins, in terms of agreements for multiple or joint doctorates and that, it is hoped, can formally become a wider PhD School on the basis of a successful outcome to this first programme.

## 8 Directory

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

| First Name | Last Name | Affiliation | Address | e-mail |
|---|---|---|---|---|
| Diana | Boraschi | Consiglio Nazionale delle Ricerche – Institute of Protein Biochemistry - IBP | Via Pietro Castellino, 111 – 80131 – Napoli – Italy | d.boraschi@ibp.cnr.it |
| Valeria | Matranga | Consiglio Nazionale delle Ricerche – Institute of Biomedicine and Molecular Immunology «A. Monroy» - IBIM | Via Ugo La Malfa, 153 – 90146 – Palermo – Italy | valeria.matranga@ibim.cnr.it |
| Albert | Duschl | Paris-Lodron Universität Salzburg - Department of Molecular Biology | Hellbrunner Strasse, 34 – 5020 – Salzburg – Austria | albert.duschl@sbg.ac.at |
| Laura | Canesi | Università di Genova – DISTAV – Department of Earth, Environment and Life Sciences | Corso Europa, 26 – 16132 – Genova – Italy | Laura.Canesi@unige.it |
| Petra | Procházková | Academy of Sciences of the Czech Republic – Laboratory of Cellular and Molecular Immunology | Videnska 1083 – 14220 – Praha 4 – Czech Republic | kohler@biomed.cas.cz |
| Birgit | Kemmerling | Eberhard-Karls-Universität Tübingen – ZMBP Plant Biochemistry | Auf der Morgenstelle, 32 – 72076 – Tübingen - Germany | birgit.kemmerling@zmbp.uni-tuebingen.de |
| Damjana | Drobne | University of Ljubljana – Biotechnical faculty - Biology Department | Večna pot, 111 – 1111 – Ljubljana – Slovenia | Damjana.Drobne@bf.uni-lj.si |
| Peter | Kille | Cardiff University – Cardiff School of Biosciences | Museum Avenue – CF10 3AT – Cardiff – United Kingdom | Kille@cardiff.ac.uk |
| Claus | Svendsen | Natural Environment Research Council – Centre For Ecology and Hydrology – Wallingford | Benson Lane, Crowmarsh Gifford – OX10 8BB – Wallingford – United Kingdom | csv@ceh.ac.uk |
| Colin | Wilde | AvantiCell Science Ltd. | Gibbsyard Building – KA6 5HW – Ayr – United Kingdom | Colin.Wilde@avanticell.com |
| Victor | Puntes | Fundaciò Institut Català de Nanociencia i Nanotecnologia – Inorganic Nanoparticles Group | Campus de la UAB – 08193 – Bellaterra - Spain | victor.puntes@icn.cat |

# 9 Copyright

# ProSafe

## Promoting the Implementation of Safe by Design

Promoting the Implementation of Safe by Design

Contract Agreement: 646325 Website: http://www.h2020-prosafe.eu/
Coordinator: Tom van Teunenbroek, Dutch Ministry of Infrastructure and the Environment

*Table 1 Consortium List.*

| No. | Beneficiary name | Short name | Country |
|---|---|---|---|
| 1. | MINISTERIE VAN INFRASTRUCTUUR EN MILIEU | Min I&M | Netherlands |
| 2. | INSTITUTE OF OCCUPATIONAL MEDICINE | IOM | United Kingdom |
| 3. | JRC -JOINT RESEARCH CENTRE- EUROPEAN COMMISSION | JRC | Belgium |
| 4. | CENTRE NATIONAL DE LA RECHERCHE SCIENTIFIQUE CNRS | CNRS CEREGE | France |
| 5. | ISTITUTO SUPERIORE DI SANITA | ISS | Italy |
| 6. | *INTERNATIONAL LIFE SCIENCES INSTITUTE EUROPEAN BRANCH AISBL* | *ILSI* | *Belgium* |
| 7. | TEMAS AG TECHNOLOGY AND MANAGEMENT | TEMAS | Switzerland |
| 8. | NANOTECHNOLOGY INDUSTRIES ASSOCIATION | NIA | Belgium |
| 9. | *VENETO NANOTECH SOCIETA CONSORTILEPER AZIONI* | *VN* | *Italy* |
| 10. | Umweltbundesamt | UBA | Germany |
| 11. | Fundacao Para a Ciencia e a Technologia | FCT | Portugal |
| 12. | Institutul De Chimie Fizica Ilie Murgulescu | IPC | Rumania |
| 13. | ECAMRICERT SRL | ER | Italy |
| 14. | RIJKSINSTITUUT VOOR VOLKSGEZONDHEID EN MILIEU | RIVM | Netherlands |

Note: beneficiaries listed in grey font, left the Consortium before the end of the project

# Contents



## 1 Summary

The duration of the project was 27 months; the end date was 30 April 2017.

PROSAFE CSA is funded by the H2020 Programme with about € 2.5 million euros.

## 2 Background

ProSafe has been designed to coordinate and support the aims of EU Member and associated states in their EU and international efforts (OECD, COR, EU-US) regarding risk assessment, management and governance by streamlining data acquisition, collection and management on regulatory orientated toxicology

testing of nanomaterials, exposure monitoring, LCA, and disposal and treatment of waste nanomaterials. Consideration is also given to regulatory policy developments on both the national and international level, including challenges raised by the convergence between nano- and biotechnologies.

## 3 Objectives

A serious threat to the capitalization of the innovative and economic potential of Nanotechnology is the limited understanding of the Environmental, Health and Safety (EHS) aspects of nanomaterials (NMs). This limited understanding leads to uncertainty on how to judge the EHS aspects of these materials in a regulatory context. This has a negative impact on the investment climate and on societal appreciation of products containing NMs.

Reducing and eliminating these uncertainties and developing ways to incorporate nanosafety in the design of nanomaterials is an objective of a great number of nanosafety projects funded by the EC or national authorities. The ProSafe project aimes at coordinating and supporting part of these efforts by bringing together and where possible aligning the results of these projects. The main aims and resulting products of the ProSafe project are:

1. The Joint Document (JD): This document compiles the results of an extensive evaluation of the results of NANoREG and other nanosafety cluster (EU) projects and the results of the OECD sponsorship programme by a Task Force of independent senior experts. The document served as a reference document for the OECD-ProSafe Joint Scientific Conference in November 2016. The Joint Document also serves as the technical annex to the EU policy-oriented White Paper.
2. The White Paper: This document provides building blocks for regulators and industry to cover Environment, Health and Safety (EHS) aspects of manufactured NMs (MNMs) including evaluated methods for testing and assessing risks of nanomaterials and including Safe by Design (SbD).
   Contributing aims and activities for the White Paper are:
   - Analysis and synthesise of what will come in the next 3-10 years for nanomaterial product development and its risk management ("foresight study").
   - Establishing standard approaches for (EHS) data management.
   - Acceptance and further elaboration of the NANoREG safe innovation and safe-by-design concept.

## 4 Major Outcomes from project

**Coordination and collaboration (WP1)**

ProSafe contributed to the accession of several new partners to the NANoREG project thus expanding NANoREG's research capacity and filling gaps in specific knowledge and experience of the NANoREG Consortium. The implementation of the collaboration agreements between those new partners and the NANoREG Consortium was also supported by the ProSafe project.

Contacts between representatives of the US Nano Initiative and the EU during the meeting of the Society of Risk Analyses in Washington have resulted in rough contours for a common EU – EC research programme for the mid- and long term. These contours encompass the following topics:

- Reference materials and standards.
- Data management; curation, big data; mining of existing data.
- High Throughput Screening and new testing techniques.

At this moment it is unsure whether the funds for these promising future topics can be organised on US as well EU side.

With 5 US, South-African and Australian partners a Non-Disclosure Agreement was concluded in order to involve them in the work of NANoREG WP3 on exposure. Among others they reviewed and contributed to several NANoREG WP3 deliverables. With CEINT – Duke University a collaboration has been established focussed on data management, curation and collaboration with the US-nanoinformatics program as to determine a strategic planning for data standardization, templates and guidance documents for data harmonization between Europe and the US.

**Joint Document, Scientific conference and White Paper (WP5+ WP1)**

The ProSafe Joint Document summarises the results of an evaluation of the regulatory relevance of methods for testing and assessing nanomaterials. This evaluation was carried out by a group of experts that covered the most relevant scientific fields of nanosafety on the basis of the "ProSafe roadmap for reviewing data, protocols, report and guidance notes for regulatory relevance". The draft Joint Document was discussed during a three day scientific conference co-organised by ProSafe and the OECD. The conference was attended by about 180 experts and policy makers from all over the world. It was concluded that there is a need to continue to work towards the further harmonisation of test methods in order to create a solid base for testing nanomaterials and to fulfil the conditions for mutual acceptance of data.

The Joint Document was finalised in March this year. It gives an impressive overview of the state of the art of methods and strategies to test and assess the risk of nanomaterials and their regulatory relevance. It is one of the building blocks for the ProSafe White Paper. A number of papers, related to the areas of concern as mentioned above, are currently written to be published in a special issue of NanoImpact.

The White Paper comes forward with recommendations for policy makers and regulators aimed at a more effective and efficient governance and regulation of nanomaterials. The main focus of these recommendations is on the application of REACH to nanomaterials, since this regulation has the broadest coverage. But also other topics are addressed such as the harmonisation of

test methods, quality of nano-EHS data, the infrastructure for advanced information management and Safe by Design. Important building blocks for the document are the previously mentioned Joint Document, the NANoREG Regulatory Framework including the proposal for a "new approach towards nanospecific prioritisation and risk assessment" and several NANoREG deliverables. A draft of the White Paper is circulating among a core group of stakeholders for written consultation -June 2017-.

**Exploiting Synergies (WP2)**

The Synergy Scan of nanosafety projects (task 2.1) has resulted in an overview of projects that contribute to the implementation of SbD. Most of the initiatives appear to be indirectly connected with SbD itself, since their main aim is to provide and transfer knowledge, for instance in the form of data, protocols, etc. A further refinement was aimed at understanding which information needs to be extracted and provided to enable the adoption of SbD, and to foster its implementation in a regulatory perspective. The information has been -and still can be- used as input for projects further elaborating the Safe by Design concept.

The attempt to get insight in future applications of nanomaterials and national strategies in relation to nanotechnology was not successful. It proved to be difficult to identify specific trends and timelines of appearance on the market of new products and applications.

The evaluation of the equipment needs for implementing appropriate risk assessment and management procedures was incorporated in the Delphi Poll (see below). The experts participating in the poll tended to say that the need to develop methods to assess exposures from actual uses of NMs, rather than toxicity methods at present is more pressing. However, comments in the forum also indicated that this attention to exposure methods does not rule out a need for development of new toxicity methods. Rather, it simply speaks to the need to sequence methods development in such a way that toxicity assessment methods can focus on what is measured in exposure assessments of actual uses of NMs in products. In addition to sequencing methods development starting with the general class of real-world exposures, an instrumentation "class" is emerging that combines data analytics with a capability to generate multiple measurements across forms, time scales, and sampling points.

Foresight about whether technical methods will be ready to support Safe by Design (SbD) risk management approaches for uses of manufactured nanomaterials (MNMs) in the R&D pipeline was gained by means of a Delphi forum process. The forum process used a multi-stakeholder expert steering group, two web surveys, and a discussion panel workshop as interaction and feedback points. The overall process took 18 months and included detailed participation by over 250 experts in Europe and North America. This approach provided useful information about prevailing opinions of experts and it provided useful experience on what works to understand such a complex risk management challenge. The results of the Delphi forum process are extensively described in ProSafe Deliverable 2.03.

**Streamlining data acquisition, collection and data management (WP3)**

Information from FP7 and H2020 projects on database implementations and development has been collected and collated, fleshed out with information from the literature and interactions with other ongoing developments and topical activities. The information has been used for the other tasks within WP3.

The work on ontology, methods for data logging and linking databases has been executed in close collaboration with eNanoMapper and NANoREG and was very fruitful. It resulted in the development and adoption by the NANoREG project of ISA-TAB-Nano like templates for logging of experimental nano-EHS data. These templates are now available for the whole nanosafety community. ProSafe also paved the road for the transfer of NANoREG experimental data to the NANoREG – eNanoMapper database in order to make this set of "good quality data" available for other parties. Noteworthy to mention that caLIBRAte and NanoReg$^2$ will build on this data set.

The ontology work was mainly led by eNanoMapper at EU NSC level, with full collaboration of ProSafe -and NANoREG-.

The collaboration with DUKE University (US) has a great potential for a further transatlantic collaboration on the field of data management, for example data entry, data curation, exchange of data, etc.

**Liaisons with Member and Associated States (WP4)**

Liaisons and contacts with different stakeholders of the Member States, Associated States and Third Countries have been established. However the interaction with this group -under the umbrella of the Strategic Policy Development Group)-has been limited due to the fact that concrete results of the NANoREG and ProSafe project only came available at a late stage of the ProSafe project.

To stimulate collaboration and information exchange on the field of Safe by Design, a transnational call was developed in collaboration with funding agencies of several countries. The call comprised four topics with focus on the Safe by Design concept developed within the NANoREG project and their integration in industry's innovation process.

The result of this activity is rather disappointing; only one project was selected for funding.

The work on "Harmonization of national regulatory oriented protocols, procedures, data and Safe-by-Design approaches" has resulted in an impressive "state of the art document" (D4.04) that lists what is required to implement a SbD approach in terms of protocols, tools, datasets, etc, what is already available and what needs to be (further) developed. The latter has been input for the NanoReg$^2$ project.

The SbD concept developed by NANoREG has been complemented with "preparation of industry for regulation". Within NanoReg$^2$ the SbD concept will be combined with Regulatory Preparedness -

regulators being prepared for innovation- into the NanoReg² Safe Innovation Approach (SIA).

The activities aimed at promoting the SbD approach for industrial innovation processes focussed on a dialogue with industry during business meetings. On top of that, the SbD approach was communicated to industry during seminars, workshops and by means of newsletters and email contacts.

**Knowledge management, Dissemination and Exploitation (WP5)**

At the start of the project the ProSafe website was launched; several newsletters and press releases have been issued. A ProSafe Results Repository will be developed; a hyperlink will be provided via the ProSafe website in due time.

## 5 Impact beyond the project lifetime

**√ Relevance and quality of data in a regulatory context**

The extensive evaluation of the results of NANoREG and other nanosafety cluster (EU) projects and the results of the OECD sponsorship programme for the Joint Document confirms the importance of robust nano-EHS data in terms of reliability, comparability, exchangeability thus confirming that the quality of nanoEHS data is crucial for their usefulness in a regulatory context.

**√ Accessibility of results**

Open access to results of nanosafety projects is key to the effectiveness and efficiency of nanosafety research. Just like for the NANoREG project the ProSafe Consortium partners decided to make all relevant results public available. The decision follows the example set by NANoREG, based on the conviction that It is the only way to build on the results of previous projects.

**√ Collaboration**

The ProSafe project gave an additional boost to collaborations already started within the NANoREG project. The new collaborations comprised the exchange of data, the review of scientific work on exposure and data management, curation and collaboration with the US-nanoinformatics program. The NANoREG National Coordinators, most of them also participating in the ProSafe Strategic Policy Development Group, have played - and will play- an important role in the science- and policy-oriented dialogue regarding nanosafety at EU level and beyond.

The expected socio-economic impact and the wider societal implications of the project are of the long-term and strongly related to effectiveness of the Joint Document and the ProSafe White Paper and its recommendations.

**√ Joint Document**

The impact of the Joint Document can be considered as substantial. Partly, due to its content and partly due to the process of developing this document. To start with the latter, the Scientific Conference co-organized by OECD and ProSafe strongly contributes to the credibility of the Joint Document and its conclusions and recommendations.

The "ProSafe roadmap for reviewing data, protocols, report and guidance notes for regulatory relevance", that forms the basis for the Joint Document sets a standard for the evaluation of methods for testing and assessing the risks of nanomaterials. The applicability of this roadmap goes beyond the ProSafe project. It can be used more in general to evaluate the quality and regulatory relevance of data used in nowadays risk assessment of nanomaterials.

The core of the Joint Document gives guidance to industry as well as regulators, which respect to the applicability of methods, approaches, etc. to test the effects of nanomaterials and to assess their risk. The recommendation regarding harmonisation will have effect on the OECD harmonisation programme.

**√ White Paper**

The ProSafe White Paper Process is crucial for achieving impact of the NANoREG project, since it will integrate main NANoREG results (Framework and Toolbox) and the ProSafe Joint Document into the Policy Recommendations of the White Paper. The OECD-ProSafe Major Scientific Conference held at the end of 2016 in Paris contributed to the credibility of this document. The consultation procedure for the draft White Paper and the workshop with policymakers and innovators foreseen for autumn 2017 will also contribute to the support for the recommendations.

The ProSafe White Paper will have impact on several fields. Its recommendations on "a more effective and efficient governance and regulation of nanomaterials" will probably be input for discussion between Member States and the EC on topics such as:

- Adjusting REACH and its annexes and Guidance Documents to make it better fit for nanomaterials with respect to information requirements, categorisation and methods for testing and assessing the risks of these materials.
- The quality of nanoEHS data. Recommendations in the White Paper will follow up on the Joint Document with respect to harmonization and a more stringent evaluation of the quality of data to be used for risk assessment of nanomaterials.
- Safe by Design approach; The White Paper will address some of the dilemmas linked to this approach such as its role in a regulatory context, the lack of data, etc.
- Advanced data management. The recommendations in the White Paper on this topic will deal with standardization of data logging, opening up results of nanosafety projects, the accessibility of nanoEHS data, data curation.
- Harmonised occupational exposure limits.
- Innovation in risk assessment aimed at "keeping pace with innovation".

## 6 List of Publications from the project

Not applicable.

# 7 Copyright

# SKHINCAPS

## SKin Healthcare by Innovative NanoCAPsuleS

Contract Agreement: 685909 Website: http://skhincaps.eu
Coordinator: CeNTItvc - Centro Nanotecnologia Materiais Técnicos, Funcionais e Inteligentes

*Table 1 Consortium List.*

| No. | Beneficiary name | Short name | Country |
|---|---|---|---|
| 1 | CeNTItvc - Centro Nanotecnologia Materiais Técnicos, Funcionais e Inteligentes | CTI | Portugal |
| 2 | BIONANOPLUS SL | BIO | Spain |
| 4 | UNIVERSITAT POLITECNICA DE CATALUNYA | UPC | Spain |
| 5 | INSTITUT FUER VERBUNDWERKSTOFFE GMBH | IVW | Germany |
| 6 | DEVAN – MICROPOLIS S.A. | DEV | Portugal |
| 7 | TELIC SAU | TEL | Spain |
| 8 | PRO-ACTIVE | PRO | Belgium |
| 9 | TEKNOLOGIAN TUTKIMUSKESKUS VTT OY | VTT | Finland |

## Contents



## 1 Summary

Project Duration: 4 years

Project Funding: European Commission | Horizon2020

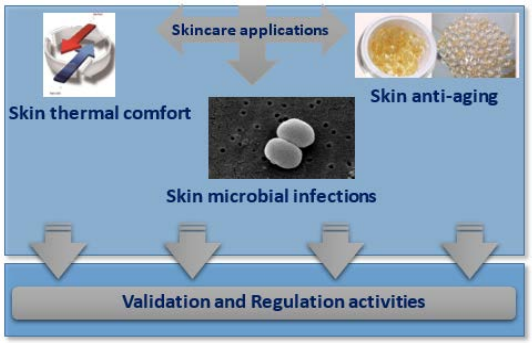
SKHINCAPS project is a research and innovation project aiming to develop customised and safe nanocapsules, using an innovative and sustainable in situ self-assembly nanoencapsulation technology, to deliver novel products for skin healthcare applications, with increased efficiency and cost benefits, leading to ground-breaking innovations on the actual products.

Different demonstrators will be produced with SKHINCAPS customised and safe 'smart' nanocapsules and fully tested for their safety and performance, for skin healthcare applications such as thermal comfort, anti-ageing and natural antimicrobial control.

## 2 Background

In the current nanotechnology context, and despite the uncertainties facing the global economy, there are certain consumers trends that are inevitable. Among the macro trends that are key to the future growth of consumer markets, the ageing population, the 'go-green' consciousness and consumer awareness, the quest for sustainability and the growing interest in natural and organic products have pressured companies to provide a personalised, adapted and increasingly sophisticated technological response to their products and processes. As such, consumer product companies face a tremendous task of bringing newer, safer, natural and more added-value products into the market, within a much shorter time frame

Nanotechnology, a Key Enabling Technology (KET) in Horizon 2020, can help to accomplish these challenges since it allows for cheaper production, less energy- and resource-intensive production processes and more effective products through the exploitation of unique properties achieved at nanoscale. Within this context, the SKHINCAPS project aims to develop the knowhow and processes to manufacture novel, sustainable, safe, cost-effective and highly stable nanocapsules containing different encapsulated active principles at their core, for their incorporation into 'smart' skin care products such as textiles and cosmetics.




## 3 Scientific and technological challenges

The main challenges that the project addresses are:

i) Nanocapsules customization for encapsulating the selected active ingredients;

ii) Achievement of the different release profiles; and

iii) Technology optimization for enabling incorporation in distinct industrial chains.

## 4 Objectives

SKHINCAPS uses a safe, sustainable and easily scalable technology (patented) to nanoencapsulate different actives, namely:

i) Phase-change materials (PCMs);

ii) A cocktail of vitamins and antioxidants; and

iii) Natural essential oils.

These novel customised nanocapsules are being engineered to achieve three possible release mechanisms as a function of application, enhancing actives efficiency.

The SKHINCAPS nanocapsules will then be used to produce different demonstrator products for skin healthcare applications:

- First layer garments containing nanoencapsulated phase-change materials (PCMs) for thermal management and skin comfort;

- Anti-ageing creams containing nanoencapsulated cocktails of vitamins and antioxidants to improve skin anti-ageing effect;

- Antimicrobial lotions and textiles containing nanoencapsulated natural essential oils to prevent or mitigate bacterial infections on the end-users' skin.

The demonstrators will be fully tested for their safety and performance to fulfil the present regulation requirements. Their efficacy and safety will also be determined and compared with currently available cosmetic and textile products.

## 5 Progress and outcomes to date

During the first 18 months, several NCs formulations have been successfully prepared and scaled-up through the nanoencapsulation technology from partner BIO and selected for further developments, using two different biocompatible and biodegradable polymers (one synthetic and other from a natural origin), and the selected active principles, according to their envisaged application. The active selection was based on the intended properties, cytotoxicity and performance. The polymers have also been modified to enhance the encapsulation efficiency and the release mechanism of the actives, with achievement of very promising results. Also, detection methods for assessing the presence of NCs, specifically on textiles, have also been developed. In parallel, the lab-scale life cycle assessment (LCA) screening has started in this period, being at this point optimised for the different SKHINCAPS applications.

The monitoring of external developments, an agreed "Canvas" methodology, and the outline of the value chains needed for the development of SKHINCAPS business plans has been carried out. Furthermore, the exploitation and/or commercialization strategy plan started to be outlined, being already defined the business plans and pilot lines for the envisaged products. In terms of communication and dissemination, SKHINCAPS consortium has been actively conducting several activities towards a broad dissemination of the project and the developments so far performed. Nevertheless, these are always in line with the agreed methodology for the protection of the Intellectual Property Rights.

## 6 Expected Impact

Based on the specific products foreseen, SKHINCAPS project will have a strong socio-economic impact, due to the increase of consortium revenues (particularly for SME partners) and creation of jobs due to the wide range of market niches and companies considered, as well as wider societal implications, since the cost-effective and sustainable skincare products will improve the individual wellbeing, contributing to the healthcare costs control, and reduce the environmental impact related to their manufacturing process and use.

By delivering products non-existing in the market or if existing, still needing to be upgraded to present superior benefits, SKHINCAPS anticipates a strong market impact of its advanced nanocarriers systems. The project goals will contribute to overcome the challenges Europe is facing on the global competition and efficient use of energy and resources, due to the wide applicability and high growth markets that will be addressed and to the sustainability of the technology involved.

By combining the expertise fields involved, SKHINCAPS intends to bring the innovative technologies needed to increase the commercial potential of smart technical textiles and cosmetics, to improve the European competitiveness and create a new growth engine for economic development.

In particular, the proprietary nanoencapsulation technology that will serve as the platform for the novel nanocapsules' development, detaches from the coexisting nanoencapsulation processes by its safety, sustainability, easy scalability and cost effectiveness. With this technology, the project will provide an innovative solution for the development of personal skin care products (textiles and cosmetics) containing the customised and sustainable SKHINCAPS nanocapsules.

# 7 Directory

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

| First Name | Last Name | Affiliation | Address | e-mail |
|---|---|---|---|---|
| Carla | Silva | CTI | Rua Fernando Mesquita, 2785, 4760-034 V.N. Famalicão, Portugal | csilva@centi.pt |
| Dora | Coelho | CTI | Rua Fernando Mesquita, 2785, 4760-034 V.N. Famalicão, Portugal | dcoelho@centi.pt |
| María | Llorente | BIO | Poligono Industrial Mocholi – Plaza Cein N° 5, nave B14, 31110 Noáin, Navarra, Spain | mllorente@bionanoplus.com |
| Susana | Sánchez | BIO | Poligono Industrial Mocholi – Plaza Cein N° 5, nave B14, 31110 Noáin, Navarra, Spain | ssanchez@bionanoplus.com |
| Tzanko | Tzanov | UPC | Campus UPC Terrassa, Edifici Gaia TR 14, Rambla Sant Nebridi 22, 08222 Terrassa, Barcelona, Spain | tzanko.tzanov@upc.edu |
| Bernd | Wetzel | IVW | Erwin-Schroedinger-Strasse 58, 67663 Kaiserslautern, Germany | bernd.wetzel@ivw.uni-kl.de |
| Sergiy | Grishchuk | IVW | Erwin-Schroedinger-Strasse 58, 67663 Kaiserslautern, Germany | sergiy.grishchuk@ivw.uni-kl.de |
| Alexandre | Beirão | DEV Portugal | Rua Engenheiro Frederico Ulrich 2650, 4470-605 Moreira da Maia, Portugal | alexandre.beirao@devan-pt.com |
| Roberto | Teixeira | DEV - Belgium | Klein Frankrijkstraat 6, 9600 Ronse, Belgium | Roberto.Teixeira@devan-be.com |
| Laura | Delgado | TEL | Poligono Industrial Can Barri, C/ Molí d'en Barri 7-9, 08415 Bigues (Barcelona), Spain | laura.technical@telic.es |
| Diana | Rivera | TEL | Poligono Industrial Can Barri, C/ Molí d'en Barri 7-9, 08415 Bigues (Barcelona), Spain | diana.technical@telic.es |
| Thomas | Zadrozny | PRO | Rue Beeckman 119, 1180 Brussels, Belgium | t.zadrozny@mail.be |
| Tiina | Pajula | VTT | Vuorimiehentie 3, 02150 Espoo, Finland | tiina.pajula@vtt.fi |
| Catharina | Hohenthal | VTT | Vuorimiehentie 3, 02150 Espoo, Finland | Catharina.Hohenthal@vtt.fi |

# 8 Copyright

# SmartNanoTox

## Smart Tools for Gauging Nano Hazards

Contract Agreement: 686098 Website: http://www.smartnanotox.eu
Coordinator: Vladimir Lobaskin, School of Physics, University College Dublin, Belfield, Dublin 4, Ireland

*Table 1 Consortium List.*

| No. | Beneficiary name | Short name | Country |
|---|---|---|---|
| 1 | National University of Ireland, Dublin / University College Dublin | NUID UCD | Ireland |
| 2 | Stockholms Universitet | SU | Sweden |
| 3 | Helmholtz Zentrum München | HMGU | Germany |
| 4 | National Research Centre for the Working Environment | NRCWE | Denmark |
| 5 | French National Institute for Occupational Health | INRS | France |
| 6 | Jozef Stefan Institute | JSI | Slovenia |
| 7 | Imperial College London | Imperial | UK |
| 8 | University of Lorraine | UL | France |
| 9 | Dassault Systemes Biovia Ltd. | Biovia | UK |
| 10 | Finnish Institute of Occupational Health | FIOH | Finland |
| 11 | Vitrocell Systems | Vitrocell | Germany |

## Contents



## 1 Summary

Project Duration: 48 months

Project Funding: 8 Mio. EUR

A definitive conclusion about the dangers associated with human or animal exposure to a particular nanomaterial (NM) can currently be made upon complex and costly procedures including complete NM characterisation with consequent careful and well-controlled in vivo experiments. A significant progress in the ability of the robust nanotoxicity prediction can be achieved using modern approaches based on one hand on systems biology, on another hand on statistical and other computational methods of analysis. In this project, using a comprehensive self-consistent study, which includes *in vivo*, *in vitro* and *in silico* research, we address main respiratory toxicity pathways for representative set of NMs, identify the mechanistic key events (KE) of the pathways, and relate them to interactions at bionano interface via careful post-uptake nanoparticle characterisation and molecular modelling. This approach will allow us to formulate novel set of toxicological mechanism-aware end-points that can be assessed in by means of economic and straightforward tests. Using the list of end-points and pathways for the selected NMs and exposure routs, we will enable clear discrimination between different pathways and relate the toxicity pathway to the properties of the material via intelligent quantitative structure-activity relationships (QSARs). If successful, this approach will allow grouping of materials based on their ability to produce the pathway-relevant key events, identification of properties of concern for new materials, and will help to reduce the need for blanket toxicity testing and animal testing in the future.

## 2 Background

Protecting citizens from health threats arising from novel technology-enabled products is one of the objectives of the EU's health strategy and an opportunity for added value at the European level. Nanotechnology is affecting EU citizens due to its numerous applications in all aspects of human and societal life including industrial, manufacturing, agricultural, food, and medicinal sectors. Thousands of NMs are already on the consumer market, and in many cases, the risks of personal or environmental exposure to these materials are unknown or poorly understood. In particular, use of NMs and nanotechnology-enabled products in the field of medicine provides us with new opportunities and tools to detect, diagnose, and treat many of the intractable diseases such as cancer or genetic disorders. However, the small size and novel properties of NMs resulting from advanced engineering pose new challenges and unforeseen risks to the immune system, which the human body is not equipped to deal with. Under REACH rules, manufacturers, importers and downstream users are obliged to ensure that the NMs used in their products do not adversely affect human health or the environment yet there is no way of reliably predicting such effects. Concern has been voiced that the safety of NMs has not been addressed satisfactorily. On the economical side, a study of DG Enterprise and Industry has shown that REACH rules may cause administrative burden, affect time to market especially for SMEs.

In an attempt to tackle this problem, a great amount of nanotoxicological data has been generated for NMs using *in vitro* models. However, it is now evident that much of the *in vitro* data are of questionable value: one often can find almost any result published for a given NM, ranging from "overt toxicity" to "no observable toxicity". A general concern with common *in vitro* models is the lack of ability to control or to characterize the physical state of the NM before and after the exposure within the environmental or biological system. Moreover, the distribution of the NM in the living organism is essential for toxic effects and cannot be accurately mimicked in *in vitro* models for most exposure scenarios. Many of the end-points determined using *in vitro* testing models have little or no relevance for the physiological and pathological changes occurring in humans or animals. While animal experiments should be avoided where possible, they are required in order to relate the design of *in vitro* assays to responses at the various levels of the whole organism and to essential events of the relevant toxicity pathways and adverse outcomes. Thus, there is a need for the design and development of efficient *in vitro* and *in silico* models that are predictive of *in vivo* responses. The development and validation of an effective and reliable predictive model should be based on a detailed understanding of interactions of NMs with various components of the biological systems and on identifying the specific properties of NMs that are relevant to such interactions.

## 3 Scientific and technological challenges

Relating the NM descriptors to the toxicity end point is at present practically unfeasible. Most approaches use black-box search for statistical correlations between the descriptors and toxicity indicators. We consider that progress can be made using adverse outcomes (AO) pathway-based mechanism-aware intelligent QSARs.

Another challenge is to determine all the hazardous effects of engineered NMs using common *in vitro* studies of the toxic effects, which address only acute responses and cytotoxicity. A more complete picture can be obtained using *in vivo* study of the acute and chronic toxic effects, with relation to the adverse outcome, in depth analysis of the pathologies.

Understanding of the mechanisms underlying the observed adverse effects from engineered NMs is not possible in most cases as the state of the NM inside the tissues is not known. The state of the NM after the initial contact, in particular their biomolecular corona, must be analysed. NMs must be tracked inside the biological fluids and molecules involved in bionano interactions identified.

Therefore, a progress in the field will depend on whether the following challenges can be answered:

- *Identification of underlying pathways ('toxicity pathways') for NM interactions with living organisms*

In response to this, we plan to perform comprehensive analysis (transcriptomics, proteomics, histopathology) of *in vivo* pulmonary toxicity and use systems biology for a number of representative engineered NMs and extensive data mining to identify the TPs and related AOs.

- *Development and demonstration of a mechanism-based understanding of the toxicity*

We plan to identify and analyse *in vivo* pulmonary toxicity pathways for a number of engineered NMs, track the path of the NMs inside the organism to identify the molecular initiating events/key events (MIE/KE) for each pathway understand how the NM triggers or steers the AOP via inducing the corresponding KEs. The triggering effect will be verified experimentally for the affected pathways.

- *Linkage of the potential for adverse effects to specific physical or chemical nanoscale properties*

We aim to analyse the whole set of molecular interactions at the bionano interface after the NM uptake in the lungs and into individual cells and their distribution beyond.

- *Creation of a basis for grouping of engineered NMs by the toxic action*

We plan to systematically study, *in silico*, *in vitro*, and *in vivo*, the main groups of engineered NMs to identify the properties responsible for triggering the toxicity pathways (TP) and determine the groups of NMs by their ability to cause particular MIE/KE.

- *Development of tools that maximize read-across and determine properties that are applicable across the board*

To achieve this we plan to create a bionano interactions database to classify the NMs according to the type of change of the BM state and type of molecular event each particular property can be related to.

## 4 Objectives

Without the mechanistic understanding of the toxicity, one can only rely on statistical correlations between the NM properties and the toxicity endpoints. Thanks to the development of systemic approaches to analysis of biological systems (systems biology) and new methods of characterization and data generation in toxicology in the recent years, we are now in a position to make a step forward from the black-box-type statistical approaches and formulate a new paradigm in the toxicology – a **mechanism-aware NM toxicity screening**.

We contend that the game-changing screening approach should be based on the detailed understanding of the response of the organism to exposure to NMs from the initial contact to the adverse outcome.

We propose that to construct a predictive model for gauging the toxicological and biological impacts of NMs one should address

- *in vivo* toxicity pathways for NMs that are of regulatory importance
- molecular initiating events (MIE) and key events (KE) steering the toxicity pathways finally leading to an adverse outcome
- molecular mechanisms of NM involvement in these KEs
- structure and content of NM-biomolecule complexes after NM uptake

The key step in the development of the smart screening approach is to combine the systems biology analysis of the responses of organisms to the NM exposure, resulting in a clear identification of the resulting pathways and key events, with the analysis of the whole chain of bionano interactions involving the NM inside the organisms.

The central goal of our project is the construction of a quantitative model that connects the NM descriptors (properties) detrimental to their interaction with biomolecules that causes the molecular initiating events and key events leading to the development of an adverse outcome.

**SmartNanoTox objectives:**

- To identify main pulmonary adverse outcomes induced by common NMs, and identify associated MIE, KEs and toxicity pathways leading to AO.
- To establish relationships between physicochemical properties of NMs and KEs steering the TP leading to AO, and suggest descriptors for grouping of NMs according to their toxicological mode-of-action.
- To create a database of bionano interactions that will enable development of read-across and QSAR tools for the toxicity assessment of new NMs.
- To develop a smart screening approach, where predictions of toxicity of a NM can be made on the basis of purely computational or limited in vitro screening tests focused on crucial bionano interactions.

## 5 Progress and Outcomes to date

The research programme combines a broad range of experimental and theoretical methods covering all stages of NM interaction with an organism from the initial uptake to the (post contact) adverse outcome. We attempt to identify all possible short-term and long-term adverse effects related to inhalation for a range of selected NMs. We focus on a relatively small number of materials and exposure routes to be able to achieve the mechanistic interpretation of the NM transport, crucial bionano interactions that can trigger the adverse outcome pathway (AOP), and thus relate the basic NM descriptors to the adverse effects. We expect that the results of this research will enable the subsequent formulation of intelligent QSAR systems based on key nanoparticle descriptors. Based on the analysis of the NM-induced pathways, the project will develop toxicity pathway-based QSARs and map the NM physicochemical properties to the MIE/KE and thus to the specific AO for any NM.

*WP1 Identification and classification of the entry mechanisms and adverse effects of NMs in vivo and in vitro.*

This WP deals with *in vivo* exposure supplemented with *in vitro* experiments: inhalation, instillation of rodents, analysis of the gene expression and proteomics and supplies the data about the NM entry routes and endpoints for WP2 and WP3.

WP2 *Identification and classification of the state of nanoparticles after initial contact with the organism.*

This WP contains *in vitro* experiments, either complementing the *in vivo* studies of WP1 or standalone and aims at characterising the NM state after entering the organism, or specific molecular mechanisms of the uptake, transport or toxicity

*WP3 Identification of end-points and classification of the possible TPs, identification of the MIEs and KEs for selected pathways.*

This WP is devoted to analysis and integration of the gene expression and/or proteomics data as obtained in WP1 and WP2, data mining and statistical modelling aiming to establish the adverse outcome pathways and identify the MIE/KEs

*WP4 Database and QSAR for NM-biomolecule interactions.*

This WP is computational and deals with modelling biomolecules in contact with nanoparticles and building a database of bionano interactions.

*WP5 Assessment and validation of the AOPs and MIE/KE, test of NM descriptors as predictors.*

WP5 assesses the candidate MIE/KEs as extracted in WP3 either in silico, using the models from WP4, or in vitro and validates the identified pathways and MIE/KE by modifying either the NM or the target system

*WP6 Development of a smart test addressing the molecular key events.*

Here we finally construct a simplified in vitro or in silico test for the identified AOPs using the database from WP4 combined with findings of WP5.

*WP7 Dissemination and exploitation.*

*WP8 Management.* The overall research programme thus combines molecular modelling tools, systems biology, *in vivo* and *in vitro* studies.

The outline of the project concept is presented in Fig. 1.

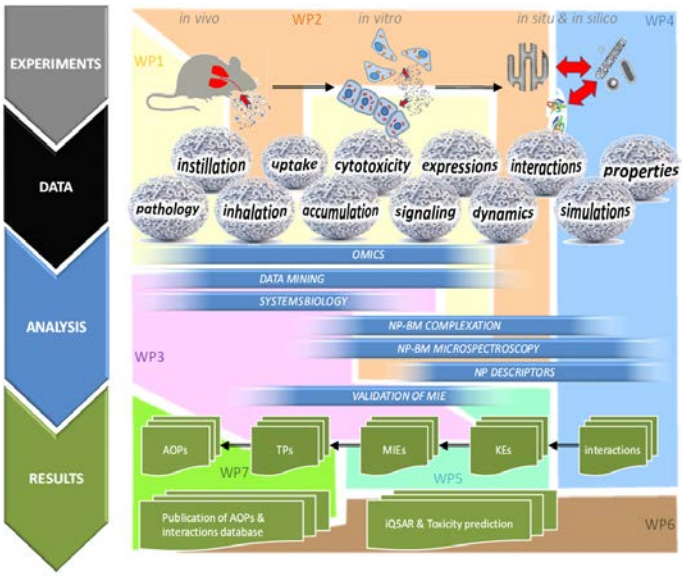



*Fig. 1. Outline of the concept and approach. Abbreviations used: WP – work package, BM – biomolecule, NP – nanoparticle, AOP – adverse outcome pathway.Content of the work packages:*

The data flow between the work packages and their interdependence are schematically shown in the diagram below:

We use the materials deposited in the EC, Joint Research Centre and a series of 61 materials (the NRCWE series) available at the NRCWE as the base for the investigations. The list includes carbonaceous materials (including more than 30 types of carbon nanotubes), about ten different titanium dioxides, iron-, zinc-, cerium oxides, metals, asbestos, and other. About 50 of these have been tested for biological effects after instillation in mice and effects have been measured up to one year for some of them. There is also available comparative *in vitro* data for a subset and for about 15 of these global gene expression data have been published (NRCWE) or is currently underway.

The first 12-month period of the project was focused on an analysis of the existing information and a development of an optimum strategy to identify in vivo AOPs for respiratory exposure to NM. We also developed, tested and validated methods for NM tracking inside the biological samples, post-uptake characterisation and bionano interface modelling. Our **main achievements** for the period are as follows:

Work Package 1:
- Identified 5 respiratory AOPs that can be addressed within the project:
- Selected a set of relevant NMs suitable for aerosolisation and study of the chosen AOPs
- Performed transcriptomics analysis of lung samples after in vivo exposure to MWCNT from NANoREG

Work Package 2:
- Developed a protocol to assess fluorescent probe desorption from NM
- Applied the protocol for ensuring the quality of labelled NM at three major phases of experiments: functionalisation of NM, labelling and free probe removal
- Developed a NM labelling technique by embedding europium atoms into the TiO2 crystal lattice during TiO2 NP synthesis, which enables fluorescent imaging of NP after long-exposure in vivo experiments
- Developed a set of in vitro tests consisting of pristine NPs and model membranes to study the evolution of NP wraps and alleviate the determination of MIEs
- Refined the procedure for the analysis of NM protein corona

Work Package 3:
- Selected NMs, doses and protocols to identify the relevant TPs
- Isolated coated nanoparticles from in vivo samples from mice and identified proteins in corona by mass-spectrometry
- Performed proteomics analysis of in vivo samples after exposure to asbestos and CNTs
- Performed pathway reconstruction for several in vivo samples with MWCNTs

Work Package 4:
- Developed a multiscale method of modelling of NM-biomolecule interaction using two-layer NM model and potentials of mean force NM-aminoacid from atomistic simulations
- Calculated adsorption free energies of aminoacids to various CNTs
- Predicted 3D structures for over a 100 plasma proteins identified in NM protein corona

Dissemination:
- Launched a project website
- Published 5 papers in refereed journals
- Presented 17 talks at conferences
- Published 5 press releases and created a twitter account to advertise the project activities
- Participated in OECD-ProSafe meeting on Nanosafety

## 6 Expected Impact

- *SmartNanoTox will develop new screening tools to enhance the efficiency of end-rate, at which NM hazard profiling can be performed*

We will develop reliable *in vitro* and *in silico* tools based on the knowledge of the mechanisms of the pulmonary toxicity of NMs and bound to novel endpoints. We will validate the predictive power of *in vitro* models/endpoints and *in silico* models by *in vivo* strength of evidence. Such tools will facilitate a fast risk assessment and support faster market introduction of new NM products.

- *SmartNanoTox will facilitate faster definition of NM toxicity mechanisms*

We aim at identifying the KEs and MIEs for each selected TP. Assessment of hazards and risks and the design of new test systems will be facilitated by this. We will develop tools that predict the likelihood for the given NM to cause these events and trigger the particular TP and AO. Knowledge of KE and MIE will enable identification of biomarkers for exposure and health effects, it will reduce the need for assessing morbidity and mortality and defines new strategy for the assessment endpoints based on the KE and bionano interactions. This will reduce the need of animal experiments by supplying more sensitive/frequent

endpoints or parametric instead of stochastic endpoint variables. New biomarkers will provide analytical tools for human exposure and diagnostic tools for detecting early indications of human health effects.

- *SmartNanoTox will facilitate development of "safer by design" approaches, tailored to stakeholders' needs (modellers, industry and regulators)*

By systematically studying interactions between the NMs and all the building blocks of biomolecules, we will enable a prediction of the outcome of interaction of arbitrary key molecules with the NM and the content of NM-BM complexes for any NM with known physicochemical properties.

By scanning main groups of engineered NMs, we will identify the NM properties that might be responsible for causing a particular toxic effect and lead to a particular AO, and thus should be modified or avoided. This will provide means of grouping and read-across characterization of NMs and enable development of materials that are safe by design.

- *SmartNanoTox will produce data in a recognized and accessible database for use beyond the lifetime of the project*

We will create a bionano interactions database to classify the NM according to the type of change of the BM state and type of event each particular property can be related to. The gene profiling results from in vivo and in vitro exposure experiments will be uploaded to GEO database, and the AOPs submitted to OECD AOP project.

The experimental results will be presented in the ISA-TAB-Nano format and made available to the community using the framework provided by EU FP7 eNanoMapper project. The consortium will harmonise data with other EU projects such as H2020 ProSafe and NanoReg II.

- *SmartNanoTox will provide solutions to the long-term challenges of nanosafety and nanoregulation:*
    - develop and validate a novel testing strategy that can be used for risk assessment of new materials
    - develop computational tools for testing the NMs for regulatory purposes
    - design cost-effective predictive in vitro assays targeted to MIE/KE based on reporter gene
    - contribute to NCBI GEO database with MIAME-compliant NM-induced gene expression profiles
    - contribute to OECD database with exhaustive description of new TPs (or AOPs).

# 7 Directory

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

| First Name | Last Name | Affiliation | Address | e-mail |
|---|---|---|---|---|
| Vladimir<br>Boris<br>David | Lobaskin<br>Kholodenko<br>Gomez | NUID UCD | School of Physics, Belfield, Dublin 4, Ireland | vladimir.lobaskin@ucd.ie<br>boris.kholodenko@ucd.ie<br>david.gomez@ucd.ie |
| Alexander | Lyubartsev | SU | Department of Materials and Environmental Chemistry, Svante Arrhenius väg 16C, Stockholm, Sweden | alexander.lyubartsev@mmk.su.se |
| Tobias<br>Otmar | Stöger<br>Schmid | HMGU | Helmholtz Zentrum München: Deutsches Forschungszentrum für Gesundheit und Umwelt Gmbh, Ingolstädter Landstrasse 1, Neuherberg 85764, Germany | tobias.stoeger@helmholtz-muenchen.de<br>otmar.schmid@helmholtz-muenchen.de |
| Ulla<br>Kristina | Vogel<br>Bram Knudsen | NRCWE | Det Nationale Forskningscenter For Arbejdsmiljø, Lersø Parkalle 105, København 2100, Denmark | ubv@arbejdsmiljoforskning.dk<br>kbk@arbejdsmiljoforskning.dk |
| Laurent<br>Frederic | Gaté<br>Cosnier | INRS | Institut National de Recherche et de Securite, Boulevard Richard Lenoir 65, Paris 75011, France | laurent.gate@inrs.fr<br>frederic.cosnier@inrs.fr |

| First Name | Last Name | Affiliation | Address | e-mail |
|---|---|---|---|---|
| Janez | Štrancar | JSI | Institut Jozef Stefan, Jamova 39, Ljubljana 1000, Slovenia | janez.strancar@ijs.si |
| Nicholas | Quirke | Imperial | Department of Chemistry, Exhibition Road, South Kensington Campus, London SW7 2AZ, UK | n.quirke@imperial.ac.uk |
| Olivier<br>Luc<br>Bertrand | Joubert<br>Ferrari<br>Rihn | UL | Universite De Lorraine, Cours Leopold 34, Nancy CEDEX 54052, France | Olivier.Joubert@univ-lorraine.fr<br>Luc.Ferrari@univ-lorraine.fr<br>Bertrand.Rihn@univ-lorraine.fr |
| Marc | Meunier | Biovia | Biovia Ltd, Unit 334 Cambridge Science Park, Cambridge CB4 0WN, UK | Marc.MEUNIER@3ds.com |
| Henrik | Wolff | FIOH | Työterveyslaitos, Topeliuksenkatu 41a, Helsinki 00250, Finland | Henrik.Wolff@ttl.fi |
| Tobias | Krebs | Vitrocell | Vitrocell Systems Gmbh, Fabrik Sonntag 3, Waldkirch 79183, Germany | t.krebs@vitrocell.com |

# 8 Copyright


# Updates from FP7 projects that are currently running

# FIBRALSPEC

## Functionalised Innovative Carbon Fibres Developed from Novel Precursors With Cost Efficiency and Tailored Properties


Contract Agreement: 604248 Website: http://www.fibralspec.net/
Coordinator: Prof. Dr. Constantinos Charitidis, National Technical University of Athens

*Table 1 Consortium List.*

| No. | Beneficiary name | Short name | Country |
|---|---|---|---|
| 1 | National Technical University of Athens | NTUA | GRE |
| 2 | Politecnico di Torino | POLITO | ITA |
| 3 | University of Birmingham | UOB | UK |
| 4 | Euromobilita S.R.O. | EUMO | CZ |
| 5 | Thales Research and Technology | TRT | FR |
| 6 | GlobalSafeGuard Ltd | GSG | UK |
| 7 | Open Source Management Ltd | OSM | UK |
| 8 | Anthony, Patrick & Murta Exportação | AP&M | POR |
| 9 | Frantsevich Institute for Problems of Materials Science | IPMS | UKR |
| 10 | CTM Ltd | CTM | UK |
| 11 | CB Yuzhnoye | YUZ | UKR |

## Contents



## 1 Summary

Project Duration: 48 months, Project Funding: 6,083,991.00 €

FIBRALSPEC initiative addresses new technologies, which have a clear market potential and a strong impact and as such promotes European Union's (EU) wide cooperation. FIBRALSPEC focuses on conducting innovative processes with streamlining and improved control through Unit for Continuous PAN-based carbon fiber Pilot Production. Testing of laminates and prepregs production based on the new developed carbon fibers followed by manufacturing of laminates/coupons and high- performance filament wound tubes. The project also efforts on functionalization, mainly focused on cost reduction and improvement of mechanical and chemical properties of carbon fibers. During the life of project, novel carbon fiber precursors such as lignin are being developed. Life cycle assessment assists in possible commercial risks that are continuously estimated during the project and quantify/assess the environmental impact of the materials that will be used. As for recycling and used utility of recycled carbon fibers, new techniques are applied to provide commercially-relevant products that are manufactured from waste carbon Fibers.

Keywords: Carbon Fibers, Novel Precursosrs, Surface Treatment, Oxidation, Life Cycle Analysis, Supercapacitors, Rapid Deployment Secure Emergency Shelter

## 2 Background

Carbon fibers (CFs) are one of the most important reinforcement materials used for composite manufacturing, as they combine an exceptional set of properties (low density, high stiffness etc.) that makes them ideal for high performance composites; usually CFs are combined with thermo set resin matrices, yet thermoplastic matrix composites have begun to increasingly appear. Even

though the industrial scale production of CFs started from the late 1960s/early to 1970s, due to several challenges in their manufacturing the production volume has not grown on par with the increasing demand; as a result, the price of the CFs based materials remains still high. Thus, there are still a lot of opportunities for improvement in this field, with the focus being on the precursor material used for carbonization and optimization of stabilization process.

Currently, the CFs precursors of choice are polyacrylonitrile (PAN) and pitch fibers; both of them originate from petroleum and as such they follow its price trends. There are several alternate possible precursors that have been proposed; the most significant is lignin, a natural polymer existing in all plants. Lignin is considered as a sustainable and renewable source, given that large amounts are separated as byproduct from wood during paper pulp production.

FibralSpec's main motivations include :

- The improvement of production of carbon fibres from green precursors such as lignin and renewable resources
- The manufacturing of fibre reinforced composites via ecofriendly-production techniques
- The use of the reinforced composites in different applications, such as: flexible supercapacitors and Rapid Deployment Secure Emergency Shelters
- LCA and LCC analyses will quantify the green credentials of the reuse and recycling strategies from the start - precursor development-, till the carbon fibre waste.

## 3 Scientific and technological challenges

Many other polymers have been proposed as alternate carbon fibres precursors to PAN and pitch (e.g. polyvinylchloride, PVC), but none of them worked as good as these two materials and now they are abandoned. The properties of the carbon fibres (and especially their high mechanical strength and stiffness) are exploited through their composites (Carbon Fibre-Reinforced Composites, CFRC). However, the full exploitation of the outstanding carbon fibre properties has not yet been achieved. This is mainly due to the inherently inert and smooth surface of carbon fibres, which does not permit a strong anchoring of the matrix onto the surface of the fibres; thus, the fibres are not loaded with the stress that they can carry, but with a smaller load which is defined by the winkle out of the fibres from the matrix (pull out effect)[1]. Moreover, a class of materials with similar structure that have been commercialized are the polyimides (which also have high char yield), but these belong to the "engineering polymers" class and are quite expensive materials, thus they are not considered as carbon fibre precursors. Nevertheless, the potential to improve the quality and the effectiveness of the PAN-based carbon fibre processes should not be underestimated. So, apart from developing novel precursors, there are also other still open research fields on PAN precursors which are as important, in both production procedures as well as in composites manufacturing. New carbon fibre precursor need to have at least as good properties and be as cost-effective as PAN.

Furthermore, carbonaceous nanomaterials are widely used as electrodes materials for **supercapacitor** applications, because of their high specific surface area and high electrical conductivity. Among carbonaceous nanomaterials, activated carbon are widely used in commercial supercapacitors and several scientific works have reported the properties of CNTs, graphene, onion like carbons for electrode materials. Carbon NanoFibres (CNFs) have been less described although presenting a lot of interesting physical properties like high specific surface, high electrical conductivity and low cost. CNFs are a carbonaceous material of interest for the development of supercapacitors devices as a standalone materials or in combination with others nanomaterials. CNFs are also a promising nanomaterial for the development of pseudocapacitors after chemical modification and grafting of electroactive organic or inorganic materials.

***Medium technology – large scale: Rapid Deployment Secure Emergency Shelter (RDSES)***

The existing concept "Rapid Deployment Secure Emergency Shelter-FibreGlass" (RDSES-FG) which is designed and developed by applying traditional composite manufacturing techniques is be extended; employing the use of carbon fibre, the unit mass will be reduced while at the same time increasing rigidity, durability and end user usability, resulting in Rapid Deployment Secure Emergency Shelter Carbon Fibre (RDSES-CF).

***Figure 1.*** *Rapid Deployment Secure Emergency Shelter (RDSES)*

The unit will be formed of a bottom unit and a top unit, internal sleeping platforms and a door unit. The idea behind this project is that top and bottom units would be a common part, with the aim of reducing manufacturing costs. The units and all other components can be stacked / nested inside each other.

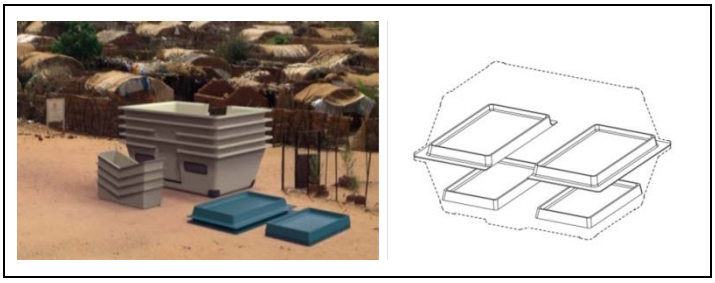


***Figure 2.****Unique unit and stacking ability to facilitate transportation*

[1]Morgan, P.; Carbon fibres and their composites, Taylor & Francis, Boca Raton, 2005, p. 65-324, 501-550.

After more than 5 decades of research, carbon fibres and their composites have reached maturity and they are currently not just a 'high-end' costly solution for low rate production, but represent a growing industry with a multitude of applications. Their success is due to their high strength-to-weight ratio and to the fact that in composites they exhibit a combination of valuable properties that may provide a solution in complex problems of materials science and technology. In 2013 carbon fiber demand was abound 46,500 tons, with expected growth rate of around 12.5 % for the following years. Some of their most important applications are in the sports and leisure industry with articles for many sports (tennis racquets, golf clubs, bicycles etc.), in the aerospace industry (the newest and largest commercial airplanes, Boeing 787 and Airbus A380, demonstrate a large part of their airframe built using carbon fibre composites) and for the blades used in wind turbines[2,3]. The state of the art precursor (in terms of production volume) is polyacrylonitrile (PAN) fibres; PAN-based carbon fibres represent more than 70 % of the total carbon fibre (CF) production[4]. A point to note here is the supply and availability of carbon fibres in the future where there is the need for EU States to be independent of the current supply chain. Moreover, there is also a need to consider for 'green' CF precursor which do not derived from petroleum. Other important precursor materials are pitches of various forms like petroleum-, coal tar- or mesophase- pitch.

## 4 Major Outcomes to date

The use of lignin makes the material carbon-neutral (CO-emission vice). More importantly, the energy needed for making CF from lignin is reported to be only 10% of making PAN-based CF (which typically is 270MJ/kg). Hence, energy efficiency (allowing low price for customers) in combination of non-use of fossils provide a route for a sustainable carbon fibre feed-stock (importance of recycled CF). Again the energy needed for reclaiming the used CF from CFRP-composites (Recycled CF) is only 10% of that needed for making new fibres. This in combination with the fact that the mechanical properties of the recycled CF are practically unaffected opens a route for cheap, sustainable, R-CFRP materials.

Within the framework of FibralSpec the main achievements include:

- A prototype of advanced flexible supercapacitor based on CNFs for next generation energy storage devices has been delivered (Fig. 3).
- Concerning on fiber spinning process a prototype of Operational Mechano Electro spinner has been designed tested and delivered during the previous period (Fig.4). The software has also been developed and the maintenance manual has been delivered as well. This software controls the flow of each component in a full time closed loop model. After the precursor spinning stage thermal treatment follows in order to form carbon fibers.
- Novel bio-based precursor materials have been melt spun by blending lignin with various polymeres (PLA, HDPE, PP) (Fig.5) in order to reduce the precursor cost, improve the mechanical properties and find new applications of lignin ; for the time being it is an undervalued product and moreover to create a sustainable, renewable bio-based material (reducing the cost of the carbon fibre precursor is feasible through the use of side product from cellulosic ethanol and paper industries).
- Concerning the stabilization procedure the fully automated continuous line has been tested on several different profiles and after several modifications finally setted (Fig. 6).

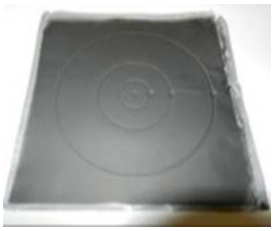


***Figure 3.*** *Electrode fabricated using the dynamic spray-gun method and composed of mixtures of graphene/graphite and CNTs*

**Figure 4.** *Operational Mechano Electro Spinner*

***Figure 5.*** *Melt spinning of lignin with polymers*

[2]Azarova, M.T.; Kazakov, M.E., Fibre Chemistry, 42 (2011), 271-277.
[3]Ishikawa, T.;, Advanced Polymer Science, 178 (2005), 109-112.
[4]Vaidya, U.K.; Chawla, K.K.;, International Materials Reviews, 53 (2008), 185-218.

***Figure 6.*** *Continuous Stabilization line*

One of the important industrial problems is the production of complex geometrical shapes including the dual curvature ones. Together with the method of winding, the production method based on prepreg manufacturing is very useful for aerospace industry. Prepregs are obtained by means of binder (epoxy and other resins) impregnating CF textile yarns preliminary produced on textile machines. One of the main features of carbon textile production is the processing of high strength and high modulus fibers with enhanced brittleness. During the yarn formation, it is necessary to exclude small bend radius. For these purposes it is possible to combine CF from PAN fibers situated without bends with more technological CF form HC-fibers. The last ones can form for example knitted loops around high strength CF from PAN-fibers. Furthermore, if another filaments are used (glasses, metals, kevlar, basalt) they form loops around CF from PAN-fibers. Carbon and textiles in composites lead to increase shear characteristics and transverse (relative to CF direction) strength.

## 5 Expected Impact

**The expected impacts listed in the work program**

The technology has clear market potential and will have a strong impact on the economic prospects the SME participants via two routes:

- The industrial partners will use the technology directly in their own manufacturing operations and/or directly in the services they provide.
- The industrial partners will market the technology through process licensing to other manufacturing organisations (via product type, market area, geographical region).

The FIBRALSPEC proposal well addresses all the impacts listed in the work programme of the Call. The project aims in increase of competitive power of European CF sector and especially that of the industrial partners. Industrial partners are more sensitive in the conditions of growing competitiveness because of the limited resources and access to modern RTD facilities.

**Benefits for the involved SMEs and downstream producers**

The existing concept "Rapid Deployment Secure Emergency Shelter- fibreglass" (RDSES-FG) designed and developed applying traditional composite manufacturing techniques will be extended through FIBRALSPEC by employing the use of carbon fibre, reducing the unit mass while at the same time increasing rigidity, durability and end user usability, resulting in Rapid Deployment Secure Emergency Shelter carbon fibre (RDSES-CF). The current RDSES-FG unit concept and all of its components weigh in at 500 kg. The target is to reduce the current design weight by 60% to approx. 200kg per RDSES-CF unit. A reduction of wall thickness by 50% from 60mm to 30mm would reduce mass per unit. This would allow greater numbers of stacked/nested units to be transported per shipment, therefore making significant gains in transport efficiency. An increase in components ability to resist damage would reduce the maintenance required during and after installation. Any increase in component rigidity would allow more efficient on-site installation. Less flexing would reduce the requirement for time consuming site preparation / ground levelling. As these RDSES-CF units will be sited in circumstances that may not have the use of lifting equipment, the target of 200kg reduces the amount of on-site manpower / manual handling required for unit assembly. Additionally, the use of carbon fibre would greatly assist in the end-of-life recycling. The shelter design could be made out of GRP sandwich; using strips of the new carbon fibre to stiffen the GRP panels will have significant impact for a carbon/glass combination where the structure is inflated from a packed state (deployable structure which can be parachuted to the disaster area and then inflated).

**Economic impact**

Supercapacitors technology is being developed through this project and this fact creates huge economical potentials as well because this market looks promising with opportunities in transportation, electronics and energy industry The knowledge gained from this project could ceate qualified personell, decrease unemployment and contribute to EU's economy. Another aim of this project is to enhance competitiveness and the exports of European industry by defining new international standards in CFs field. The increase of employment of high qualified personnel is also expected.

**Environmental impacts**

The replacement of less environmental friendly technologies will occur with more intelligent systems. Material waste losses will be reduced due to reliability and in service performance of components and reduced corrosion activity. Safer working conditions will also be ensured and carbon dioxide emissions will be limited due to savings in materials.

**Social Impact**

Within the area of Humanitanan aid the issue of protection of vulnerable people in disasters worldwide is critical. RDSES design and manufacturing process also could develop onsite manufacture right in the heart of the affected territories using containerized factories. This will allow a suitable programme of production using local labour, local resources were available and therefore stimulating local economic activity.

# 6 Directory

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

| First Name | Last Name | Affiliation | Address | e-mail |
|---|---|---|---|---|
| Costas | Charitidis | NATIONAL TECHNICAL UNIVERSITY OF ATHENS | Heroon Polytechniou Street 9<br>15780 Athens - GREECE | charitidis@chemeng.ntua.gr |
| Alberto | Tagliaferro | POLITECNICO DI TORINO | Corso Duca degli Abruzzi 24<br>10129 Torino - ITALY | alberto.tagliaferro@polito.it |
| Hanshan | Dong | THE UNIVERSITY OF BIRMINGHAM | Edgbaston<br>B15 2TTBirmingham - UNITED KINGDOM | H.DONG.20@bham.ac.uk |
| Ales | Loncaric | Euromobilita s.r.o. | Taborska922<br>29301Mlada Boleslav - CZECH REPUBLIC | ales@euromobilita.com |
| Paolo | Bondavalli | THALES SA | Rue de Villiers45<br>92200 Neuilly Sur Seine - FRANCE | paolo.bondavalli@thalesgroup.com |
| Glen | Monaghan | GLOBAL SAFEGUARD LTD | Todburn Moor FarmCottages2<br>NE65 8RXLonghorsley Morpeth<br>Northumberland - UNITED KINGDOM | glen@globalsafeguard.com |
| Emmanuel | Sofianopoulos | OPEN SOURCE MANAGEMENT LIMITED | COWLEY ROAD<br>CB4 0WSCambridge - UNITED KINGDOM | emmanuel@osm.eu.com |
| Guy | Simmonds | ANTHONY, PATRICK & MURTA-EXPORTACAO LDA | Sitio Da Falfeira EN 120<br>8601 903Lagos - PORTUGAL | simmonds.guy@gmail.com |
| Iryna | Belan | FRANTSEVICH INSTITUTE FOR PROBLEMS OF MATERIALS SCIENCE OF NATIONAL ACADEMY OF SCIENCE OF UKRAINE | Krzhyzhanovsky Street 3<br>03142 Kiev- Ukraine | belanira@bk.ru |
| Ruth | Wootton | C.T.M. EQUIPMENT LIMITED | Whaley Road Zenith Park<br>Unit E<br>S75 1HTBarnsley South Yorkshire - UNITED KINGDOM | ruth@ctmukltd.com |
| Irina | Husharova | YUZHNOYE DESIGN OFFICE NAMED AFTER MIKHAIL YANGEL | Krivorozhskaya 3<br>49008Dniepropetrovsk - Ukraine | space@yuzhnoye.com |

# 7 Copyright

# FutureNanoNeeds

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

Contract Agreement: NMP.2013.1.3-3 Website: http://www.futurenanoneeds.eu/

Coordinator: Kenneth Dawson, Centre for BioNano Interactions, University College Dublin, Belfield, Dublin 4, Ireland

*Table 1 Consortium List.*

| No. | Beneficiary name | Short name | Country |
|---|---|---|---|
| 1 | University College Dublin, National University Of Ireland | NUID UCD | Ireland |
| 2 | Fundacio Privada Institut Catala De Nanotechnologia | ICN | Spain |
| 3 | Philipps Universität Marburg | PUM | Germany |
| 4 | Ecole Polytechnique Federale De Lausanne | EPFL | Switzerland |
| 5 | Institut Für Energie Und Umwelttechnik Ev | IUTA | Germany |
| 6 | Heriot-Watt University | HWU | United Kingdom |
| 7 | Luxembourg Institute of Science and Technology - Centre De Recherche Public - Gabriel Lippmann | LIST | Luxembourg |
| 8 | The University Of Birmingham | UoB | United Kingdom |
| 9 | Trinity College Dublin | TCD | Ireland |
| 10 | Nederlandse Organisatie Voor Toegepast Natuurwetenschappelijk | TNO | Netherlands |
| 11 | Istituto Di Ricerche Farmacologiche Mario Negri | MNI | Italy |
| 12 | Rijksinstituut Voor Volksgezondheiden Milieu | RIVM | Netherlands |
| 13 | Vlaamse Instelling Voor Technologisch Onderzoek N.V. | VITO | Belgium |
| 14 | Commissariat A L Energie Atomique Et Aux Energies Alternatives | CEA | France |
| 15 | Technicka Univerzita V Liberci | TUL | Czech Republic |
| 16 | Comitee Des Donnees Scientifiques Et Technologiques | CODATA | France |
| 17 | Nanofutures Asbl | NfA | Belgium |
| 18 | Filarete Servizi Srl | FILARETE | Italy |
| 19 | Nanogap Sub-Nm-Powder Sa | NANOGAP | Spain |
| 20 | Solarprint Limited | Solarprint | Ireland |
| 21 | Nanonica Europe Sl | Nanonica | Spain |
| 22 | Centro Ricerche Fiat Scpa | CRF | Italy |
| 23 | Universidade De Santiago De Compostela | USC | Spain |
| 24 | G24Power Ltd | G24 | United Kingdom |
| 25 | Fundacio Hospital Universitari Vall D'Hebron – Institut de Recerca | VHIR | Spain |

## Contents

## 1 Summary

Project Duration: *Starting in January 2014 for 48 months*

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

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

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

## 2 Background

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

Furthermore, even if a well-developed policy-level strategy for technology transfer into the market had been available; one has to face the real facts on the ground, during that period. Scientists were not prepared to provide the information, let alone deeper insights, into the new issues involved. Indeed, when called upon to respond, the challenge of new science requiring a highly integrated interdisciplinary approach lead to a fragmented scientific response from a community that was itself just assembling. Some of the information published was simply factually incorrect, some over-interpreted (or, sometimes worse, not at all) and the discussion turned into a debate with 'sides' taken by different stakeholders. In the end, the scientific response admixed with a broader societal debate, with significant mutual misunderstanding of what science could, and could not, do on the time-scales available.
It must be considered a remarkable achievement of policy makers, European (US, and other) institutions, and a scientific community gaining increasing confidence, that the situation has now sufficiently stabilized to allow for a rethink of the overall approach. There is still uncertainty (some significant) about some aspects of the legacy materials, but certainly the most severe concerns about acute hazard that were widely publicized several years ago are (for most common materials) no longer considered justified. A more systematic and thorough scientific investigation of the legacy materials is now underway, as is also a systematic effort to address the outstanding issues.

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

## 3 Scientific and technological challenges

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

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

are exposed along their life cycle, for specific value chains (Figure 1). While it is true that many of these new features will not lead to novel impacts, certainly some will.


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

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

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

## 4 Objectives

FutureNanoNeeds focusses on the following objectives:

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

## 5. Progress and Outcomes to date

Since its beginning in January 2014 the FutureNanoNeeds project has made some significant progress on the various tasks and activities outlined in the project. This development is to no small part due to the close collaboration between partners in various Work Packages. Communication is facilitated by a series of teleconferences and annual meetings. The ability of the individual WP leaders to develop strategies to address specific issues, supported by the project coordinator, has proven especially beneficial.

At the forefront of the project, WP 3 has put considerable effort into the identification of potential value chains as well as assessment of the material life cycle, from synthesis, through manufacturing to the end of the lifetime of the nano-based

material. Since the conception of the project over seven separate value chains have been identified. A list of those is presented in Table 1.

| Area/Sector | Nr | Value Chains | (Nano)materials |
|---|---|---|---|
| ***ENERGY*** | VC1 | Nanomaterials for Energy Harvesting | Reference material: **Perovskites, Zinc Oxides with different dopants** Other possible materials: Titanium dioxides, different doping |
| ***ENERGY*** | VC2 | Nanomaterials for Litium-Ion Batteries | Reference materials for Anode: **Metal oxides, amorphous Si, crystalline Si** Other possible materials for the anode: graphene Possible materials for the cathode: spinel-like materials, for example LiMn1.5Nio.5O4. |
| ***ENERGY / TRANSPORTATION*** | VC3 | Silicon based nanomaterials for Thermoelectrics | Reference material: **doped silicon** Other possible materials: Chalcogenides ($Bi_2Te_3$) Skutterudites Half-Hueslers Silicides and other silicon based materials |
| ***TRANSPORTATION/ INDUSTRY*** | VC4 | Inorganic fullerenes for Nano-based Lubricants | Reference material: **Inorganic fullerenes ($MoS_2$)** Other possible materials: Inorganic fullerenes ($WS_2$) Graphene flakes, graphite oxide, Doped ceria Boron nitride |
| ***MANUFACTURING PROCESSES*** | VC5 | Nanomaterials for Additive Manufacturing | Photopolymers Thermoplastics Metals and alloys |
| ***ELECTRONICS*** | VC6 | Quantum Dots for Display Technologies | Semi-conductive, Luminescent **Quantum dots** |
| ***ELETRONICS*** | VC7 | Silver nanowires for electronic applications | **Silver nanowires** Other possible materials: Iron nanowires |
| ***FUNCTIONAL COATINGS*** | VC8 | Nano-enabled antimicrobial coatings[1] | Reference material: **Titanium dioxide ($TiO_2$), doped or different shape** Other possible materials: Cerium ions and oxides, Silver nanoparticles |

**Table 1 Identified value chains in WP3**

Especially valuable is the development of a framework to forecast exposure to manufactured nano-based products as shown in Figure 1. A two-tiered iterative approach is proposed to prioritize the focal point and to forecast the potential release and emissions at different levels of detail. A framework to forecast exposure of the next generation of nanomaterials using Bayesian networks (BN) was completed

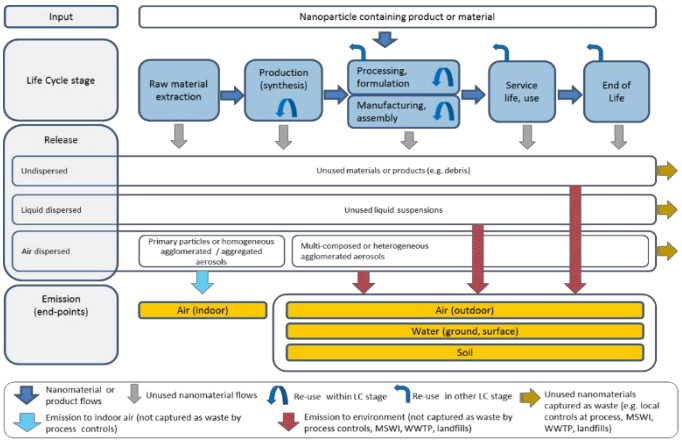



*Figure 1 Framework with nanomaterial releases and emissions along different life cycle stages*

Next steps involve the development of BN of an identified main release source (or focal point). The main objective of these assessments is to identify focal points, that flag potential specific areas of concern in the nanomaterial life cycle and may relate to substantial release, emission or exposure, or where unknown changes and alterations of the material are expected (also referred to as 'hotspots').

Once identified, materials included in a value chain were highlighted to the partners in WP4 for synthesis. This was done in an effort to further study the physio-chemical properties of those materials in different media, their long-term behaviour (stability, degradation, etc.) and their possible biological impact. During 2015 over 80 distinct types of materials of various shapes and doping were synthesised by different WP4 partners and distributed for various studies. A small sample of a shape controlled $TiO_2$ synthesis developed by ICN can be seen on Figure 2. At present the issue of possible contamination and its effects has been highlighted within the project. Special emphasis is now being put into the assessment and prevention of impurities compounds during synthesis. This was further reinforced by a discussion at the 2016 Annual Meeting. Materials synthesised by WP4 partners were analysed in a variety of conditions using several different characterisation methods, including dynamic light scattering, transmission electron microscopy, differential centrifugal sedimentation and LPS assay.

The materials were then submitted to partners in WP5 and WP6 where they were a vigorous study of their in vitro toxicity was conducted. This included a considerable effort by several partners using different cell lines. One of the main focal points was to elucidate the role of particle shape on the NP biodistirubution both in vitro and in vivo. Noteworthy is the large-scale study at VITO where the particle uptake of gold shaped particles was studied in A549 and Caco-2 cells using ICP-MS. In total over 300 particle samples were studied in the two cell lines in different conditions. Another large effort was put into establishing the biological impact of these future materials using different toxicity assays. Those include MTS assay, ROS generation and high content analysis, which were carried out by partners in different exposure scenarios.

Some progress has been made in terms of material classification where several approaches to shape quantification have been studied. The combination of defining of what comprises a shape and biological studies of various samples has enabled the development and implementation of the concept of safe-by-design

nanomaterials. This has been further supported by the exchange of ideas with other institutions, such as the Advanced Materials Industrial Association (AMIA), and an information discrimination effort. Special interest is placed on the risk assessment of new materials and their environmental impact.

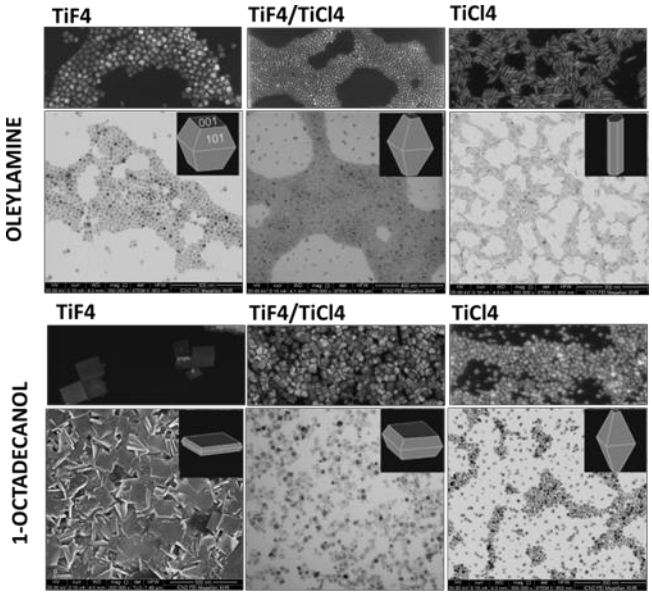



*Figure 2 - TEM/STEM images $TiO_2$ NCs synthesized using the precursor $TiF_4$ (left), a mixed precursor of $TiF_4$ and $TiCl_4$ (middle), and $TiCl_4$ (right) synthesized in the presence of OLAM (upper panel) and 1- ODOL (bottom panel).*

The focus within the project has shifted to the search for potential sources of hazard from entirely novel materials and a deeper study of potentially harmful complex behaviours, including novel shape specific phenomena and possible new diseases prompted by complex bio – nano interactions (e.g. the Trojan horse effect). In implementing this strategy we face many challenges related to the synthesis, analysis and classification of such novel materials, the presence of contamination which would impact hazard studies, and the complication of novel or uncommon biological studies. The synthesis of such new materials had not previously been optimized for biological use and suffered from problems due to the contamination by chemicals, catalysts, and (almost universally) biological contamination. Comprehensive analysis of such materials, anisotropic or composite, presents several hurdles. Most notably there is a lack of consensus in both naming and categorizing, a problem which will be discussed in more detail below. This leads to a lack of tools and understanding to assess fundamental physico-chemical properties such as size and shape, properties which are essential to biological and toxicological studies. In the last period we worked on a series of solutions, not only for a basis for particle classification and accurate assessment of fundamental properties, but also analysis of anisotropic particles in complex media. A significant amount of attention was given to developing endotoxin free and surfactant free synthesis procedures or alternatively quantifying endotoxin content in samples so that appropriate conclusions are made. Even after all of these precautions to avoid confounding contamination, we have observed several new biological effects that could relate to the nature of their shape. These effects will be studied in more depth to eliminate their role in any potential form of toxicity

## 6 Expected Impact

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

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

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

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

*Still, the facts remain that neither within the Nanosafety community, nor nanomedicine, nor indeed any of the other contiguous communities, have we really begun to develop an understanding of the connection between the properties of*

*radically new materials, and their biological impacts, let alone provide a deep basis for safety.*

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

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

*FutureNanoNeeds actively promotes the alliance between Nanosafty Cluster and other relevant NMP programs, regulatory agencies and programs like NanoReg and industry by ongoing communication.*

# 7 Directory

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

| First Name | Last Name | Affiliation | Address | e-mail |
|---|---|---|---|---|
| Derk | Brouwer | TNO | Utrechtseweg, Zeist 3704 HE, Netherlands | derk.brouwer@tno.nl |
| Miroslav | Černík | TUL | STUDENTSKA, LIBEREC 1, 46117, Czech Republic | miroslav.cernik@tul.cz |
| Simon | Clavaguera | CEA | Rue des Martyrs, Grenoble Cedex 9 38054 France | simon.clavaguera@cea.fr |
| Aurora | Conill | Nanonica | Corcega, Barcelona 08025, Spain | aconill@nanotargeting.eu |
| Kenneth | Dawson | NUID UCD | School of Chemistry, Centre for BioNano Interactions, Belfield, Dublin 4 | kenneth .a.dawson @cbn i.ucd.ie |
| Teresa | Fernandes | HWU | Riccarton, EDINBURGH EH14 4AS, UK | t.fernandes@hw.ac.uk |
| Daniel | Fernandez Mosquera | NANOGAP | RUA DA XESTA NAVE, AMES A CORUNA 15895, Spain | d.fernandez@nanogap.es |
| Bertrand | Fillon | CEA | Rue des Martyrs, Grenoble Cedex 9 38054 France | bertrand.fillon@cea.fr |
| Henk | Goede | TNO | Utrechtseweg, Zeist 3704 HE, Netherlands | henk.goede@tno.nl |
| Michael | Graetzel | EPFL | Lausanne, CH-1015, Switzerland | michael.graetzel@epfl.ch |
| Yuri | Gunko | TCD | College Green, Dublin 2, Ireland | igounko@tcd.ie |
| Arno | GUTLEB | LIST | Rue du Brill, Belvaux 4422, Luxembourg | arno.gutleb@list.lu |
| Mura | McCafferty | NUID UCD | School of Chemistry, Centre for BioNano Interactions, Belfield, Dublin 4 | mura.mccafferty@cbni.ucd.ie |
| Thomas | Kuhlbusch | IUTA | Bliersheimer Strasse, Duisburg D-47229, Germany | tky@iuta.de |
| Manuel Arturo | Lopez Quintela | USC | Faculty of Chemistry, Avda. Das Ciencias, Santiago de Compostela 15782, Spain | malopez.quintela@usc.es |
| Iseult | Lynch | UoB | Edgbaston, BIRMINGHAM B15 2TT, UK | i.lynch@bham.ac.uk |
| Paolo | Milani | FILARETE | Viale Ortles, MILANO 20139, Italy | paolo.milani@fondazionefilarete.com |
| Inge | Nelissen | VITO | Boeretang, MOL 2400, Belgium | inge.nelissen@vito.be |
| Wolfgang | Parak | PUM | Renthof, Marburg 35037, Germany | Wolfgang.parak@physik.unimarburg.de |
| Peter | Pechy | EPFL | Lausanne, CH-1015, Switzerland | peter.pechy@epfl.ch |

| First Name | Last Name | Affiliation | Address | e-mail |
|---|---|---|---|---|
| Willie | Peijnenburg | RIVM | Antonie Van Leeuwenhoeklaan, BILTHOVEN 3721 MA, Netherlands | willie.peijnenburg@rivm.nl |
| Victor | Puntes | ICN | Campus UAB, Building CIN2 – Office 2040, Bellaterra 08193, Spain | victor.puntes.ing@icn.cat |
| John | Rumble | CODATA | RUE AUGUSTE VACQUERIE, PARIS 75016, FRANCE | jumbleusa@earthlink.net |
| Mario | Salmona | MNI | Via Giuseppe La Masa, MILANO 20156, Italy | mario.salmona@marionegri.it |
| Maoro | Sgroi | CRF | Strada Torino, ORBASSANO (TO) 10043, Italy | mauro.sgroi@crf.it |
| Thomas | Zadrozny | NfA | Rue de la Presse, Brussels 1000, Belgium | t.zadrozny@mail.be |

# 8 Copyright

# NanoDefine

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


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

*Table 1: Consortium List*

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

# Contents



## 1 Summary

Project Duration: 1 November 2013 – 31 October 2017

Project Funding: 6.9 Mio. EUR

Nanotechnology is a key enabling technology. Still, existing uncertainties concerning EHS need to be addressed to explore the full potential of this new technology. The European Commission has recently recommended a definition of NM as a reference to determine whether an unknown material can be considered a 'nanomaterial' (2011/696/EU). One challenge consists in the development of methods that reliably identify, characterize and measure the size of nanomaterials (NM) both as substance and in various products and matrices. NanoDefine will explicitly address this question. A consortium of European top RTD performers, metrology institutes and nanomaterials and instrument manufacturers has been established to mobilize the critical mass of expertise required to support the implementation of the definition. Based on a comprehensive evaluation of existing methodologies and a rigorous intra-lab and inter-lab comparison, validated measurement methods and instruments will be developed that are robust, readily implementable, cost-effective and capable of reliably measuring the size of particles in the range of 1–100 nm, and above, with different shapes, coatings and for the widest possible range of materials, in various complex media and products. Case studies will assess their applicability for various sectors, including food/feed, cosmetics etc. One major outcome of the project will be the establishment of an integrated tiered approach including validated rapid screening methods (tier 1) and validated confirmatory methods (tier 2), with the NanoDefiner eTool and a user manual to guide end-users, such as manufacturers, regulatory bodies and contract laboratories, to implement the developed methodology. NanoDefine will be strongly linked to standardization bodies, such as CEN, ISO and OECD, by actively participating in TCs and WGs, and by proposing new ISO/CEN work items, to integrate the developed and validated methodology into the current standardization work.

## 2 Background

Nanotechnology is expected to stimulate industrial growth, innovation and development in many fields such as medicine, electronics, automotive, energy, construction, food, and cosmetics. But uncertainties about its safety are currently hampering more widespread exploration and exploitation. The recommendation of a definition of nanomaterial as suggested by the European Commission (EC) (2011/696/EU) is an important step to clearly determine if a material is a nanomaterial, or not. The definition concerns natural, incidental or manufactured particulate materials and focuses on particle size. According to the given definition, a material is a nanomaterial if 50% or more of the constituent particles (by number) have one or more external dimensions in the size range 1 - 100 nm.

## 3 Scientific and technological challenges

Recent research has shown that the reliable detection, characterization and quantification of nanomaterials – as raw material as well as in final products - is one of the big challenges in implementing nanospecific regulatory legislations. Another challenge is that the definition goes beyond advanced materials and encompasses e.g., pigments for paints, coatings and cosmetics, minerals and fillers for composite plastics, paper and packaging, construction materials or catalysts, and so has a broad impact on industry since most types of particulates now need to be re-assessed. Indeed, proper implementation of the definition may need to adapt already existing sector-specific provisions, such as Regulations on Cosmetic Products, Biocidal Products, Food Information and Plastic Food Contact Materials and the review and amendment of the European Chemicals Regulation REACH, to ensure a consistent approach across legislation which helps industry, regulators and consumers alike. It requires, in particular, validated detection, measurement and monitoring methods for nanomaterials because producers must be able to reliably classify an ingredient as a "nanomaterial" and control authorities must be able to test final products for the appropriateness of the labeling. To implement the definition in a legally clear and enforceable way, analytical tools and measures need to be developed for monitoring compliance with the definition.

This means that the EC Definition needs clear guidance on which methodology may be best suited best fit to classify a material according to the definition. Such guidance should be flexible and easily extendable to allow a sector-specific implementation and adaptation for changing regulatory environments and the continuous integration of new scientific and technological progress, but also provide method-independent performance criteria to ensure the applicability of methods for next generation (nano) materials as well.

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

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

- is based on a "number size distribution"

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

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

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

## 4 Objectives

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

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

The NanoDefine working concept is based on three pillars:

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

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

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

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

S**pecific objectives** are:

**1. Development of a decision framework and classification procedure**

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

The classification procedure we will develop will guide the user to select the most appropriate methods for a specific case by means of a uniform decision tree (see Fig. 1), taking into account already available information on the material as well as the requirements on the quality of result and the availability of instruments and methods at the respective laboratory. At each decision node the system will evaluate the obtained information and data and guide the user through the next steps. This can either be an additional measurement by a tier 1 method or a tier 2 method, or may directly lead to the decision if a given material can be considered nano or non–nano according to the definition.

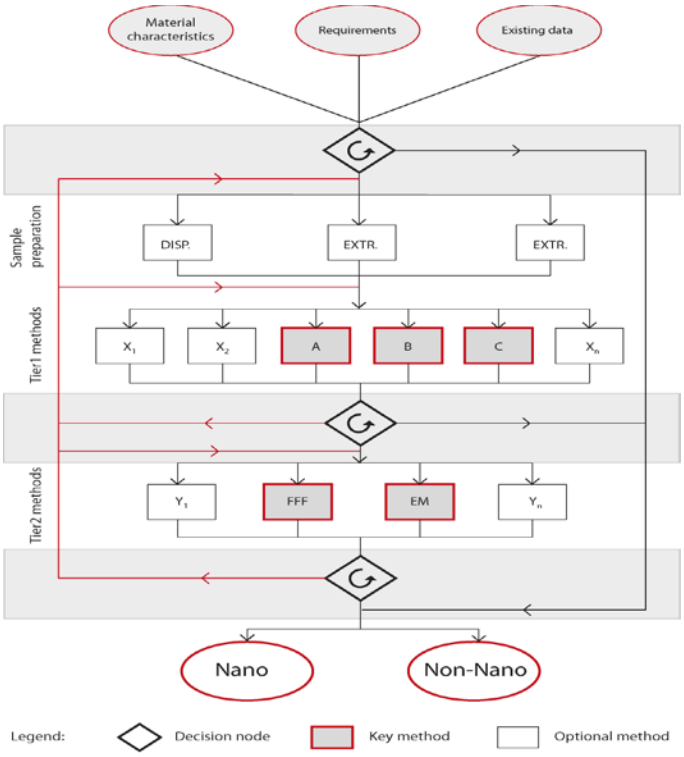



Fig.1: The NanoDefiner decision flow scheme that will integrate all project results and guide users by means a decision tree. At each decision node the available data will be evaluated to guide to the next step until the classification into nano/non-nano according to the definition is achieved.

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

**2. Systematic method evaluation and development**

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

**3. Standardisation and implementation of methods**

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

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

To achieve these ambitious goals, NanoDefine has mobilized ae critical mass of top expertise and skills available in Europe in the field of measurement, characterization and standardization of nanomaterials, including research performers, metrology and reference material producing and certifying institutes, industrial (nano)material manufacturers, and instrument developers, as well as sector organisations of the most affected industries.

**Selection of test materials:**

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

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

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

# 5 Progress and Outcomes to date

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

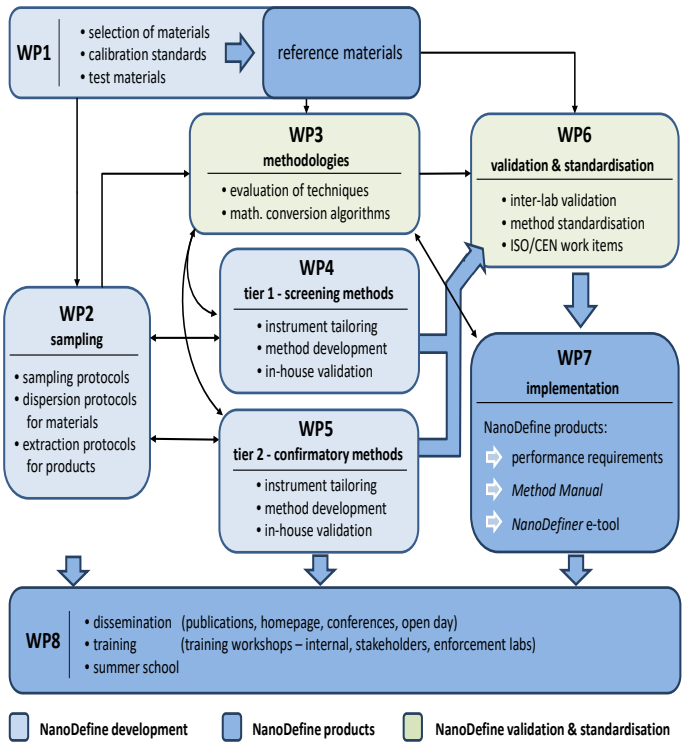



Fig. 2: Interdependencies and interactions between WP1-8

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

NanoDefine started on 1 November 2013 and was launched at a kick-off meeting in Wageningen, The Netherlands, on 19-20 November 2013. Work is being carried out in all work packages and many results have been already achieved after the first 18 months. In the following, a short overview is given of the main objectives and tasks to be performed in the different work packages during the course of the project (2013-2017).

## 5.1 *Test and reference materials (WP1)*

Main objective of WP1 in the first part of the project was to provide the participating labs with calibration standards and test materials. Beside the distribution of 5 mono- and trimodal calibration materials covering different sizes and material systems, a review on commercially available reference materials was given. 15 test materials ware carefully selected and distributed within the labs within NanoDefine. A matrix of requirements covering all types of morphology (irregular particles, homogeneous particles, platelets, fibres) and application fields/manufacturing techniques (mined materials, pigments, fillers, food and engineered particles) was set up and used for judging the relevance of potential test materials. Furthermore, the particles were selected to cover a broad field of different chemical compositions and crystal structures. To challenge sample preparation and purification, pure powders and suspensions (substances) as well as formulations (products) were sourced. The selection process and descriptions of the materials as well as preliminary characterization results from the substances and products were reported to the project. In order to track the extraction efficiency from complex matrices and thus increase method robustness and traceability of results, labelled nanomaterials with stable isotopes or rare elements were synthesized and distributed to test their suitability.

## 5.2 *Sample preparation, dispersion and sampling methods (WP2)*

The aim of WP2 if to identify representative sampling strategies and to develop and optimize methods to disperse the NM in such a way that the resulting dispersions of nanoparticles from substances and products are stable and contain a maximum of mainly primary constituent particles. EM will be used to distinguish between large primary constituent particles and non-dispersable aggregates. An additional challenge is the possible dissolution and/or reactivity of some materials under certain conditions. The success of sample preparation techniques will be evaluated by a coordinated application of the methods of analysis that are developed in WP4 and 5. WP2 has developed tentative dispersion protocols for 10 nano-scaled substances. Additionally, the WP will develop rather generic protocols that can deal with a majority of the most consumer-relevant products such as sun screen, food, tooth paste and a polymer containing pigment nanoparticles.

## 5.3 *Evaluation and selection of techniques and methodologies (WP3)*

WP3 is the NanoDefine methods evaluation hub. It provides continuous support for the method development in WPs 2, 4, 5 and surveillance of the developmental progress by the application of reference and test samples against unambiguous, quantifiable method performance criteria. A systematic critical review of the performance parameters of a broad set of suitable methods will be performed and results balanced against cost-per-analysis. This literature and expertise-based evaluation will be – for the first time - followed by competitive practical evaluation of methods on non-ideal, realistic samples from WP1. Input from on-going nanometrology projects and standardization institutes will be requested and liaisons established. The quantitative establishment of method performance defines the required improvements and

therefore directly affects the further work in WPs 4 and 5 where the NanoDefine core methods will be established. The two-step process of state-of-the-art based selection of candidate methods and continuous practical evaluation of the developed beyond-state-of-the-art methods in WP3 is tightly aligned with WP7 for development of the NanoDefine Manual. Evaluation will be by categorization using true counting methods (aerosol and liquid), non-counting ensemble methods, and imaging and separation methods.

## 5.4 *Screening methods (tier 1) (WP4)*

WP4 is performing tasks on improving particle counting methods (SMPS, NTA, spICP-MS) and adapting analytical centrifugation (AC) as screening method for NanoDefine. These rapid screening methodologies are being developed and improved into validated methods for the cost-efficient nanoparticle analysis according to the definition. One important outcome of the first 18 month period is a comprehensive guide on the operation and performance of AC for nanoparticle analysis. It includes new routes to considerably enhance the signal quality with respect to noise level or baseline stability and thus supports the accurate determination of number weighted size distributions. The document also compares and discusses the different types of AC and gives advice for their application to calibrants and real-life substances. As a further important outcome of the period, a platform independent software for inductively coupled plasma-mass spectrometry ICP-MS has been developed. This software allows the data acquisition and evaluation of nanoparticle size and number concentration using ICP-MS operated in the single particle mode (spICP-MS). The software is designed to evaluate data sets that were acquired using dwell times in the range of 1-10 ms. The task regarding NTA consisted of SOP development and an anticipated software release for improved routine application. The accuracy and precision for number concentration measurement will be shown e.g. on Au reference materials (NIST 8013). A prototype for HR-mobility spectrometer is well on the track (expected in November 2015). In-house validation will establish the methods as fully quantitative in the sense of the definition.

## 5.5 *Confirmatory methods (tier 2) (WP5)*

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

## 5.6 *Validation and standardization (WP6)*

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

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

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

The NanoDefiner e-tool, a specific software based classification approach and decision support framework, will pool results and conclusions together from method evaluation and development WPs with findings obtained from validation and case studies. This tool, with options based on material type, purpose, required data quality (including confidence level) and economic parameters, will guide the user to the most reliable and cost-efficient measurement method to identify/classify any substance or mixture according to the definition. The user will be provided with precise guidance which will allow extending the measurement to the widest possible range of substances, mixtures, and encompass also products and matrices. This will be accompanied by the NanoDefine Methods Manual with detailed information on the capabilities as well as strengths and weaknesses of each method addressed in the project. The first edition of the Methods Manual will be available by June 2015. The Manual includes the NanoDefine Material Classification System, Methods Performance Criteria, and detailed performance descriptions of commonly available counting, ensemble, fractionation and integral methods. A decision criteria and ranking system for the NanoDefiner decision support system has been developed that takes into account the purpose (legal, screening, quality check, … ), required data quality and cost of the analysis. Both NanoDefiner (decision support framework) and NanoDefine Methods Manual will be available as software (e-tool) and as guidance documents, suitable to be integrated into the web platform on nanomaterials and nanotechnology announced by the European Commission. Case studies will finally

demonstrate the real-world applicability of the developed and validated methods and the performance of the NanoDefiner by means of materials of industrial origin.

### 5.8 *Dissemination, training and technology transfer (WP8)*

This WP is providing the project results in a suitable format for the wider community to access and use; through regular news and information streams, reports, new standardised protocols, training events, and networking opportunities. It will provide the means by which to engage with different target groups: standardization bodies, industry, academia, regulatory agencies, policy-makers, relevant NGOs, and the wider public. It will create opportunities to exploit project results and assess their intellectual property value. It will also afford the wider community the opportunity to interact with the consortium and influence the development of the consortium's work. All public deliverables will be uploaded on the project website www.nanodefine.eu. A first joint workshop has been organized at NRCWE in Copenhagen on 6 June 2014 with other relevant projects within the Nanosafety cluster, to identify and share synergies existing in the use of test materials, sample preparation and measurement protocols. And a first public workshop will take place in Brussels on the 17 June 2015, to discuss goals and outcomes achieved so far (M18) with other experts, policy makers, industries and regulators. NanoDefine will also engage in the EuroNanoForum 2015 international conference (ENF2015) in Riga (Latvia) from 10-12 June 2015. The project has been selected among the ten best projects within the ENF2015 and will showcase the project at the Nanotech Europe exhibition (included in the ENF2015) to demonstrate the most prominent outcome and results to a wider community.

## 6 Expected impact

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

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

### 6.1 *Regulatory and legislative impact*

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

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

### 6.2 *Economic innovation and method standardization*

The EC definition of a nanomaterial has an immediate and broad impact on industry. This extensive impact is because European industry has portfolios of 100,000s of conventional particulate products that a priori could fall under the regulatory nano definition. Their size characterisation itself generates costs that are considerable: With EM as the only broadly accepted method, the costs incurred can stretch to reach billions of euros for classification. NanoDefine has the potential to cut these costs up to 80%. This has a huge impact on the competitiveness of European industries. In addition, the development of instruments that are mandatory in order to run internationally standardised methods (CEN/ISO) will be a major competitive market advantage for European instrument manufacturers. In particular, the preparation of the NanoDefine guidance and classification procedure (The NanoDefiner) and the submission of developed and validated key methods as work items to international standardization organisations (CEN/ISO) as standard methods will remove existing uncertainties among industrial and regulatory stakeholders on which methodology has to be used in the regulatory approval of substances.

## 6.3 Scientific impact

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

## 6.4 European and international integration

A number of efforts are currently underway both on the European and international levels to develop and validate characterisation methods for nanomaterials. Consortium partners are already involved in these initiatives, providing a robust route for NanoDefine to carefully evaluate the results/progress of completed and on-going projects to avoid duplication of work and start at the most advanced state-of-the-art. In particular, the results from the FP7 projects NanoValid, NanoLyse, MARINA, and SMARTNANO will be taken into account. Close collaboration with the NanoReg project, in particular with WP2 has been established to share bench materials, sample preparation and measurement protocols. Active involvement in the EU NanoSafetyCluster, in particular in WG1 (materials) will help to integrate knowledge and experience gained from other relevant projects into NanoDefine in a timely manner, and also to disseminate results among the Cluster members. At international level, close cooperation is also foreseen with the North American NanoRelease project and within the Communities of Research (CoRs) of the US-EU collaboration on nanosafety.

## 6.5 Knowledge transfer

The project outcome will be continuously presented to the scientific community, regulators, authorities, industry and the general public via well established and novel means of communication, in particular through the NanoDefine public website www.NanoDefine.eu, presentations in journals and at conferences, flyers and brochures, e-Newsletter, professional electronic network platforms and specific knowledge transfer activities.

To increase the impact and to ensure the presence of relevant audience, the specific knowledge transfer activities will be organised in parallel with major stakeholder events. At least two workshops will be organised in the course of the project and participants will include national EU Member State standardization bodies, national regulatory authorities, e.g. DEFRA, (UK), Ministry of Ecology and Sustainable Development (FR), UBA (DE), Dutch Food and Consumer Products authority NVWA (NL), industry associations outside the project partners (e.g. CEFIC, VCI, CIA-UK etc.) as well as international organizations such as OECD (participation from WPMN and WPN), WHO/FAO (Codex Alimentarius) and authorities such as NIST, EPA and FDA.

## 6.6 Technology transfer and training

There is an urgent need for methods that support the classifying of materials and products according to the EC definition as these form the basis for several upcoming regulations. NanoDefine therefore implements the direct transfer of suited methods to those laboratories that need them. These include:

- **enforcement laboratories**: responsible for the monitoring of consumer products and food for the presence of nanomaterials,
- **industry laboratories**: for classifying their own or purchased materials according to the definition as well as for quality assurance purposes,
- **contract laboratories**: providing analytical services to enforcement agencies and companies that have not the required analytical facilities.

This approach will guarantee the fastest and most widespread availability of the required methods for the affected stakeholders. NanoDefine will provide method transfer specifically targeted to the respective end-user group. Enforcement laboratories will receive hands-on training workshops that are focused on the analysis and classification in products and food. The respective training modules will be designed by those partners that are involved themselves in enforcement tasks to make sure that the developed modules are tailor-made to this target group. The needs of industrial laboratories differ per sector. Sector associations of the most affected industries present in the consortium are in charge of pitching and organising training workshops according to the needs of their members. Contract laboratories will be invited to those workshops that best fit their customer structure. The active involvement of one of the largest European analytical service provider (Eurofins) assures that the developed methods are also fit for use in highly routine settings with a high sample throughput.

## 6.7 Exploitation

NanoDefine has drafted and will further develop an exploitation plan which has already identified a variety of key exploitable results and which will continuously guide project partners in assessing the exploitation potential of the outcome they produced in terms of new knowledge, instruments and methodologies, also in the light of new external developments. It will also provide guidance on how to engage effectively with end-users and the assessment of intellectual property rights. The plan will cover ownership of results among partners, implementation of innovation related activities, including financial considerations for commercialisation. The plan will be refined continuously as the project results evolve and their exploitation potential becomes clearer. Where appropriate, the developed foreground knowledge will be filed for patents.

## 6.8 Knowledge management and Intellectual Property Rights

One of the objectives of the Seventh Framework Programme is to ensure open access to make knowledge generated within projects available to the wider community. However, this must be balanced

with another of the Programme's objectives: to ensure adequate protection and exploitation of intellectual property rights (IPR). The IPR assessment will closely work together with services provided by the European IPR Helpdesk. NanoDefine will balance the dissemination of project results with the need to protect IPR. A consortium agreement (based on the DESCA model agreement) has been established which clearly describes the procedures to be followed by beneficiaries. The consortium agreement will protect beneficiaries' pre-existing (background) know-how, and establish mechanisms by which confidentiality is maintained and new IPR for the newly generated data (foreground) is managed (e.g. publishing versus patenting). It will be supplemented within the first three months of the project starting with a document describing the processes to be followed by all beneficiaries (led by the project coordinator and supported by the WP leaders) to ensure that new knowledge is managed appropriately (including dates, individuals responsible, relation to existing IPR, etc.) and exploited in the most appropriate manner for the socio-economic benefit of the consortium and the wider community.

To further support routes to commercialization, beneficiaries that generate new IPR will be able to access confidential, expert advice through other beneficiaries and their networks, and EC appointed experts (e.g. from the IPR helpdesk).

It is envisaged that the following information will be made freely available to all stakeholders:

- ✓ production methods for standardised reference materials;
- ✓ details of the most appropriate parameters of the reference materials to be measured;
- ✓ protocols and SOPs for measuring the size and size related parameters for the tested ENs
- ✓ the NanoDefine Method Manual;
- ✓ the NanoDefiner e-tool
- ✓ results from the case studies.

The following expected developments will be commercially exploited by the consortium:

- ➢ new certified reference materials to validate size measurement methods;
- ➢ new or improved measurement instruments and the associated software to detect and quantify the size and number based size distribution of nanoparticles in materials, products and environmental matrices.

# 7 Directory

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

| First Name | Last Name | Affiliation | Address | e-mail |
|---|---|---|---|---|
| Stefan | Weigel | RIKILT | RIKILT Wageningen UR, (Institute of Food Safety), P.O. Box 230, 6700 AE Wageningen, NL | stefan.weigel@wur.nl |
| Hans | Marvin | RIKILT | RIKILT Wageningen UR, (Institute of Food Safety), P.O. Box 230, 6700 AE Wageningen NL | Hans.marvin@wur.nl |
| Wim | Beek | RIKILT | RIKILT Wageningen UR, (Institute of Food Safety), P.O. Box 230, 6700 AE Wageningen NL | wim.beek@wur.nl |
| Anna | Undas | RIKILT | RIKILT Wageningen UR, (Institute of Food Safety), P.O. Box 230, 6700 AE Wageningen NL | anna.undas@wur.nl |
| Ruud | Peters | RIKILT | RIKILT Wageningen UR, (Institute of Food Safety), P.O. Box 230, 6700 AE Wageningen NL | ruudj.peters@wur.nl |
| Claudia | Cascio | RIKILT | RIKILT Wageningen UR, (Institute of Food Safety), P.O. Box 230, 6700 AE Wageningen NL | claudia.cascio@wur.nl |
| Rudolf | Reuther | NOMI | Strandvägen 2, 671 29 Arvika Sweden | rudolf.reuther@enas-online.com |
| Rune | Karlsson | NOMI | Strandvägen 2, 671 29 Arvika Sweden | rune.karlsson@enas-online.com |
| Lesley | Tobin | NOMI | Strandvägen 2, 671 29 Arvika Sweden | lesleytobin@hotmail.com |
| Tina | Stewart | NOMI | Strandvägen 2, 671 29 Arvika Sweden | tina1@screenscape.co.uk |
| Francois | Rossi | JRC-IHCP | Joint Research Centre, Nanobiosciences Unit, IPR-20i 008, I-21027 Ispra Italy | francois.rossi@jrc.ec.europa.eu |
| Hubert | Rauscher | JRC-IHCP | Joint Research Centre, Nanobiosciences Unit, IPR-20i 008, I-21027 Ispra Italy | Hubert.rauscher@ec.europa.eu |

| First Name | Last Name | Affiliation | Address | e-mail |
|---|---|---|---|---|
| Douglas | Gilliland | JRC-IHCP | Joint Research Centre, Nanobiosciences Unit, IPR-20i 008, I-21027 Ispra Italy | douglas.gilliland@jrc.ec.europa.eu |
| Ana | Boix-Sanfeliu | JRC-IRMM | Retieseweg 111, 2440 Geel Belgium | Ana.boix-sanfeliu@ec.europa.eu |
| Robert | Koeber | JRC-IRMM | Retieseweg 111, 2440 Geel Belgium | Robert.koeber@ec.europa.eu |
| Frank | vdKammer | UNIVIE | Department of Environmental Geosciences, Althanstrasse 14 UZAII 1090 Vienna, Austria | frank.von.der.kammer@univie.ac.at |
| Erik | Huusfeldt Larsen | DTU | Technical University of Denmark, National Food Institute, Building B, MoerkhoejBygade 19, DK-2860 Soeborg, Denmark | ehlar@food.dtu.dk |
| Katrin | Löschner | DTU | Technical University of Denmark, National Food Institute, Building B, MoerkhoejBygade 19, DK-2860 Soeborg, Denmark | kals@Food.dtu.dk |
| Manuel | Correira | DTU | Technical University of Denmark, National Food Institute, Building B, MoerkhoejBygade 19, DK-2860 Soeborg, Denmark | manco@food.dtu.dk |
| Jutta | Tentschert | BFR | Berlin, Max-Dohrn-Strasse 8 -10 10589 Berlin Germany | Jutta.Tentschert@bfr.bund.de |
| Ralf | Kaegi | EAWAG | Überlandstrasse 133, P.O.Box 611, 8600 Dübendorf Switzerland | Ralf.kaegi@eawag.ch |
| Toni | Uusimaeki | EAWAG | Überlandstrasse 133, P.O.Box 611, 8600 Dübendorf Switzerland | ToniMikael.Uusimaeki@eawag.ch |
| Aurelien | Auger | CEA | Gif-sur-Yvette, Paris France | Aurelien.auger@cea.fr |
| Frank | Babick | TUD | TechnischeUniversität Dresden AG MechanischeVerfahrenstechnik Georg-Schumann-Bau, Raum 208 MünchnerPlatz 3 01187 Dresden Germany | Frank.Babick@tu-dresden.de |
| Michael | Stintz | TUD | TechnischeUniversität Dresden AG MechanischeVerfahrenstechnik Georg-Schumann-Bau, Raum 208 MünchnerPlatz 3 01187 Dresden Germany | Michael.stintz@tu.dresden.de |
| Sven | Kreigenfeld | TUD | TechnischeUniversität Dresden AG MechanischeVerfahrenstechnik Georg-Schumann-Bau, Raum 208 MünchnerPlatz 3 01187 Dresden Germany | sven.kreigenfeld@tu-dresden.de |
| Eveline | Verleysen | CODA-CERVA | Groeselenbergstraat 99, 1180 Uccle Belgium | Eveline.Verleysen@coda-cerva.be |
| Jan | Mast | CODA-CERVA | Groeselenbergstraat 99, 1180 Uccle Belgium | Jan.mast@coda-cerva.be |
| Laura-Jayne | Ellis | UoB | University of Birmingham, School of Geography, Earth and Environmental Sciences, Edgbaston, Birmingham, West Midlands B15 2TT UK | EllisLA@adf.bham.ac.uk |
| Eva | Valsami-Jones | UoB | University of Birmingham, School of Geography, Earth and Environmental Sciences, Edgbaston, Birmingham, West Midlands B15 2TT UK | e.valsamijones@bham.ac.uk |
| Christoph | Friedrich | FHDO | Emil-Figge-Str. 42, D-44227 Dortmund Germany | christoph.friedrich@fh-dortmund.de |
| Hans-Gerd | Lipinski | FHDO | Emil-Figge-Str. 42, D-44227 Dortmund Germany | lipinski@fh-dortmund.de |
| Marcus | Ingenfeld | FHDO | Sonnenstraße 98, D-44139 Dortmund Germany | marcus.ingenfeld@fh-dortmund.de |

| First Name | Last Name | Affiliation | Address | e-mail |
|---|---|---|---|---|
| Monika | Schmidt | FHDO | Sonnenstraße 98, D-44139 Dortmund Germany | monika.schmidt@fh-dortmund.de |
| Thorsten | Wagner | FHDO | Emil-Figge-Str. 42, D-44227 Dortmund Germany | wagner@biomedical-imaging.de |
| Andreas | Thuenemann | BAM | BAM, Division 6.8 Surface Analysis and Interfacial Chemistry, Unter den Eichen 87, 12205 Berlin Germany | Andreas.Thuenemann@bam.de |
| Dan | Hodoraba | BAM | BAM, Division 6.5 Polymers in Life Science and Nanotechnology, Unter den Eichen 87, 12205 Berlin Germany | dan.hodoroaba@bam.de |
| Johannes | Mielke | BAM | BAM, Division 1.3 Structure Analysis, Richard-Willstätter-Str. 11, 12489 Berlin-Adlershof Germany | Johannes.mielke@bam.de |
| Kerstin | Brademann-Jock | BAM | BAM, Division 1.3 Structure Analysis, Richard-Willstätter-Str. 11, 12489 Berlin-Adlershof Germany | Kerstin.brademann-jock@bam.de |
| Ralf | Bienert | BAM | BAM, Division 1.3 Structure Analysis, Richard-Willstätter-Str. 11, 12489 Berlin-Adlershof Germany | Ralf.bienert@bam.de |
| Franziska | Emmerling | BAM | BAM, Division 1.3 Structure Analysis, Richard-Willstätter-Str. 11, 12489 Berlin-Adlershof Germany | Franziska.Emmerling@bam.de |
| Michael | Schmitt | DIN | Burggrafenstr. 6, 10787 Berlin Germany | Michael.Schmitt@din.de |
| Sandra | Johae | DIN | Burggrafenstr. 6, 10787 Berlin Germany | Sandra.johae@din.de |
| Roman | Ruettinger | DIN | Burggrafenstr. 6, 10787 Berlin Germany | roman.ruettinger@din.de |
| Michael | Roeper | BASF | 67056 Ludwigshafen, Carl-Bosch-Straße 38, Building M300, Room 202, Berlin Germany | michael.roeper@basf.com |
| Philipp | Mueller | BASF | 67056 Ludwigshafen, Carl-Bosch-Straße 38, Building M300, Room 202, Berlin Germany | philipp.mueller@basf.com |
| Hermann | Feise | BASF | 67056 Ludwigshafen, Carl-Bosch-Straße 38, Building M300, Room 202, Berlin Germany | Hermann.feise@basf.com |
| Wendell | Wohlleben | BASF | 67056 Ludwigshafen, Carl-Bosch-Straße 38, Building M300, Room 202, Berlin Germany | wendel.wohlleben@basf.com |
| Klaus | Kund | Clariant | Am Unisys-Park 1, 65843 Sulzbach am Taunus, Frankfurt Germany | klaus.kund@clariant.com |
| Antoine | Ghanem | SOLVAY | Rue de Ransbeek, 310 - 1120 Brussels | Antoine.ghanem@solvay.com |
| Alvise | Bianchin | MBN | via Bortolan 42, 31050 Vascon di Carbonera, TV - Italy | research@mbn.it |
| Paolo | Matteazzi | MBN | via Bortolan 42, 31050 Vascon di Carbonera, TV - Italy | matteazzi@mbn.it |
| Phillippe | Hallegot | L'OREAL | Laboratoire de MicroscopieElectronique, L'Oréal, 1 avenue EugèneSchueller, BP 22, 93601 Aulnay Sous Bois Cedex, Clichy | phallegot@rd.loreal.com |
| Patrick | Hole | NanoSight | NanoSight, Minton Park, London Road, Amesbury, SP4 7RT UK | patrick.hole@nanosight.com |
| Phil | Vincent | NanoSight | NanoSight, Minton Park, London Road, Amesbury, SP4 7RT UK | Phil.vincent@nanosight.com |
| Emilio | Ramiro | RAMEM | Sambara 33, 28027 Madrid Spain Madrid | e.ramiro@ramem.com |
| Silvia | Lopez | RAMEM | Sambara 33, 28027 Madrid Spain Madrid | slopez@ioner.net |

| First Name | Last Name | Affiliation | Address | e-mail |
|---|---|---|---|---|
| Pavla | Dohanyosova | RAMEM | Sambara 33, 28027 Madrid Spain Madrid | p.dohanyosova@ioner.net |
| Christoph | Johann | Superon | Hochstrasse 12, 56307 Dernbach Germany | Christoph.johann@superon.eu |
| Stefan | Boehm | Superon | Hochstrasse 12, 56307 Dernbach Germany | stefan.boehm@superon.eu |
| Ulrich | Roesch | Superon | Hochstrasse 12, 56307 Dernbach Germany | ulrich.roesch@Superon.eu |
| Daniel | Kutscher | THERMO FISHER | Hanna-Kunath-Strasse 11, 28199 Bremen, Germany | daniel.kutscher@thermofisher.com |
| Shona | McSheehy | THERMO FISHER | Hanna-Kunath-Strasse 11, 28199 Bremen, Germany | shona.mcsheehy@thermofisher.com |
| Philipp | Bruening | Eurofins | Neuländer Kamp 1, 21079 Hamburg Germany | PhilippBruening@eurofins.de |
| David | Carlander | NIA | 101 Avenue Louise, 1050 Brussels, Belgium | David.carlander@nanotechia.org |
| Heike | Liewald | VdMi | MainzerLandstrasse 55, 60329 Frankfurt Germany | liewald@vdmi.vci.de |
| Florian | Schellauf | Cosmetics Europe | Cosmetics Europe - The Personal Care Association, Avenue Herrmann Debroux 40, 1160 Brussels Belgium | fschellauf@cosmeticseurope.eu |
| Georges | Favres | LNE | 1, rue Gaston Boissier, Paris France | georges.favre@lne.fr |
| Tatiana | Mace | LNE | 1, rue Gaston Boissier, Paris France | tatiana.mace@lne.fr |
| Charles | Motkus | LNE | 1, rue Gaston Boissier, Paris France | charles.motzkus@lne.fr |
| François | Gaie-Levrel | LNE | 1, rue Gaston Boissier, Paris France | françois.gaie-levrel@lne.fr |

# 8 Copyright

# NanoToxClass

**ERANET SIINN NanoToxClass: Establishing NM grouping/ classification strategies according to toxicity and biological effects for supporting risk assessment**

Website: www.nanotoxclass.eu
Coordinator: Dr. Andrea Haase, German Federal Institute for Risk Assessment (BfR), Berlin, Germany

*Table 1 Consortium List.*

| No. | Beneficiary name | Short name | Country |
|---|---|---|---|
| 1 | German Federal Institute for Risk Assessment | BfR | Germany |
| 2 | Helmholtz Centre for Environmental Research | UFZ | Germany |
| 3 | Institute of Energy and Environmental Technology | IUTA | Germany |
| 4 | University Hospital Hamburg- Eppendorf | UKE | Germany |
| 5 | Tel Aviv University | TAU | Israel |
| 6 | University of Namur | UNamur | Belgium |
| 7 | StratiCell | StratiCell | Belgium |
| 8 | Institute of Public Health, University of Porto | ISPUP | Portugal |
| 9 | University of Bucharest | UB | Romania |
| 10 | BASF SE (associate partner) | BASF | Germany |
| 11 | Robert Koch Institute (associate partner) | RKI | Germany |
| 12 | Evonik Resource Efficiency GmbH (associate partner) | Evonik | Germany |

## Contents



## 1 Summary

Project Duration: 1.12.2015- 30.11.2018

Project Funding: 1 572 421 EUR

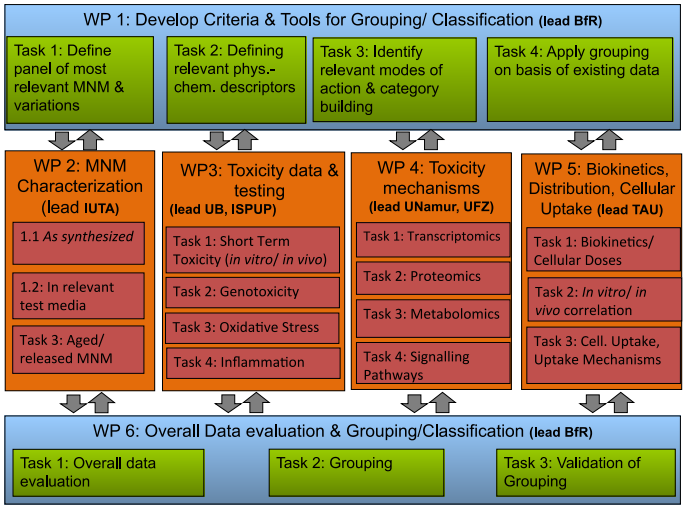
## 2 Background

Nanotechnology is one of the key technologies for the 21st century. Already by now, many products containing manufactured nanomaterials (MNM) have entered the market and many more are expected to follow. Applications range from energy storage, waste remediation, different types of consumer products, to medicine. The majority of the current industrial applications are based on just a few NM types, mostly metals, metal oxides, silica, and carbon. However, by combining different core materials and by variations in size, shape and surface already by now the possible number of NM variants is very large. Currently, each NM variant is tested and assessed individually in a case-by-case approach. Grouping principles are, apart from a few exceptions, not established and are only beginning to emerge.

## 3 Scientific and technological challenges

The number of NM and NM variants on the market is already very high and is steadily increasing. In addition, each NM is changing several of its properties during the lifecycle. Depending on the environment, NM may agglomerate, or agglomerates may disintegrate, NM may dissolve, or the surface properties may change due to aging. Considering the number of different possible biological effects it is unfeasible to test each single variant of nanomaterial in each stage of its life cycle individually. Thus, grouping approaches would be highly supportive for different reasons. In order to establish grouping and categorization

principles for NM it is firstly needed to describe structural similarity. For chemicals, the concept of structural similarity is already well established. In contrast, for NM we have a good knowledge that several intrinsic physico-chemical parameters like size, surface charge/ area and surface chemistry as well as several extrinsic or system-dependent properties (i.e agglomeration, dissolution or changes on the NM surface due to protein corona formation) are important and influence the biological behaviour. However, still it appears rather challenging to describe structural similarity for NM and we currently don't know which parameters we need to take into account. Going one step further, for grouping it is also required or advisable to demonstrate the common mode of action. Again, for NM the knowledge on specific modes of action is only beginning to evolve. Taken together, apart from a very few exceptions grouping principles for NM are not yet established and still a matter of research.




## 3 Objectives

NanoToxClass will develop grouping principles for NM based on their physico-chemical properties under consideration of their toxicity and toxicity mechanisms. The grouping principles will be developed for a set of selected industrially relevant NM. As far as possible, we will use already existing data, i.e. from literature or from other, already finished projects. However, a major pillar of this project will be the targeted generation of new data based on modern systems biology based techniques in combination with established toxicological endpoints. We will perform transcriptomics, metabolomics, and proteomics analyses of in vitro and in vivo samples. Omics approaches are in particular useful to investigate the mode of action or the underlying toxicity mechanisms. Therefore, for establishing grouping principles in NanoToxClass we will consider toxicity mechanisms of NM. Furthermore, by comparing results from in vitro and in vivo testing, this project will enhance our understanding of in vitro - in vivo correlation. In addition, a few selected NM will be tested during different stages of their life cycle, i.e. after thermal aging and release from a nanocomposite. Grouping criteria and principles will be verified and validated with a few additional NM.

## 4 Organisation

The project consists of 6 different work packages (Figure 1).

Within WP1 the grouping principles are established and relevant parameters are identified. WP2 deals with the physico- chemical characterization of the NM. In WP3 the toxicity of the NM is tested in vitro as well as in vivo with respect to established toxicological endpoints. WP4 is investigating the changes on the level of the transcriptome, proteome, and metabolome, as well as alterations of signalling pathways. In WP5 we study biokinetics, cellular uptake and we perform in vitro - in vivo correlations. WP6 is responsible for overall data analysis, for data integration, and for grouping.

For external advice and input, NanoToxClass has established an International External Advisory Panel.

## 5 Expected Impact

Although currently most industrial applications focus on only a few types of different MNM (mainly silver, carbon based MNM, titanium/titanium dioxide, silicon dioxide, zinc/zinc oxide or gold) the total amount of available MNM variants is incredibly high. Each of these MNM can be produced in different sizes, shapes, and with different surfaces.

Toxicity of NM can be tested with a set of 59 endpoints (OECD WPMN 2010), representing a huge test list. Currently, risk assessment of NM is done on a case-by-case basis. Each NM variant is tested and assessed individually. Moreover, only for a few selected NM and a few selected applications the data sets are more or less complete enough to perform a full risk assessment. For most NM and NM variants severe data gaps remain. In addition, given the large number of NM variants together with the long list of potential endpoints, it is obvious that it will not be feasible to test each NM variant for each endpoint. Therefore, in order to overcome this roadblock we need to develop grouping and read-across principles and approaches for NM, which may then be used to predict toxicity for NM and to fill data gaps by other means then testing. Grouping and read-across will support prioritization and decision making for whether and which further tests are needed or which tests may be waived. It may also help in deciding which tests are sufficient and necessary, and thereby largely reducing the long list of currently required data for physico-chemical as well as for toxicological characterization. NanoToxClass will develop grouping approaches for MNM. This is expected to have a high impact on regulation and risk assessment of NM. Moreover, also for industrial use and further commercialization of nanotechnology established grouping approaches will have a major impact.

NanoToxClass also enhances our understanding of modes of action for NM and can give guidance to the large set of possible toxicity endpoints for NM by selecting the most predictive ones (which will be then used as a basis for grouping). Omics techniques will enable to assess NM hazards on a mechanistic basis and will enable the determination of adverse outcome pathways (AOP). Finally, this knowledge may be applied as a tool to create safer nanoparticles (so called "safe-by-design" approaches).

# 6 Directory

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

| First Name | Last Name | Affiliation | Address | e-mail |
|---|---|---|---|---|
| Anne | Bannuscher | BfR | Berlin, Germany | anne.bannuscher@bfr.bund.de |
| Fabrice | Berger | StratiCell | Les Isnes, Belgium | fberger@straticell.com |
| Ulrich | Brinkmann | Evonik | Hanau, Germany | ulrich.brinkmann@evonik.com |
| Joseph | Brinkmann | Evonik | Hanau, Germany | joseph.brinkmann@evonik.com |
| Florence | Chainiaux | UNamur | Namur, Belgium | florence.chainiaux@unamur.be |
| Anca | Dinischiotu | UB | Bukarest, Romania | adin@bio.unibuc.ro |
| Sonia | Fraga | ISPUP | Porto, Portugal | teixeirafraga@hotmail.com |
| Andrea | Haase | BfR | Berlin, Germany | andrea.haase@bfr.bund.de |
| Bryan | Hellack | IUTA | Duisburg, Germany | hellack@iuta.de |
| Harald | Jungnickel | BfR | Berlin, Germany | harald.jungnickel@bfr.bund.de |
| Carsten | Kneuer | BfR | Berlin, Germany | carsten.kneuer@bfr.bund |
| Rafi | Korenstein | TAU | Tel Aviv, Isreal | korens@post.tau.ac.il |
| Thomas | Kuhlbusch | IUTA | Duisburg, Germany | tky@iuta.de |
| Robert | Landsiedel | BASF | Ludwigshafen, Germany | robert.landsiedel@basf.com |
| Andreas | Luch | BfR | Berlin, Germany | andreas.luch@bfr.bund.de |
| Monika | Maier | Evonik | Hanau, Germany | monika.maier@evonik.com |
| Nicole | Neubauer | BASF | Ludwigshafen, Germany | nicole.neubauer@basf.com |
| Carmen | Nickel | IUTA | Duisburg, Germany | nickel@iuta.de |
| Peter | Nollau | UKE | Hamburg, Germany | nollau@uke.de |
| Jean-Pascal | Piret | UNamur | Namur, Belgium | jean-pascal.piret@unamur.be |
| Bernhard | Renard | RKI | Berlin, Germany | RenardB@rki.de |
| Christian | Riebeling | BfR | Berlin, Germany | Christian.riebeling@bfr.bund.de |
| Michel | Salmon | StratiCell | Les Isnes, Belgium | msalmon@straticell.com |
| Joao Paulo | Teixeira | ISPUP | Porto, Portugal | jpft12@gmail.com |
| Janina | Tomm | UFZ | Leipzig, Germany | janina.tomm@ufz.de |
| Olivier | Toussaint | UNamur | Namur, Belgium | olivier.toussaint@unamur.be |
| Martin | von Bergen | UFZ | Leipzig, Germany | martin.vonbergen@ufz.de |
| Wendel | Wohlleben | BASF | Ludwigshafen, Germany | wendel.wohlleben@basf.com |

# 7 Copyright


# Updates from FP7 projects that ended in 2017

# eNanoMapper

## A Database and Ontology Framework for Nanomaterials Design and Safety Assessment

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

*Table 1 Consortium List.*

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

## Contents



## 1 Summary

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

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

Started in February 2014, eNanoMapper developed a computational framework for nanomaterials toxicological data, which is based on open standards, open source, common languages, and an interoperable design, enabling a more effective and integrated approach in risk assessment. eNanoMapper has supported the collaborative safety assessment for nanomaterials by creating a modular, extensible infrastructure for transparent data sharing, data analysis, and the creation of computational toxicology models.

The infrastructure developed within eNanoMapper project aims to support the data management in the area of nano safety research and to enable an integrated approach for the risk assessment of nanomaterials. To achieve these, eNanoMapper developed an ontology, a data infrastructure and modelling tools with applicability in risk assessment of nanomaterials. By merging the expertise of partners in statistical and data mining tools and in predictive toxicology, biology and nanotechnology research, eNanoMapper developed resources and tools for predicting toxicity of nanomaterials and worked towards improving the standards in risk assessment of nanomaterials. The ontology includes common vocabulary terms used in nanosafety research and aims to provide a clear explanation of nanostructures based on information relating to their characterization, relevant experimental paradigms, biological interactions, safety indications and the integration of data from existing nanotoxicology sources. To support a collaborative safety assessment approach an infrastructure for data management was developed, with a database which includes functionalities for data protection, data sharing, data quality assurance, search and interfaces for different needs and usages, comparability and cross-talk with other databases. Further, a collection of descriptors, computational toxicology models and modelling tools were developed, to enable the use and integration of nanosafety data from various sources. The project also provides a rich library of information and documentation (tutorials, webinars, reports and publications) to support and guide the users.

## 2 Background

The eNanoMapper project started in February 2014 with the goal to create a common language in the form of an ontology and computational standards as well as infrastructure for nanomaterials design and safety assessment for the European NanoSafety Cluster (NSC), as well as for the international nanomaterial science community in general. The main challenge of establishing standards and setting up infrastructure lies in the relatively new scientific field of nanomaterial safety: the complexity of toxicology, inherited from chemistry and biology and the additional complexity of characterizing engineered nanomaterials (ENMs) leads to difficulties of ENM identification and hence reproducibility. The result is uncertainty in the validity of experiments and data. This makes it very difficult for scientists, regulators and the industry to share, compare and validate data, models, protocols, and SOPs.

The consortium has assembled partners with specific experience in predictive toxicology, database and modelling resource development (DC, NTUA, IST, IDEA, UM), ontology creation and curation (EMBL-EBI, UM), and existing interactions with European nanotechnology and NanoSafety Cluster projects (IST, KI, MB). The eNanoMapper project implementation was arranged in seven Work Packages (WPs). The user-driven requirements analysis, design and application testing activities of WP1 provided extremely valuable guidance for all RTD activities. WPs 2, 3 and 4 provided the core component development parts of eNanoMapper in the areas of ontology, data management and computational processing and analysis infrastructure, with WP5 providing a regular integrated testing and release of components including GUIs for use application testing and deployment. The use cases guided all components and integrated application development focus and validation by providing software applications to users. WP6 on Dissemination and Training and WP7 on Project Management were pervasive throughout the duration of the project ensuring quality outcomes and results, and relied on the experienced EC project coordination and WP management skills of partners.

## 3 Objectives

The key goal for eNanoMapper was to enhance scientific validity by creating and establishing standards and by harmonization on different levels:

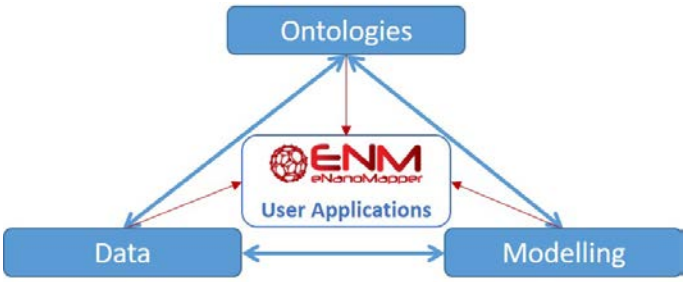
- To establish mechanisms for a sound collaboration between eNanoMapper and the nanotechnology/nanosafety community and ensure effective feedback on its objectives;
- To establish a harmonized language with the help of a standardized ontology;
- To accelerate knowledge exchange and reuse through the development of ontologies for the categorisation and characterisation of ENMs in collaboration with other projects;
- To harmonize the scientific processes by establishing an infrastructure for SOPs, protocols or templates, and to harmonize the data sets by setting up a standardized data warehouse, as well as defining standard APIs for the distributed resources of the community;
- To improve the utilisation of data through the implementation of a modular infrastructure for data storage, searching and sharing, based on open standards and semantic web technologies, minimum information standards and established security solutions;
- To develop harmonized and reproducible computational toxicology models, by establishing modelling and API standards;
- To enable the creation of new computational models in nanomaterials safety through the implementation of interfaces for toxicity modelling and prediction algorithms which may process all data made available through eNanoMapper;
- To enable the meta-analysis of nano-bio interactions supporting "safe-by-design" ENMs development by pursuing a Linked Data approach which integrates data and metadata originating from diverse sources within nanoscience, chemistry, biology and toxicology;
- To create tools for the exchange, quality assurance and reporting of research protocols and data for regulatory purposes;
- To integrate and test eNanoMapper development components into user applications;
- To disseminate its scientific results, tools and applications to the scientific and technology communities and groups, by providing and facilitating user guidance and interactions;
- To raise awareness of eNanoMapper resources with regulators and policy makers to show their enabling contribution to acceptance of predictive toxicology approaches for nanomaterials safety assessment as alternatives to animal testing;
- To create a community framework to accelerate interdisciplinary collaboration between experimental and computational scientists in the establishment and use of data management and analysis infrastructure and to mutually develop quality-driven guidelines for experimental design and optimal production and sustainable maintenance of new datasets;

Overall, eNanoMapper aimed to support the collaborative safety assessment for nanomaterials by creating a modular, extensible infrastructure for transparent data sharing, data analysis, and the creation of computational toxicology models for nanomaterials. Also, based on previous developments of consortium partners in predictive toxicology, biology and nanotechnology research, eNanoMapper aimed to develop resources, tools and standards for a scientifically sound risk assessment of nanomaterials to support the design of new safe and environment-friendly NMs as well as the assessment of existing materials. Ultimately, an important goal was to align the eNanoMapper infrastructure development with user needs and to enhance the research cohesion, integration and advancement of the EU NanoSafety Cluster agenda.

## 4 Major Outcomes from project

During the first phase of the project, the eNanoMapper team carried out a broad requirement analysis, performing detailed interviews with scientists and managers across all main projects of the NSC, including researchers, regulators and industry representatives. Based on the consolidated findings, we were able to prepare use cases, designs and development plans that were aligned with the cluster's needs on data, ontology and modelling, and to proceed with work on initial prototypes leading to early

releases and integration efforts. The findings were further exploited and used during the whole period of the project to develop, test and implement various solutions towards its primary goal of creating a common language in the form of an ontology, computational standards as well as infrastructure for nanomaterials design and safety assessment.




On the **community outreach**, the main achievements include reaching out successfully to the EU and US communities, establishment of different collaborations, identification of use cases and establishing an overall systems design. The requirements analysis, which started with interviews, followed by processing into use cases, the derived system design, and the prototypes have ensured that the standards and tools developed will fit with the requirements of the scientists and regulators. Thus, eNanoMapper performed a requirements analysis by interviewing many people involved in the NanoSafety Cluster (NSC) following a contextual design approach. This lead to a systems design and a set of use cases capturing needs from people in the NSC community. Besides dissemination, eNanoMapper has actively been seeking feedback from the community. This was partly done by collaborating with other projects in bilateral activities and by participation in various activities such as harmonization initiatives for modelling and exposure data, international activities around databases, like the NSC WG4 on databases, the US NanoWG, and the US-EU NanoEHS Communities of Research. To capture publicly received feedback, we kept track of online mention of eNanoMapper using CiteULike tool (http://www.citeulike.org/group/19781). We have also successfully collaborated with various project on the dissemination of data and knowledge from those other projects.

**Ontology Development** - the eNanoMapper ontology covers the full scope of terminology needed to support research in nanomaterial safety. It builds on multiple pre-existing external ontologies such as the NanoParticle Ontology. We have released an infrastructure for ontology development and maintenance based on a GitHub repository, a Jenkins build environment and also an automated ontology testing software. Further, with an extensive research of the domain area, as well as a survey of existing ontologies, we released the eNanoMapper ontology. We also developed and released as open source library to create slimmed versions of third-party ontologies.

- Download the ontology in .owl from: http://purl.enanomapper.net/onto/enanomapper.owl
- Download and explore on BioPortal: http://bioportal.bioontology.org/ontologies/ENM
- Download and explore on Aber-OWL: http://aber-owl.net/ontology/ENM
- Download and explore on Ontology Lookup Service: http://www.ebi.ac.uk/ols/beta/ontologies/enm
- Feedback: https://github.com/enanomapper/ontologies/issues

Version 4 of the eNanoMapper Ontology was made on 26 January 2017 (doi:10.5281/zenodo.260098). It contains 10,881 terms, an increase of over 2,400 from the previous release:

- 10,295 of the terms in the new release were reused from 22 other ontologies in the biomedical domain.
- The remaining 586 terms were not present in existing ontologies and so were created manually; 321 of these are appeared in the ontology for the final release.

For interactive queries, we have implemented a RDF database based on the Virtuoso triple store and implemented a SPARQL query interface (https://sparql.enanomapper.net). The RDF backend mirrors eNanoMapper ontologies and data and supports combined queries to ontologies and data. For the interactive visualisation of SPARQL query results we have implemented a graphical user interface which is publicly available at https://query.enanomapper.net.

**Database Development and Implementation** - the main achievements include the eNanoMapper prototype database featuring a flexible data model based on an exhaustive review of existing nano-related entries in chemical- and toxicogenomic-databases. The database REST Application Programming Interface (API) enables user friendly interface and programmatic interaction. The eNanoMapper database is publicly available at http://data.enanomapper.net. It is a public database hosting nanomaterials composition and characterization data, as well as biological and toxicological information. The database content has been continuously updated with datasets provided by project partners and NSC projects. The database software is open source and was recently described in a peer-reviewed publication (doi: 10.3762/bjnano.6.165). Installation instructions are available from http://ambit.sourceforge.net/enanomapper.html. The eNanoMapper database demonstrates the integration of data from multiple sources, using the common data model and API. Evaluations and improvements have been implemented by interactions with the eNanoMapper partners, NSC projects, ISA-TAB, US-Nano WG, publications, presentations and public GitHub issue tracker. Ontology annotation was performed with close collaboration with WP2. The import formats currently supported are: OECD Harmonized Templates, NanoWiki RDF and eNanoMapper RDF, and set of spreadsheet templates. A configurable parser was developed to enable import of data stored in spreadsheet templates, used by the EU NanoSafety Cluster projects. The export formats have been extended with the new ISA JSONv1 format, following the new ISA specification and with eNanoMapper RDF, based on the BioAssay Ontology data model.

eNanoMapper database provides various search functionality: chemically aware search, several options for searching experimental data, semantic search including SPARQL endpoint and free text search, supported by the eNanoMapper ontology and annotated database entries. The free text and faceted search services and the corresponding front end interface at http://search.data.enanomapper.net were launched Jan 2016 and since then became the most popular user interface to the eNanoMapper database. Importantly, this search interface does a federated search over two different databases (http://data.enanomapper.net and NCI caNanoLab (https://cananolab.nci.nih.gov/caNanoLab/#/). Feedback from users is gathered through a public issue tracker at GitHub and was taken into account in updated versions of the search application.

Tools for converting the internal model of the eNanoMapper database into ISA-JSONv1 as well as tools for generating ISA-JSONv1 files were developed and released. The export to ISA-JSONv1 is enabled for each data collection and material page in the eNanoMapper database, and most recently enabled in the free text search application. Converting supported input files into ISA-JSON format is also possible without import into the database and the relevant tools were developed. If needed, the ISA-JSON files can be translated into legacy ISA-TAB. A Substance/Material JSON schema which is a nanomaterial extension of ISA-JSONv1, the counterpart of the ISA-Tab-Nano format. The schema is available at the enanomapper/isa-api fork at GitHub.

On the **analysis and modelling** part of the project, various results were achieved, presented here grouped by area:

*Analysis & Modelling Specifications, APIs*

- Defined the technical specifications of the eNanoMapper analysis and modelling infrastructure. OpenTox algorithm and model APIs were extended to account for the special needs of ENM predictive toxicology and the fact that ENMs are often characterised by a multitude of assays, resulting in high-dimensional datasets

*Descriptor Calculation Algorithms and Methods*

- Developed an image analysis web application for deriving ENM descriptors from images available at http://enanomapper.ntua.gr:8880/imageAnalysis/
- Integrated the MOPAC quantum chemistry software for calculating ENM quantum mechanical descriptors from crystallographic data
- Omics data were integrated with biological pathways information to produce a new set of descriptors (named BIO descriptors) for ENMs.
- Extended the Java-based Chemistry Development Kit (CDK) with nanomaterial specific descriptors

*nQSAR Modelling infrastructure*

- Developed the Jaqpot Quattro (JQ) web application as a platform that integrates ENM modelling and analysis tools. JQ used data from the eNanoMapper database, incorporates the eNanoMapper ontology and is fully compatible with the APIs. The JQ platform has implemented and integrated many functionalities: image and quantum mechanical descriptor calculations, data merging and preprocessing, machine learning and data mining algorithms, split, cross and external validation services, interlaboratory proficiency testing techniques, read-across, and optimal experimental design as well as extensive reporting services (including the automatic generation of QPRF reports). The *Jaqpot Protocol of Data Interchange* (in short JPDI) specifies the form of data exchange between eNanoMapper services and third party algorithm web service implementations and allows developers to integrate their algorithms in the framework. Based on the JDPI protocol, the eNanoMapper computational infrastructure provides wrappers for WEKA, the R language and the Python language. In order to promote the reproducibility of calculations and the ease of dissemination of tools to users, Docker images for JQ components have been made available (https://hub.docker.com/r/jaqpot/jaqpot-core/). The capabilities of JQ have been covered thoroughly with a number of documents and video tutorials as well as with the Swagger interface and API documentation hosted at http://jaqpot.org:8080/jaqpot/swagger/. A user interface, available at http://jaqpot.org, enabling non modelling-proficient users to have easy access to all modelling JQ tools and applications, but also with the capability to act as a validated model repository has been developed.
- In order to facilitate read across predictions for nanomaterials we extended the lazar read-across framework (https://github.com/opentox/lazar) with capabilities to predict nanoparticle toxicities, additional interfaces for eNanoMapper data and ontologies and developed a novel method for predicting nanoparticle toxicities based on computed properties alone. These techniques were validated extensively and the results were submitted for publication in the Predictive Toxicology section of Frontiers in Pharmacology. In addition, we have provided the complete source code for lazar libraries, validation experiments and manuscript generation and a Docker image with all pre-installed dependencies as an example for reproducible research (https://hub.docker.com/r/insilicotox/lazar/). With the publication of our complete working environment we intend to encourage other researchers to reproduce and examine our experiments with minimal efforts, and to supply a reference even if system dependencies change in the future
- Implemented all major machine learning and data mining algorithms as API compatible web services and used them generate predictive nanoQSAR models for particular use cases, which are exposed to the public as ready to use web applications
- Developed the RRegrs R package for computer aided model selection and easy-to-use model comparison. This package automates the estimation of the best regression model by using certain cross validation schemes and by searching over many different algorithms and tuning the parameters in each algorithm
- Developed services for validating the produced predictive models (split, cross and external validation services)
- QSAR Model Reporting Format (QMRF) and QSAR Prediction Reporting Format (QPRF) services were developed and integrated in the eNM modelling infrastructure to summarise information on QSAR/NanoQSAR models and their validation results, based on the REACH objective to reduce animal testing

*Mechanism-of action Modelling Tools*

- Extended work has been done on mechanistic modelling. Particularly, an ENM portal with nanosafety-relevant pathways was initiated on WikiPathways as a basis for pathway analysis (http://nanomaterials.wikipathways.org/), and also workflows for ENMs data analysis, (mainly transcriptomics data) were established, to reveal the significantly and differently affected biological pathways by a variety of exposure scenarios and ENMs (this information was added to the eNanoMapper database)
- Omics data were integrated into an optimisation search functionality estimates the set of descriptors that best predict the toxicity dataset under consideration. Further work on integration of data has been conducted aiming to integrate omics and physicochemical nanoparticles data on a read-across predictive modelling framework. The workflow suggests filtering of the data based on biological pathway

information, and then predicts the toxicity index by using either jointly or individually omics and physicochemical ENM data. The produced toxFlow web application is available at http://147.102.86.129:3838/

*Design of experiments and inter-laboratory testing facilities*

- Developed an Experimental Design service to serve two main needs of the community, namely a Factorial Design (FD) service for experimental laboratories that would like to design their initial experiments, and an Iterative Experimental Design (IED) service for laboratories with available experimental data aiming to suggest the next experiments or 'trials' that need to be conducted
- For the integration of data analysis resources with experimental design, we considered an application to Dose-Response modelling, after carefully studying the prerequisites with two partners from the SUN NanoSafety Cluster project. A specially designed workflow was the use case based on which an R package was implemented to perform Dose-Response modelling for continuous data. As an extension, an experimental design functionality was added to the already implemented experimental design services aiming to estimate the optimal number of cases per dosage
- Deployed the Interlaboratory Proficiency Testing service, which comprises a series of calculations and statistical tests in order to assess bias in laboratories and increase repeatability of experiments according to international standards. This is carried out by processing the reported measurements of each laboratory individually against the group consensus

**User Application Development, Integration and Testing** - a collaborative working environment was set up for the eNanoMapper project including an issue tracker, a development coordination system and build environment. Based on OpenTox services, we set up standard user services as well as libraries for all eNanoMapper components in order to create the base services for all eNanoMapper products for user registration, authentication and authorization. A paper-prototype-specification for key use cases for harmonization and integration purposes was created. The tools and functionalities development followed the requirements analysis and the prioritized use cases (e.g. user application for importing NanoWiki data, user application for importing NanoSafety Cluster data and user application for free text search of nanomaterials and experimental data in the eNanoMapper and caNanoLab database, user application for conformance to reporting and curation standards, user application on QSAR and read-across for nanomaterials, etc.). Further, the testing phase aimed to provide the basis for test-driven development, ensure the interoperability of services and the developing ontology and alert developers about bugs and interoperability problems. The following **user interfaces** were developed:

- Jaqpot user interface - the JQ user interface integrates the JQ modelling and analysis tools and is offered to the community as a central modelling platform and application. The JQ can be accessed at http://www.jaqpot.org/ and its functionalities include ENM descriptor calculations, nanoQSAR modelling and validation, read-across studies, optimal experimental design and interlaboratory testing (see details under Analysis and Modelling section).
- Nano-lazar user interface - as a frontend for lazar nanoparticle predictions we have developed a web application (https://nano-lazar.in-silico.ch) that uses lazar for toxicity predictions and ontologies and data from eNanoMapper for the graphical user interface. The user can enter core and coating structures, physico-chemical properties of nanoparticles or protein interaction data and receives toxicity predictions from a local nano-QSAR (Quantitative Structure-Activity Relationship) model together with a list of similar particles and their physico-chemical and toxicological properties. Links to eNanoMapper ontologies and data as well as to external resources (e.g. BioPortal, UniProt) provide definitions for domain-specific terms and supporting information for toxicity predictions. For this reason, the nano-lazar GUI is also a showcase for the integration of eNanoMapper resources with external services and the functionality of the eNanoMapper API. An additional lazar frontend is provided by nano-lazar REST services, that exposes nano-lazar capabilities via a eNanoMapper compatible API. This interface can be used by external web services and mashups to access nano-lazar functionality.
- Knowledge-integrating Applications (Summit) - web-based applications were created as knowledge-based integrating collaborative support tools for nanosafety related activities such as case study discussions and workshops, with possible extension to other areas. Version 1.0 (https://nanoehs.enanomapper.net/) was used to support the US-EU NanoEHS workshop and its "scrimmage" workshop (Arlington, 2016), while version 2.0 (https://summit.enanomapper.net/) was used to support the EU-US NanoEHS workshop and breakout session (Rheinfelden, 2016). The functionalities of the Summit application facilitates the interactions before, during and after the event: Add and create content and resources (e.g. publications, guidance documents, tools); Add topics and questions to be discussed; Add comments and answers related to the topics; Gather information and capture the discussions; Support the reporting from the workshop.

**User Application Documentation** - in order to support the developers in this area, eNanoMapper released a broad range of documentation materials and tutorials. Most of the eNanoMapper source code is stored and documented at Github, a web-based repository hosting system with distributed version control and source code management functionality. eNanoMapper project directory on Github at https://github.com/enanomapper, and each repository has an associated issue tracker. For the archival and collecting of different versions of tutorials, there is a separate tutorial repository at https://github.com/enanomapper/tutorials. Also, to facilitate the access to various sources (applications, code documentation, publicly available reports, dissemination materials, etc.) an online publicly available library was set up at http://www.enanomapper.net/library, and we have put together a documentation map available also in GitHub, which offers direct links to all these resources.

## 5 Impact beyond the project lifetime

Establishing standards and setting up harmonised infrastructures with applicability in nanosafety, still represents a major challenge for the scientific community. The complexity of the toxicology, chemistry and biology and the additional physicochemical properties of nanomaterials, leads to increased uncertainty in the

validity of experimental data. To support these international efforts, eNanoMapper delivered an ontology, a data infrastructure and modelling tools with applicability in risk assessment of nanomaterials. The infrastructure developed for nanomaterials toxicological data is based on open standards, open source, common languages, and an interoperable design, enabling a more effective and integrated approach in risk assessment.

The main achievements of eNanoMapper towards improved standards in risk assessment of nanomaterials and with an impact on the nanosafety community are represented by an agreed language formalized in a nano ontology, an open platform for integrating different nanomaterials data sources providing access to open and confidential data and the computational infrastructure, analysis and modelling tools for predicting toxicity of nanomaterials. The eNanoMapper approach supported the integration of non-testing methods into risk assessment, and facilitated a harmonised use of existing data and knowledge, enabling a significant reduction of animals used for nanomaterials toxicity testing. Several NanoSafety Cluster projects have shown strong interest in the eNanoMapper data management and integration solutions, manifested by the NANoREG decision to transfer all data to a eNanoMapper database instance, and subsequent decisions of the new H2020 projects caLIBRAte, NanoReg2 and ACEnano to adopt the similar solutions.

Concretely, the eNanoMapper data and ontology platform can now be used to capture data and knowledge along the full lifecycle of ENMs, from research to product development to manufacturing procedures, human and environmental exposures, (eco)toxicological effects and degradation processes. Its flexible design supports the safety, environmental, regulation, and standardisation aspects of ENMs, which are all important for safety-by-design and risk assessment. The linked data approach supports real, achievable and operational interoperability with external ontologies and databases and enables the application of statistical and data mining procedures for data analysis, as well as to support the information transfer activities of the NanoSafety cluster. Thus, these deliverables of eNanoMapper are applicable to exploit and couple together many diverse data types from nanosafety research projects.

All deliverables are archived on Zenodo and available from https://zenodo.org/search?page=1&size=20&q=eNanoMapper&subtype=report.

## 6 List of Publications from the project

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Doganis P, Tsiliki G, Drakakis G, Chomenidis C, Nymark P, Kohonen P, Grafström R, Abdelaziz A, Farcal L, Exner T, Hardy B, Sarimveis H, Computational Modelling of Biological Responses to Engineered Nanomaterials. In: Nanotoxicology: Experimental and Computational Perspectives, Editors: Dhawan A, Anderson D, Shanker R, Royal Society of Chemistry. (2017) (*in press*).

## 7 Copyright

# GUIDEnano

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

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

*Table 1 Consortium List.*

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

## Contents

## 1 Summary

Project Duration: 42 months

Project Funding: 8.150.000 €

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

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

## 2 Background

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

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

## 3 Objectives

The main objective of GUIDEnano was to develop innovative methodologies to evaluate and manage human and environmental health risks of nano-enabled products, considering the whole product life cycle: synthesis of NM, manufacturing of NM-enabled products, use, and end-of-life phase (including recycling) (Figure 1).

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

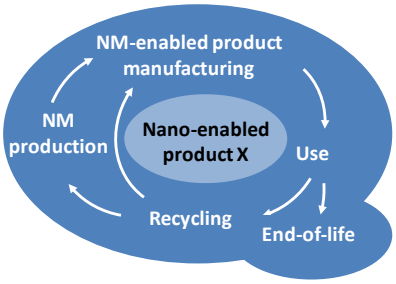


*Figure 1. Target of the risk assessment in the GUIDEnano Tool*

*Specific goals of the project:*

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

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

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

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

## 4 Major Outcomes from project

GUIDEnano was structured into 11 work packages (Figure 2) arranged by four main blocks: the Coordination block (WP1 and WP2), the Knowledge block subdivided into different technological building sub-blocks (WP3, WP4, WP5, WP6, WP7 and WP8) that generated the scientific input to the GUIDEnano Tool, the Software Development and Demonstration block (WP9 and WP10) that created the Tool itself and validated it in real life case studies, and the Dissemination, Standardization, and IPR block (WP11).

The major outcomes achieved by each WP (the technical ones: WP3-9) of the project is explained below.

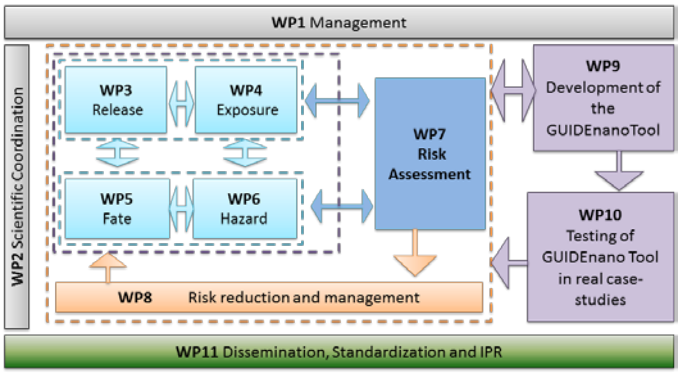


*Figure 2. Organization of the project in work packages*

**WP3: Release assessment**

The main objective of this WP was to generate and validate strategies to identify and categorize the release processes that take place during the life cycle of a NM-enabled product, and assess potential release of NM during such processes. Apart from the case studies included in GUIDEnano, which included polymeric nanocomposites, antifouling paints, anti-slip and photocatalytic tiles, nano-enabled textiles, nanocellulose based coatings and bituminous road products (see Figure 3), this WP aimed at covering the most representative applications in the market as well as those nanomaterials with current higher production volumes.

Based on mass-flow diagrams describing the life cycle stages of nano-enabled products, WP3 identified the sources and release pathways of different nanomaterials (NMs, including TiO2, Ag, ZnO, MWCNT and Cu-based nanomaterials). These release pathways or release scenarios were the starting point of the GUIDEnano Tool aiming at delivering standardized formats and default NM release values from nano-enabled products into the different environmental compartments. Release scenarios are called Activity Cards (AC) in GUIDEnano Tool. An AC library with 160 activities has been created and implemented in GUIDEnano tool, in collaboration with WP4. From this list the user can select one or more AC and start the risk assessment process. This library covers release scenarios in different life cycle stages of nano-enabled products: 1) synthesis (e.g. flame spray pyrolysis), 2) manufacturing (e.g. dumping), 3) use (e.g. use of polymeric nanocomposites outdoors) and 4) end-of-life (e.g. incineration). These activities contain information relative to the release of NMs and waste containing NMs that is generated during different processes (default release values are expressed as mass fraction). This allows predicting amounts of NMs reaching the different environmental compartments (e.g. water), and link the release / exposure module with subsequent fate and toxicity in the GUIDEnano Tool. Such environmental release factors were defined from existing literature values and information coming from the industrial partners when possible. In absence of empirical data release was defined following the ECHA R.16 Guideline and also from expert judgment.

A series of experiments were planned to reduce key uncertainties that lead to the prediction of NMs release taking into account the life cycle stages that are most likely to result in the transformation and/or to result in the release of NM from different nano-enabled products provided by the industrial partners in GUIDEnano. As a result, 7 nano-enabled products and 11 exposure scenarios were evaluated (see Figure 3) by LEITAT and CEA, investigating different parameters such as NM concentration, crystalline phase or coating. Experiments were performed to: 1) understand which are the processes promoting release, 2) how to reduce such release, 3) release kinetics, 4) release forms.

| CASE STUDY | PARTNER | NMs | Weathering | Washing | Abrasion | Incineration/ Leaching | Comments |
|---|---|---|---|---|---|---|---|
| Coated Tiles SbD | | $TiO_2$ $Al_2O_3$ | ✓ LEITAT | | ✓ CEA | | Performance also evaluated |
| Food Packaging | INNVENTIA | N-cellulose | | | ✓ CEA | | Labelling with metallic NPs |
| Antifouling paints | | ZnO, Cu | ✓ CEA | | ✓ CEA | | ZnO with different coatings |
| Polymeric composites SbD | LATI | $TiO_2$ MWCNT | ✓ LEITAT | | | ✓ LEITAT | Different $TiO_2$ crystalline phases, [NMs] |
| Photocatalytic coatings | Servia Cantó | $TiO_2$ | ✓ LEITAT | | | | Different [$TiO_2$] |
| Nano-enabled textiles SbD | inoTEX | Ag AgCl | | ✓ LEITAT | | | Different coatings |
| Self-cleaning agent | Nanesterface | $TiO_2$ | Only characterization | | | | |
| Remediation contaminated groundwater | | Fe | Experiments not performed | | | | |

*Figure 3. Release experiments performed in WP3.*

No NMs release was found during abrasion of the antislip tiles containing $Al_2O_3$, paper film containing nanocellulose and polymeric composites with embedded MWCNT. Regarding the photocatalytic coatings applied on roads ($TiO_2$), NMs were progressively removed from the surface, promoted by oxidation of bituminous compounds due to UV radiation. Interestingly, NMs release was successfully reduced by the application of safe-by-design strategies on three different case studies: photocatalytic tiles (TiO2), polymeric nanocomposites with TiO2 and textiles with Ag. These strategies consisted in modifying the interaction between matrix-nanomaterials, modifying the surface properties of the NMs and modifying the morphology of the NMs to improve adhesion on surfaces.

Based on criteria such as scalability, amount produced and functionalization properties, but also according to WP5 and WP6 requirements, WP3 supplied different nanomaterials to be tested in those WPs (fate and toxicity, respectively). These materials included $TiO_2$, $CeO_2$ and Ag with different coatings, release NMs (e.g. $Ag_2S$), or materials collected from experimental simulations (e.g. waters collected during the leaching experiment with antifouling paints).

**WP4: Exposure assessment**

The main objective of WP4 was the development of models and guidance on (human) exposure assessment for the various stages of NM-enabled product value chains (life cycle). The human exposure assessment quantifies the indoor air concentration of Nano Objects and their Agglomerates and Aggregates (NOAA) by using available release/exposure measurement data, libraries and/or models for a wide variety of exposure scenarios, and guides the user of the GUIDEnano Tool to the available exposure data (in

libraries) or to the appropriate exposure assessment model in the absence of suitable measured data.

An activity card library, containing around 150 activities, was built with associated worse case material release rates. These release rates are then automatically processed in the dispersion model resulting into a worst case estimate of the exposure.

In addition to the activity card library, exposure scenario information from ongoing and finished FP7 projects (e.g. Nanomicex, Sanowork, Nanodevice, MARINA, SUN, NanoReg, GUIDEnano) was collected using the MARINA exposure scenario template. The resulting GUIDEnano library contains over 200 exposure scenarios.

A quality, similarity and relevance scoring system were developed to rate the different information sources as to their analogy to the user's scenario.

Because the GUIDEnano Tool aims to quantitatively assess human exposure (in respirable mass concentration), currently available qualitative risk/control banding tools cannot be used, and therefore for worker's exposure it was decided to use the ART model (Advanced Reach Tool). For validation of ART for NM scenarios, high quality exposure scenarios were selected and run in ART to test the feasibility of the model. In addition, a model performance check was performed for the Advanced REACH Tool and the Stoffenmanager Nano 1.0 tool. This performance check included metric conversion methodology, analyses of nano-exposure measurements and finally the model performance check, which was designed to gain knowledge on the applicability of certain models for the use within nano-exposure assessment and thus for implementation in the GUIDEnano Tool.

Number count exposure data collected in standardized circumstances were converted to mass concentrations in order to be comparable to the model outputs. Statistical analyses were performed to examine correlation between model estimates and converted mass concentrations. Furthermore, model performance was evaluated based on the uncertainty given by the developers of the ART model.

Secondly, exposure measurement data were generated for the GUIDEnano case studies in order to refine the exposure scenarios mentioned above. Measurements were collected using the following direct-reading instruments: Condensation Particle Counter (CPC), NanoTracer, DiscMini (only Hempel and Servia Canto) and filter samples of airborne particles for SEM/EDX analysis. These measurement results gave good insight in the exposure levels during several activities in the value chain of the GUIDEnano case studies and were used to further validate the GUIDEnano model. Chamber experiments (under well controlled conditions) were also performed to simulate the scenarios of the case studies in the absence of workplace measurements.

**WP5: Environmental Fate**

The main objective of this WP was to generate strategies to understand how NMs behave in natural systems including the critical transformation reactions. Several key questions were addressed: (i) How do NM properties and their nanoscale features affect their behavior and interactions with other environmentally relevant parameters, i.e. which property-fate relationships are crucial for fate prediction? (ii) What transformations are likely to occur in natural systems? (iii) How do the transformations affect the NM's fate? These questions were quantitatively addressed to develop a conceptual fate model framework (Figure 4) focused on NM fate and behavior for implementation into the GUIDEnano Tool, and parameterized using available literature or by obtaining experimental results when data gaps were identified.

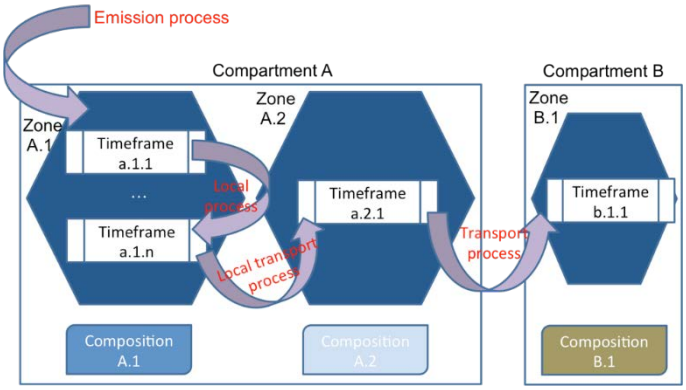


*Figure 4. Schematic overview of the approach used in the model world that should take into account the following: compartments/zones/timeframes/processes.*

The integrated exposure model that was proposed for the prediction of the environmental fate of NMs in the various natural and man-made compartments had the kinetic nature of the NM fate processes as a central endpoint. A semi-mechanistic approach was used, with a minimum number of fate descriptors for the NM. In general, environmental matrix interactions depend on NM properties and relevant environmental properties, such as organic and inorganic matter type and concentration, as well as ionic strength and the presence of mono- or divalent ions. The fate descriptors were detailed for the following compartments: wastewater treatment plant (WWTP), water, soil and subsurface, and for the following transformations: heteroaggregation, sedimentation and sulfidation. The time-dependency of chemical species concentrations is an essential feature of this model, because it serves to link exposure scenarios with hazards that change over time.
WWTP microcosms using Ag NM showed that sedimentation depends on size and total suspended solids, whereas coating, shear forces or ionic strength had no effect. No aggregation was observed in these studies. The final model for the fate in WWTP predicts the mass and speciation of NM that ends up in soils AND freshwater as a function of size and Hamaker constants.
Dissolution, heteroaggregation and sedimentation experiments in the aquatic compartment were performed using several CuO, ZnO and Ag NMs with different manufactured coatings and sizes. One of the main conclusions from the experimental work was that NMs dissolution in relevant environmental matrices (i.e., in presence of organic matter) is not significant and independent of water composition, manufactured coating or size of the NM.

**WP6: Hazard Assessment**

The main work of this WP was developing a strategy for predicting the (eco)toxicological and human health hazard of the exposure-relevant NM forms released into exposure situations throughout the lifecycle of NM-enabled products.

The eco- and human toxicologists worked together to develop a hazard assessment strategy to estimate safety limit values making the fullest use of existing information while allowing prediction of

hazard on the basis of different levels of data availability (each with an associated level of uncertainty reflecting the richness and quality of data available). Existing PNEC, OEL or DNEL values for the material under evaluation will be used, if these are available. Otherwise, generic highly conservative thresholds can be used. When necessary, these can be refined based on available toxicity studies. The strategy mostly relies on information from studies following harmonized testing guidelines such as OECD and/or ISO, but is designed to also make use of 'other non-standard' studies and toxicity information. When assessing already existing individual toxicity studies from literature the hazard assessment strategy involves the establishment of scores to inform on quality (i.e. how good and reliable a study and its reporting is), relevance (i.e. how relevant the NM study is for the respective environmental compartment or human pathway/endpoint) and similarity (i.e. how well does the NM exposure in the study to be used reflect the exposure relevant form of nanomaterial which is being assessed). These scores are used to select studies that can be included in the process to derive safety limit values. In addition, the similarity score is used to introduce a 'dissimilarity' uncertainty factor.

Hypotheses-driven experiments (different cores and coatings) were performed to evaluate the assumptions identified as most critical to reduce the most prominent uncertainties (e.g. from read across) in the process. To test the influence of the core material and the different coatings a base set of three different core materials ($TiO_2$, CeO and Ag) each with three different coatings (citrate, PEG and the hydrophobic coating DDPA) was selected for testing in a wide range of *in vivo* and *in vitro* test systems. No consistent trends were observed for the effects of coatings. These effects depended on the core material and the experimental test.

In addition, experiments were performed to characterize the hazard profile of the release and exposure relevant materials of the GUIDEnano case studies and to evaluate the efficiency of safe-by-design modifications.

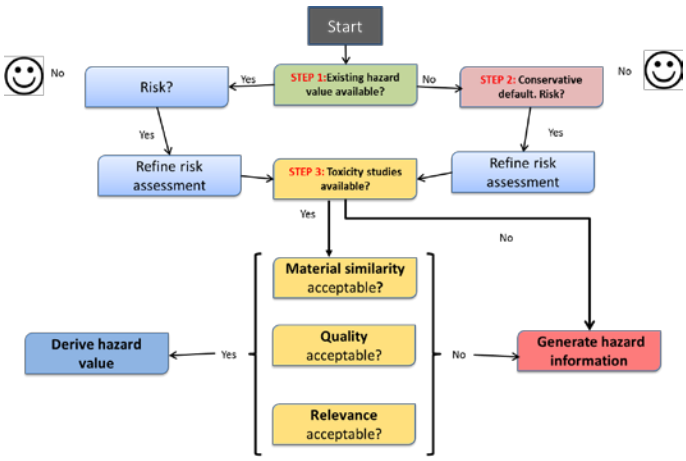


*Figure 5. GUIDEnano hazard assessment strategy for a human and environmental risk assessment.*

**WP7: Risk assessment**

The main goal of this work package was to develop a strategy for risk assessment of release- and exposure-relevant NMs in NM-enabled products throughout the various product life-cycle stages. This risk assessment strategy was incorporated in the interactive web-based GUIDEnano tool and was evaluated with hypothetical and real case studies within the project.

The initial decision flow for risk assessment incorporated in the GUIDEnano tool is presented in Figure 6. The safety limit value is comparable to the Derived No Effect Level (DNEL) for human health and Predicted No Effect Concentration (PNEC) for environment according to the REACH regulation.

Summary of the steps to derive a safety limit value:

1. Select hazard studies with associated effect levels (NOAEL, LOAEL, BMD, etc)
2. Determine modification/assessment factor for each effect level
3. Derive safety limit value by applying the assessment factors for each effect level (including a factor for dissimilarity)
4. Derive overall safety limit value per endpoint
5. Risk assessment: compare safety limit value with corresponding exposure level

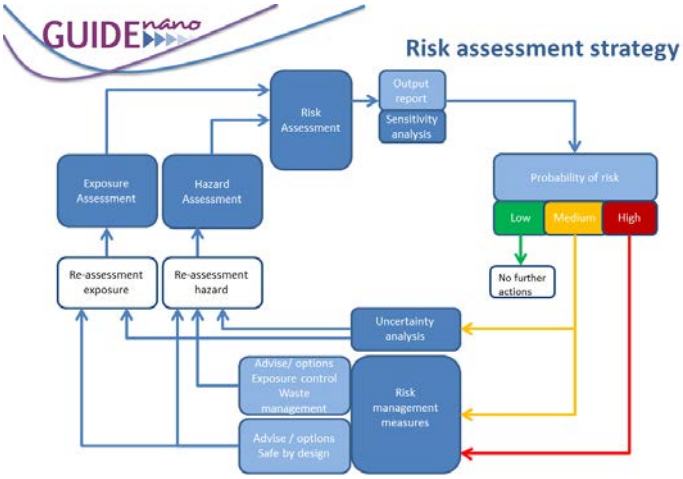


*Figure 6. Updated GUIDEnano risk assessment decision flow*

The required factors (step 2) were listed and for each factor the purpose for application and the value are included. The risk assessment in GUIDEnano Tool v3 is deterministic, but some information is provided to the user to inform on the sources of uncertainty for the hazard limits derivation. For each assessment factor, it is indicated if it represents modification, uncertainty or variability. This information is needed to determine the possibility for reduction of the uncertainty of the safety limit value.

An output report has been designed and generated by WP7 together with the tool builders. This output report consists of a detailed background report with all information generated by the Tool based on the input of the user. This will be provided to the user after using the Tool, together with an executive summary of the risk assessment result (probability of risk) and proposed follow up actions (reduction of uncertainty, mitigation of risk by for instance safe by design strategies).

Case studies reported in the literature or hypothetical were used to evaluate the version 2 of the Tool. Feedback from this evaluation and feedback from stakeholders (e.g. industry, (re)insurance communities) obtained in dissemination events and via tailored questionnaires was used in the updates towards version 3. WP7 also monitored to what extent the industrial

partners of the project were able to correctly use the GUIDEnano Tool during the evaluation of the project case studies.

**WP8: Risk Management**

Work package 8 aimed to propose, develop and validate risks mitigation measures (RMM) to reduce the potential risks identified through the risk assessment provided by the web-based Guidance Tool GUIDEnano. In such a way the final user, will obtain suggestion on the most appropriate RMM allowing to control risks highlighted and decrease them to an acceptable level, making the whole process safer. Among possible RMM, safer-by-design (SbD), occupational exposure control and advanced waste management strategies, were selected and are being evaluated within the context of GUIDEnano case studies.

**Safer-by-design strategies** were proposed taking into account the case studies and the NM employed and focusing on relevant endpoints and effects/risks to mitigate. SbD strategies were intended to :

- *re-design relevant physicochemical properties of NM* to mitigate their hazardous potential, while maintaining their characteristic functionality within the NM enabled product,
- avoid or reduce the release of NM during different life cycle stages of the nano-enabled products by *improving compatibility between NM and matrix*, to lower the possibility of environmental and/or human exposure to NM,
- avoid or reduce the environmental and/or human exposure to NM by designing and synthesizing *less reactive and/or less persistent NM*.

A set of **SbD** NM were synthesized at lab-scale level with targeted modifications of relevant physico-chemical properties and were provided to the different WPs to be evaluated for specific endpoints/ functionalities. For selected case studies, the resulting SbD NMs were implemented within their real industrial setting.

The investigation of **occupational exposure control measure** was focused on the measurement of the effectiveness of the personal protective equipment (PPEs) commonly employed by the industrial partners involved in GUIDEnano, with the objective to obtain for each of them a nominal protection factor (NPF) against NMs. The NPF of different PPEs have been determined, modified and validated using standard protocols available for bulk materials. Depending on the nature of the NM tested, two test protocols were adopted:

- a *static test protocol* were employed to evaluate the NPF of PPEs against metal oxide NMs, which are not yet classified as safe materials. These tests were performed under simulated conditions and using mannequins in substitution to individuals,
- a *dynamic tests protocol* were designed to evaluate the NPF of PPEs against NaCl nanoparticles, that being not considered hazardous materials, allowed that PPEs were worn by different individual, thus conducting the test under the more realistic dynamic conditions.

Several types of masks, suits and gloves were investigated under simulated conditions performing static tests using different NM and at different concentrations. Results demonstrated that suits and masks, especially respiratory filters, offered a quite good NPF, sometimes slightly lower than the default value. Data collected showed unlike performance of the glove depending on NM type and concentration employed, therefore further tests are needed.

Full and half masks were tested under the dynamic test protocol. Results showed high variability on the performance of the masks due to the fitting to the facial geometry, that could cause leaks where NM penetrate. From results obtained, the half-masks appeared to provide better protection than full face masks as they accommodate better different facial geometries, especially in the case of women due to smaller face geometries and men with beard.
Future activity will include a continue evaluation of existing PPEs to obtain a global protection factor and specific protection factor size-by-size.

The work performed on the **waste management strategies** was aimed to reduce the NM environmental exposure (and exposure through the environment) by application of novel and known waste management strategies and by proposing waste reduction and potential implementation of recycling processes.
The seven GUIDEnano production processes/case studies were described in collaboration with the corresponding industrial partners (Hempel, Innventia, Inotex, Lati, Torrecid, Plasmachem and Servià Cantó). Then, for each case study, a production process diagram including a list of the nanowaste fractions generated, were depicted. Being the water containing NM one of the main waste fraction generated, different waste water treatment were selected according to their relevance for industry and their NM removal efficiency, these included settling, electrocoagulation, membrane distillation and advanced oxidation processes. This kind of test will be performed also with the waste water generated when SbD strategy have been implemented.

The efficiencies of the different strategies for NM removal were evaluated together with industrial partners and were collected in a compilation table, where information on "waste fractions", "waste attributes" and "treatment process" was included. This information was incorporated into the GUIDEnano Tool.

**WP9: Tool development**

The main objective of this WP was to develop the web-based tool. The design of the tool: Structure, flow, connections of all the components of the risk assessment (exposure, fate and hazard) and risk management strategies, is the result of team work of all the technical WP experts in the different scientific areas (WP3-WP8) and the software specialists.

Three versions of the GUIDEnano Tool were delivered during the project.

**GUIDEnano Tool v1** was released to consortium in the 18th month meeting (May 2015). In v1 the focus was on implementing the main structure of the tool in an object-oriented manner. First, class-diagrams where developed together with the experts for each individual wp. Next, these wp class-diagrams where merged into one single GUIDEnano class diagram, identifying overlap and harmonizing nomenclature. Relevant class-attributes (properties) where identified and assigned to the defined classes. In parallel, v1 of the application framework was developed, allowing online usage of the tool under development. Finally, v1 allowed the user to describe the relevant nanomaterial forms and identify the potential hazard hotspots during the life cycle of a nanomaterial/ nano-enabled product due to related activities and potential release(s).

**GUIDEnano Tool v2** was released to consortium at the end of April 2016. In v2 the focus was on the implementation of the 'behavior' of the identified classes, like: rules, decision trees, algorithms. Over a hundred toxicity study classes covering both human and eco hazard were incorporated. The knowledge implemented in v2 allowed users not just to identify but also to quantify the risk of certain prioritized hotspots. This version was the basis for the internal evaluations (see WP7 and WP10).

**GUIDEnano Tool v3** was released at the Final consortium meeting. First, the feedback and related issues as a result of the evaluation were taken into account. Special focus in v3 was the improvement of the kinetic fate module, allowing now to predict the fate of nanoparticles per size-bin. A first implementation of nanomaterial similarity for the inhalation hazard endpoint was built in.

The tool has been built on a modular basis which will be easy to update in the future.

**WP10: Validation of the GUIDEnano Tool in real case studies**

Industrial partners used the GUIDEnano Tool version 2 to evaluate the risks for their case study. During this process, when needed, they received support from the experts in each part (WPs 3 to 9).

The high potential of the Tool was well recognized during this evaluation, although the current version was not considered to be sufficiently user-friendly. Another limitation that some industrial partners faced during the assessment process was the reluctance of their suppliers to provide characterisation data for the NMs.

# 5 Impact beyond the project lifetime

GUIDEnano built upon the state-of-the-art on NM risk assessment. The main product of the project has been the GUIDEnano Tool. But in addition to this, the project has provided a series of novel approaches that could be used in any offline risk assessment process.

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

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

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

# 6 List of Publications from the project

*Table 2. List of Publications*

| Publications | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Title | Author(s) | ISBN | Number, date or frequency | Publisher | Place of publication | Year of publication | Relevant pages | related WPs | partners involved |
| Mechanisms underlying the enhancement of toxicity caused by the co-incubation of ZnO and Cu nanoparticles in a fish hepatoma cell line. | Hernández-Moreno D; Li L; Connolly M; Conde E; Fernández M; Schuster M; Navas JM; Fernández-Cruz ML. | 1552-8618 | 35(10) | Environmental Toxicology and Chemistry. | | 2016 | 2562-2570 | WP6 | INIA |
| The validity and applicability of using a generic exposure assessment model for occupational exposure to nano-objects, and their aggregates and agglomerates | C. Bekker, E. Voogd, W. Fransman, R. Vermeulen | | | Annals of OccupationalHygiene | | 2016 | | WP4 | TNO |
| Incorporation of Ln-doped LaPO4 nanocrystals as luminescent markers in silica nanoparticles | Jacobine van Hest,Gerhard Blab, Hans Gerritsen, Celso de Mello Donega, and Andries Meijerink | | Accepted | Nanoscale | | 2016 | | WP8 | DEI |
| Experimental Evaluation of the Effectiveness Offered by Different Types of Personal Protective Clothing Against Nanoaerosols | M Domat, J Pla, M C Cadavid-Rodríguez, C Fito | | Accepted | IOP | | 2017 | | WP8 | ITENE, HWELL |
| Nanomaterials removal efficiency for different treatment technologies: review | Jordi Palau, Sofía Ricarte, César Aliaga, Carlos Fito | | In progress | | | **2017** | | WP8 | ITENE |
| Size Dependence of Silver Nanoparticle Removal in a Wastewater Treatment Plant Mesocosm Measured by FAST Single Particle ICP-MS | Tuoriniemi, J.; Juergens, M.D.; Hassellöv, M.; Cornelis, G. | | Accepted | Environmental Science: Nano | | 2017 | | WP 5 | SLU, UGOT, NERC |
| In vitro biological responses to nanofibrillated cellulose by human dermal, lung and immune cells: surface chemistry aspect | Lopes R V, Sanchez-Martinez C, Strømme M, **Ferraz N** | doi: 10.1186/s1289-016-0182-0 | | Particle adn Fibre Toxicology | Vol 14 | 2017 | | WP6 | UU |

# 7 Copyright

# NanoMILE

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


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

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

# Contents



## 1 Summary

Project Duration: 48 months

Project Funding: 10 M€

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

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

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

## 2 Background

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

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

## 3 Scientific and Technical Challenges

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

Here we highlight some key shortfalls and gaps in knowledge regarding nanosafety and illustrate how the NanoMILE project will address these and ultimately provide a new paradigm in

nanosafety, thus substantially advancing the field beyond the current state of the art.

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

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

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

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

***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 to address this.*

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.*

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 biota in a specific manner where toxicity is one of the outcomes of these interactions. Others may include reduced energy reserves, reduced fitness and ultimately increased vulnerability.*

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.

***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.*

NanoMILE will evaluate distribution (biokinetics) and toxicological endpoints after exposure of cells, isolated organs

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

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

NanoMILE will seek to discover and compare mechanisms and potencies of the potential harmful effects of different MNMs using an integrated Systems Biology approach, including transcriptomics, metabolomics, lipidomics and computational biology. These consortium participants are highly experienced in the application of 'omics technologies to studying biological responses to 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. The dynamical models will also enable *in silico* simulations of the toxicity responses to MNMs, which will be tested experimentally.

***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.

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

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

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

## 4 Project Objectives

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

Specific objectives, in chronological order of development, are:

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

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

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

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

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

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

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

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

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

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

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. 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.

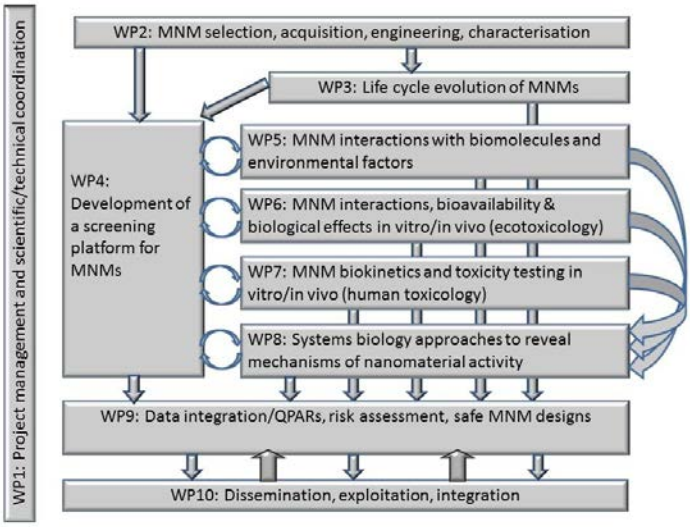


**Figure 1.** NanoMILE WP flow diagram & interdependencies

## 5 Progress and Outcomes to date

The final period of any large project is where the various strands of activity are brough together to ensure that the impact exceeds the sum of the individual parts, which is the added value and synergy expected from large-sclae collaborative EU-funded projects. NanoMILE is no exception: 6 of the 8 technical WPs have been fully operational throughout the last year of the project (WP2 continued chracterisation activities but no new MNMs were introduced during the final year), while WP3 was focused only on finalizing publications resulting from the experimental work performed in the previous periods), and all 28 partners have been actively engaged specifically in writing up the outputs and the integration activities to produce the enduring outputs such as the overall hazard framework.

All 6-monthly face to face meetings, in Edinburgh (M36), Lasko (M42) and Malaga (M48) have been exceptionally well attended with over 60 NanoMILE researchers, faciliting internal WP discussions and planning, as well as cross-WP integration and alignment, and planning for the integrated NanoMILE grouping and classification framework that forms the final project outcome. The final meeting in Malaga was also a major dissemination opportunity, as it was followed by a 3-day

conference jointly organized with NanoSOLUTIONS, GUIDEnano, SUN and eNanoMapper, attended by over 200 participants, which has also been recorded and will be available for reference and use in teacing and research well beyond the project end dates. The links to the conference presentations from NanoMILE are available here, and to the overall conference presentations are: http://www.nmsaconferencetalks.eu/.

The 10 industry partners have been fully integrated into the NanoMILE research activities, contributing actively to WP2 (PROM, N4I; particle synthesis / manufacturing), WP5 (Malvern, Attana; method development for characterization of particle-biomolecule and particle-cell interactions), WP6 (Eurofins, ecotoxicity of pristine and aged NMs), WP7 (BASF, Vitrocell; inhalation exposure, including a new Air-liquid Exposure device); WP8/WP9 (Biomax, Novamechanics; data management and QSAR development) and WP10 (EU-vRI; dissemination and exploitation). A dedicated section of the NanoMILE website is industry-facing and devoted to showcasing NanoMILE's products and services with and for industry.

Over 50 papers have been published to date, with at least another 15 submitted or in advanced stages of development. The full list of NanoMILE publications is available via the NanoMILE website and will continue to be updated over the next few months.

A summary of the final status of each WP is provided below:

**WP2 - MNM selection, acquisition, engineering, characterization** played a less central role in the final year of the project, but has continued to provide characterization information, and to under take specific characterization for individual papers or for the overall hazard assessment framework (e.g. the ECETOC dissolution study performed in the last phase of the project). WP2 also produced several MNM library synthesis papers, including the library of different cores with common shells, show schematically in Figure 2.

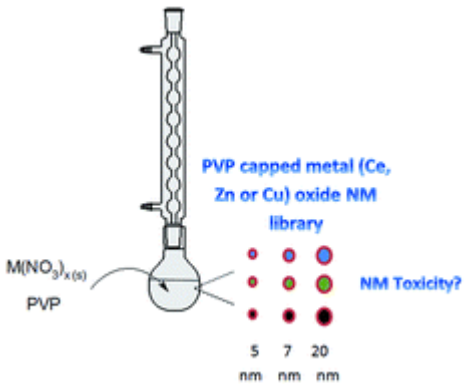


**Figure 2**. Schematic of MNMs with cores of different composition and size and PVP shells of different thickness.

**WP3 - Life cycle evolution of MNMs** - All WP3 tasks were completed as planned during by the end of period 2 and as such the focus for WP3 has been on dissemination and publications.

**WP4 - Development of a screening platform for MNMs** has carried out systematic toxicity screening of up to 100 NanoMILE MNMs in a range of cell lines and zebrafish embryos against a range of endpoints, developing novel, robust toxicity assays for HT/CS of MNMs. The cell lines represent different organs (liver, lung, colon, immune system). The assays used were based on high-throughput/-content (HT/C) techniques. End points such as cell count, cell membrane permeability, apoptotic cell death, mitochondrial membrane potential, lysosomal acidification and steatosis have been studied in cells. The zebrafish embryos were tested for hatching rate, malformations and mortality. Integrated multi-partner publications are in preparation at present. Another significant strand of activity in this WP was to understand the uptake and internalisation of MNMs including the transport of their adsorbed biomolecules from the serum, using a range of different approaches. For polystyrene (PS) MNMs, the adsorbed corona is carried with the MNMs to the lysosome and then degraded (Figure 3), whereas with $TiO_2$ MNMs the corona is retained and/or exchanged with external serum proteins depending on the coating composition, as evidenced by high resolution Stochastic Optical Reconstruction Microscopy (STORM) Imaging (article submitted).

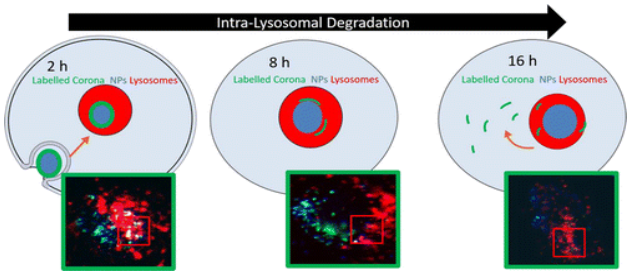


**Figure 3**. PS MNMs lose their absorbed corona (labelled green) over time in the acidic lysosomel comaprtments. *ACS Nano*, 2016, 10, 10471–10479.

**WP5 - MNM interactions with biomolecules and environmental factors** was tasked with furthering our understanding of what is presented on the surface of the NanoMILE MNMs when dispersed in relevant biological and environmental media in order to bridge from physico-chemical properties to biological effects. A significant focus has been on developing automated and higher throughout approaches to assess MNM-biomolecule interactions. One example is the development of the Attana Quartz Crystal Microbalance assay for analysis of the interactions between fixed cells and MNMs. This system has been used to investigate MNM-cell receptor interactions under in situ conditions and profiling of actual binding partners for nanoparticles in complex biological milieu. To achieve this goal, Attana with partners have optimised several parameters, including cell lines, cell seeding density, buffer conditions, biofluids used and temperature. Some of the optimisation results have been shown in the previous reports. Furthermore, hardware of the Attana system has also been carried out to tailor for MNMs interactions including use of tubes with larger diameter and modification of relevant hardware parts accordingly, in order to minimise the clogging effects occurring in the presence of MNMss. Significant effort to validate the method using using selected phase I and phase II MNMs was undertaken.

**WP6 - MNM bioavailability & biological effects in vitro/in vivo (ecotoxicology)** has tested a range of sentinel organisms to investigate the relative toxicity and organisms sensitivity to selected MNMs that are currently suspected to have biological effects (e.g. nano silver). The test organisms included a freshwater algae (Clamydomonas reinhardtii), a freshwater fish (Danio rerio), and a range of terrestrial invertebrates including Caenorhabditis elegans, earthworms (Eisenia fetida), springtail (Folsomia candida), soil mite (Hypoaspis aculeifer), and the isopod (Porcellio scaber). Several novel approaches have approaches have been developed, including a maternal transfer model, or applied, such as whole mount in-situ hybridisation (WISH) in zebrafish embryos and larvae for a suite of genes involved with detoxifying processes and oxidative stress to identify potential target tissues and effect mechanisms of AgNPs compared with a bulk counterpart and ionic silver ($AgNO_3$). AgNPs caused upregulation in the expression of mt2, gstp and gstm1 and down regulation of expression of both hmox1 and fth1 and there were both life stage and tissue-specific responses. Responding tissues included olfactory bulbs, lateral line neuromasts and ionocytes in the skin with the potential for effects on olfaction, behaviour and maintenance of ion balance. Silver ions induced similar gene responses and affected the same target tissues as AgNPs (Osbourne et al, Nanotoxicology, 2016, 10:1276-86).

**WP7 - MNM biokinetics and toxicity testing in vitro/in vivo (toxicology)** investigated selected MNMs to identify molecular mechanisms and pathways of toxicity. One hypothesis used in these studies is that the redox potential of the MNMs governs the toxicity. $CeO_2$ was chosen due to the fact that it can cycle between two redox states, $Ce^{3+}$ and $Ce^{4+}$, which endows this MNM with catalytic properties, and suggests a mechanism of activity based on oxidative stress. The use of $CeO_2$ MNMs in vehicle catalysts makes it relevant for exposure via inhalation. Doping with Zr was used to alter the redox activity by incorporating ZrO in the crystal structure of the $CeO_2$ MNMs). The differences in crystal structure and redox potential did not result in large differences in toxicity (in in vitro and in vivo studies, papers in preparation) as the toxicity of the original $CeO_2$ MNM was relatively low. For the second phase, $Fe_2O_3$ MNMs doped with different amounts of cobalt were developed which have shown some exiting in vitro and in vivo effects.

**WP8 - Systems biology approaches to reveal mechanisms of MNM activity** comprised the collection and deep analysis of "omics" Big Data associated with the biological responses of four model systems (Daphnia, Chlamydomonas, zebra fish embryos and A549 human cell line) to selected MNMs. Differential analysis, analysis of variance (ANOVA) and post hoc analysis have been perfomed to identify significantly changing genes, polar metabolites and lipids in response to the treatments. Phylogenomic mapping to the RNA-Seq data of Daphnia magna, a new model species, has demonstrated successful annotation of the genes being measured. Mass spectrometry experiments were conducted to annotate the candidate polar metabolite and lipid compounds and extensive, integrated molecular pathway over-representation analyses was undertaken to identify both the transcriptomic (regulatory) and metabolic (functional) pathways affected by the MNM exposures. Developed a MOdule Differential Analysis for weighted gene co-expression network (MODA) algorithm which was used to construct gene coexpression networks and determine the coexpression modules that are responsible for the phenotypes. Several papers are in preparation / submitted from this work.

**WP9 - Data integration/QPARs, risk assessment, safe MNM designs** has focussed on the development of *in silico* predictive models for nanosafety, including predictive models to quantitatively define the correlation of the cell association of a set of gold NPs with their physicochemical properties and available data on protein corona fingerprints. KNIME (Konstanz Information Miner) platform, a freely available and open source tool that is increasingly used for solving chemoinformatics problems (www.knime.com), was used to implement all steps required for model development and validation.

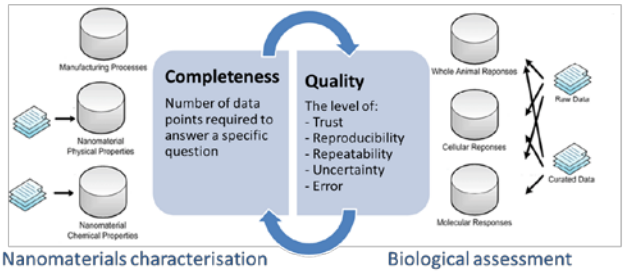


**Figure 4**: Schematic illustration of the concepts of data completeness and quality, developed in collaboration with an international consortium as part of the NDCI nanoinformatics series of papers. Marchese Robinson et al., Nanoscale. 2016, 8:9919-43.

**Dissemination activities (WP10)**

NanoMILE partners have been active in terms of disseminating their activities within NanoMILE from the outset of the project, with period 3 being especially active. The last year's dissemination activities included 29 papers and over 40 conference presentations. Table 2 shows the overall dissemination summary (executed and planned) for the NanoMILE project to the end of the project (28th February 2017). Note that dissemination and explitation will continue long after the forma end of the project.

| | Scientific community | Industry | Civil society | Policy makers | Media | **Total** |
|---|---|---|---|---|---|---|
| Done | 165 | 104 | 18 | 56 | 7 | **350** |
| Planned | 5 | 5 | 0 | 1 | 0 | **11** |
| **Total** | **170** | **109** | **18** | **57** | **7** | **361** |

**Table 2:** NanoMILE number of participations to events and audiences reached (by end February 2017).

The NanoMILE partners continued to Chair several NanoSafety cluster (NSC) working groups (WGs) including Hazard (WG2), Standardisaiton Sub-group (WG7), the new Safety-by-design (WG9), and represent WG7 (Dissemination) on the Steering

Committee. Additionally, in February 2017, UoB were voted as the overall coordinator of the NSC for the next 2 years, with partner RIVM as a member of the coordination team.

## NanoMILE Cooperation with other projects/programmes

### NanoSolutions (FP7)

NanoSolutions is our sister project, addressing the same call topic as NanoMILE, meaning there are losts of potential synergies. A joint meeting was organised at the UoB office in Brussels in July 2015, and work is underway between the two projects to draft a joint paper on the selecton procecess the projects unferwent to determine which NMs, which model systesms and which methods/assays to utilise, which will form an important part of the scientific record at the present time in nanosafety.

Additionally, the two projects co-arganised their final meetings (together with GuideNano and SUN) in February 2017 in Malaga, providing an important opportnty for data and knowledge sharing and consensus building.

### NanoFASE (Horizon 2020)

Given NanoMILE's focus on hazard and NanoFASE's focus on exposure and fate, there is a natural complementarity between the projects, which is being harnessed through common MNMs where possible (e.g. from Prometian particles, a partner in both projects), and through the extension of the NanoMILE KnowledgeBase to cover NanoFASE particles and datasets in additioan to NanoMILE's datasets. This will support integration across data for complete risk assessment in due course. NanoMILE and NanoFASE are organsing a joint workshop / Training School for Autumn 2016.

### SHYMAN (FP7)

There have been crossovers between NanoMILE and another FP7 project which PROM is involved in - SHYMAN (Sustainable Hydrothermal Manufacture of Nanomaterials, Grant Agreement Number 280983). As part of this project, selected materials which PROM currently manufactures will be destined for scale up to be produced at industrial quantities – up to 1000 tonnes per annum. It is in the interest of the NanoMILE project to study nanomaterials which are industrially relevant, while it is in the interest of the SHYMAN project to consider the toxicological impact of these engineered MNMs on living systems and the environment (especially when considering accidental release or disposal during the production phase). Therefore, PROM has been acting to feed information and samples between the two projects which has resulted in toxicology data, obtained by the JRC within NanoMILE, being used in a Deliverable Report within SHYMAN (Deliverable 6.4: Identify potentially high environmental and economic impacts of the hydrothermal synthesis process to be submitted by Czech Technical University). The JRC and the NanoMILE project have been fully credited for their work.

### eNanoMapper (FP7)

NanoMILE is working with eNanoMapper in temrs of developing the ontology for MNMs ageing, MNMs coatings etc., as well as supporting the development of the database and its sustainability. NanoMILE gave a stimulus presentiaton at the eNanoMapper databases workshop in Brussles in January 2016, and hosted a joint meeting with eNanoMapper team members as part of the M36 meeting in Edinburgh. UoB and eNanoMapper partner have jointly developed a starting communities bid for a research infrastructure (submitted April 2016) as well as for an e-infrastructure (1 stage proposal, submiited April 2016).

### NanoDefine (FP7)

NanoMILE is actively contributing to NanoDEefine's project networking activities, including presenting the project's activities and potential contributions to community activities at the NanoDefine meeting in February 2016. NanoMILE completed the templates and other reporting requested, and will continue to enagage as new initiatives emerge.

### Nanosafety Cluster

NanoMILE are playing a leadership role in the NanoSafety Cluster (NSC), as one of the larger projects running at present. To this end, we continue to contribute to NSC activities, such as editing the NSC compendium for 2014 (Iseult Lynch), the contributing to the organisation of the 2nd NSC young-researchers meeting in Visby (Profs. Valsami-Jones and Lynch are on the organising committee), as well as providing leadership of specific WGs: Flemming Cassee leading WP2, Benoit Hazebrouck leading WG7 sub-gorup on Standardiation, Eva Valsami-Jones leading the new Safety-by-Design Wg, and Isuelt Lynch representing Dissemination on the NSC Steering Committee.

### EU-US Communities of Research

NanoMILE have also been active in the EU-US CORs, with two NanoMILE parnters presenting at the 2013 CORs workshop in the US (Denise Mitrano (EMPA) and Francesco Falcini (University of Birmingham / University of Liverpool). NanoMILE partners are actively involved in the regulatory, databases & curation and exposure CORs, and will be involved in the methods and characteriation one in due course.

### Nanotechnology Data Curation Initiative

NanoMILE have signed-up to act as a Stakeholder Liaison for the EU-US CoR Database & Curation / NCIP Nanotechnology Working Group "Nanotechnology Data Curation Initiative". As part of this, NanoMILE will provide input and responses to six themed questionnaires over the next 18 months, which will (along with the inputs from the other liaisons) collectively form the "landscape" of nanomaterial data curation. This also helps to ensure that NanoMILE has international visibility and that the approacjes being pioneered within the NanoMILE knowledge base are linked to international efforts in this arena. The first co-authored paper has just been accepted in NanoScale.

## 6 NanoMILE's Impact beyond project lifetime

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

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

NanoMILE will contribute significantly to the efforts to reduce the many uncertainties about the potential impact of MNMs on health and the environment, which is urgently needed for the development of a sound regulatory framework. It is crucial to learn what the parameters are that govern the toxicity of nano-sized objects and what the underlying mechanisms are for the sustainable development of MNMs. It is also important to note that regulatory uncertainly leading to delays in commercialisation is more costly to business than clear additional regulatory requirements. 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.

The NanoMILE consortium have already identified a number of key outputs that will have significant impact for the various stakeholders involved in the nanosafety and nano-commercialisation question, including numerous potential candidates for standardization, as follows:

- Descriptors for grouping / classification of MNMs (including aged MNMs)
- Algorithms and predictive models (& the associated Standard Operating Procedures, SOPs)
- New High-throughput (HT) assays for screening MNM impacts (cell-based, cell free) (& the associated SOPs), including 2 industry-led demonstration models
- Data management tools to link physico-bio-impact data from point of generation to mining ability (& the associated SOPs)
- 'Omics' datasets for the 4 test species in response to systematic sets of MNMs
- Design rules for tailoring MNM impacts – novel MNMs as Reference Materials etc.
- Data on controlled human and organism exposure & comparison to models of increasing complexity (& the associated SOPs)
- Nanoparticle libraries (including protocols for synthesis, functionalization and purification) and safety dossiers for SME partners on their MNMs for use in business to business marketing.

Based on our selction criteria (existence of a need, e.g. for industry and regulators; technical readiness; organisational readiness) 3 work items are being progressed towards Standardisation: Stable-isotope labelling of MNMs, Isopods as a model for bioaccumulation of MNMs, and Air Liquid Exposure Systems as a replacement for animal testing.

The overall NanoMILE hazard assessment framework, which guides users through the various test approaches, discusses their use and limitations, and links to the relevant *in silico* predictive models, offers a first step towards a universal framework for MNMs hazard assessment.

The strong links with ongoing Horizon 2020 projects NanoFASE, which will provide the exposure and environmental fate information to complement NanoMILE's hazard data, NanoGenTools which is developing safety by design approaches, and ACEnano (coordinated by UoB) which is developing a toolbox of MNMs characterisation approaches and a decision tree to support industry and regulators in their use, ensure that the NanoMILE approaches, knowledge and datasets are being built-upon and continue to be utilised beyond the lifetime of the project.

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# 7 Directory

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

| First Name | Last Name | Affiliation | Address | e-mail |
|---|---|---|---|---|
| Antreas | Afantitis | NovaMechanics Ltd. | John Kennedy Avenue 62-64<br>Lefkosia 1046, Cyprus | afantitis@novamechanics.com |
| Teodor | Aastrup | Attana AB | Grev Tureg 14 3 1/2 TR<br>Stockholm 114 46, Sweden | teodor.aastrup@attana.com |
| Paul | Borm | Nano4Imaging GMBH | Pauwelsstrase 17,<br>Aachen 52024, Germany | pbo@nano4imaging.com |
| Marie | Carriere | Commissariat a l'Energie Atomique et aux Energies Alternatives | 15 rue des Martyrs, GRENOBLE 38054, France | marie.carriere@cea.fr |
| Flemming | Cassee | Rijiksinstituut voor Volksgezondheid en Milieu | RIVM, PO Box 1, 3720 BA Bilthoven<br>The Netherlands | Flemming.cassee@rivm.nl |
| Kenneth | Dawson | University College Dublin | Belfield, IE-4 Dublin, Ireland | kenneth.a.dawson@cbni.ucd.ie |
| Damjana | Drobne | University of Ljubljana | Department of biology, Biotechnical faculty, , Vecna pot 111 , Ljubljana | damjana.drobne@bf.uni-lj.si |

| First Name | Last Name | Affiliation | Address | e-mail |
|---|---|---|---|---|
| Peter | Gooden | PROMETHEAN PARTICLES LTD | University Boulevard, Nottingham Science Park, Faraday Building 6 Nottingham NG7 2QP, UK | Pete.Gooden@proparticles.co.uk |
| Patrick | Hole | Nanosight Ltd. | London Road Minton Park, Wiltshire SP4 7RT, United Kingdom | Patrick.hole@nanosight.com |
| Tobias | Krebs | Vitrocell Systems GmbH | Fabrik Sonntag 3, Waldkirch 79183, Germany | t.krebs@vitrocell.com |
| Iseult | Lynch | University of Birmingham | School of Geography, Earth and Environmental Sciences, Edgbaston,Birmingham B15 2TT, UK | i.lynch@bham.ac.uk |
| Dieter | Maier | BIOMAX INFORMATICS AG | Robert Koch Strasse 2 Planegg 82152, Germany | dieter.maier@biomax.com |
| Jutta | Muether-Paul | Eurofins Agroscience Services GMBH | Eutinger Strasse 24 Niedern Oschelbronn 75223, Germany | JuttaMuetherPaul@eurofins.com |
| Mark | Miller | University of Edinburgh | Centre for Cardiovascular Sciences Little France Crescent 47, Edinburgh, EH16 4TJ, UK | mark.miller@ed.ac.uk |
| Andre | Nel | The Regents of the Uni9versity of California | UCLA Medicine, 10833 Le Conte Ave 52-175 CHS, Los Angeles 90095-1736, USA | anel@mednet.ucla.edu |
| Bernd | Nowack | Eidgenössische Materialprüfungs- und Forschungsanstalt | Technology and Society Laboratory, Lerchenfeldstrasse 5, St. Gallen CH-9014, Switzerland | nowack@empa.ch |
| Francois | Rossi | Joint Research Centre of the European Commission | Institute for Health and Consumer Protection – Unit Nanobiosciences Via E. Fermi 2749, Ispra 21027, Italy | francois.rossi@jrc.ec.europa.eu |
| Joachim | Rädler | Ludwig-Maximilians Universität München | Geschwister-Scholl-Platz 1 Munich 80539, Germany | joachim.raedler@lmu.de |
| Olivier | Salvi | European Virtual Institute for Integrated Risk Management | 19 Haus der WirtschaftWilli-Bleicher-Strasse, Stuttgart 70174, Germany | salvi@eu-vri.eu |
| Kristin | Schirmer | Eidgenössische Anstalt für Wasserversorgung, Abwasserreinigung und Gewässerschutz | Environmental Toxicology Ueberlandstrasse 133 Duebendorf 8600, Switzerland | kristin.schirmer@eawag.ch |
| Roel | Schins | Institut fur Umweltmedizinische Forschung an der Heinrich-Heine-Universitat Dusseldorf GMBH | Auf M Hennekamp 50, Dusseldorf 40225, Germany | roel.schins@uni-duesseldorf.de |
| Serge | Stoll | University of Geneva | Institute Forel, Physical Chemistry Group, route de Suisse, CP 416 10 Versoix 1290, Switzerland | serge.stoll@unige.ch |
| Gert | Storm | University of Utrecht | Universiteitsweg 99 Utrecht 3584 CG, Netherlands | g.storm@uu.nl |
| Charles | Tyler | University of Exeter | Hatterley Laboratories, Prince of Wales Road Exeter EX4 4PS, United Kingdom | euresearch@exeter.ac.uk |
| Eugenia | Valsami-Jones | University of Birmingham | School of Geography, Earth and Environmental Sciences, Edgbaston,Birmingham B15 2TT, UK | e.valsamijones@bham.ac.uk |
| Håkan | Wallin | National Research Centre for the Working Environment | Lerso Parkalle 105, Copenhagen 2100, Denmark | hwa@nrcwe.dk |
| Carsten | Weiss | Karlsruher Institut fuer Technologie | Institute of Toxicology and Genetics, Hermann-von-Helmholtz-Platz 1, Eggenstein-Leopoldshafen 76344, | carsten.weiss@kit.edu |
| Karin | Wiench | BASF SE | Carl Bosch Strasse 38, Town 15 Ludwigshafen 67056, Germany | karin.wiench@basf.com |
| Mark | Wiesner | Duke University | Department of Civil and Environmental Engineering, 324 Blackwell Street, Suite 920, Durham 27701, USA | wiesner@duke.edu |

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# NANoREG

## a common European approach to the regulatory testing of nanomaterials

A common European approach to the regulatory testing of nanomaterials

Contract Agreement: NMP-LA-2013-310584 Website: www.nanoreg.eu
Coordinator: Tom van Teunenbroek, Dutch Ministry of Infrastructure and the Environment

*Table 1 Consortium List.*

| No. | Beneficiary name | Short name | Country |
|---|---|---|---|
| 1. | MINISTERIE VAN INFRASTRUCTUUR EN MILIEU | Min I&M | Netherlands |
| 2. | JRC -JOINT RESEARCH CENTRE- EUROPEAN COMMISSION | JRC | Belgium |
| 3. | BUNDESANSTALT FUER ARBEITSSCHUTZ UND ARBEITSMEDIZIN | BAuA | Germany |
| 4. | DET NATIONALE FORSKNINGSCENTER FORARBEJDSMILJO | NRCWE | Denmark |
| 5. | RIJKSINSTITUUT VOOR VOLKSGEZONDHEIDEN MILIEU | RIVM | Netherlands |
| 6. | BUNDESINSTITUT FUER RISIKOBEWERTUNG | BFR | Germany |
| 7. | CENTRE NATIONAL DE LA RECHERCHE SCIENTIFIQUE CNRS | CNRS CEREGE | France |
| 8. | AIT AUSTRIAN INSTITUTE OF TECHNOLOGY GMBH | AIT | Austria |
| 9. | INSTITUTE OF OCCUPATIONAL MEDICINE | IOM | United Kingdom |
| 10. | TEMAS AG TECHNOLOGY AND MANAGEMENT | TEMAS | Switzerland |
| 11. | FUNDACION GAIKER | GAIKER | Spain |
| 12. | NANOTECHNOLOGY INDUSTRIES ASSOCIATION | NIA | Belgium |
| 13. | BASF SE | BASF | Germany |
| 14. | THE PROVOST, FELLOWS, FOUNDATION SCHOLARS & THE OTHER MEMBERS OF BOARD OF THE COLLEGE OF THE HOLY & UNDIVIDED TRINITY OF QUEEN ELIZABETH NEAR DUBLIN | TCD | Ireland |
| 15. | KAROLINSKA INSTITUTET | KI | Sweden |
| 16. | NORSK INSTITUTT FOR LUFTFORSKNING | NILU | Norway |
| 17. | ISTITUTO SUPERIORE DI SANITA | ISS | Italy |
| | *3RD PARTY FOR PARTNER 17: ISTITUTO NAZIONALE ASSICURAZIONE INFORTUNI SUL LAVORO* | *INAIL* | *Italy* |
| 18. | AGENZIA NAZIONALE PER LE NUOVE TECNOLOGIE, L'ENERGIA E LO SVILUPPO ECONOMICO SOSTENIBILE | ENEA | Italy |
| 19. | STATENSARBEIDSMILJOINSTITUTT | STAMI | Norway |
| 20. | ACONDICIONAMIENTO TARRASENSE ASSOCIACION | LEITAT | Spain |
| 21. | INSTITUT NATIONAL DE RECHERCHE ET DE SECURITE | INRS | France |
| 22. | UNIVERSITE DE NAMUR ASBL | UNamur | Belgium |
| 23. | COMMISSARIAT A L ENERGIE ATOMIQUE ET AUX ENERGIES ALTERNATIVES | CEA | France |
| 24. | *GEOCHEM RESEARCH BV* | *GeoChem* | *Netherlands* |
| 25. | DEPARTMENT OF HEALTH | DH-PHE | United Kingdom |
| 26. | CENTRUM VOOR ONDERZOEK IN DIERGENEESKUNDE EN AGROCHEMIE - CODA | CODA-CERVA | Belgium |
| 27. | UNIVERSITAT AUTONOMA DE BARCELONA | UAB | Spain |
| 28. | FONDAZIONE ISTITUTO ITALIANO DI TECNOLOGIA | IIT | Italy |
| | *3RD PARTY FOR PARTNER 28: UNIVERSITA DEGLI STUDI DI ROMA TORVERGATA* | | Italy |
| 29. | ASOCIACION DE INVESTIGACION DE LAS INDUSTRIAS DE LA CONSTRUCCION | AIDICO | Spain |
| 30. | STICHTING WAGENINGEN RESEARCH | DLO-RIKILT | Netherlands |
| 31. | CONSIGLIO NAZIONALE DELLE RICERCHE | CNR | Italy |
| | *3RD PARTY FOR PARTNER 31: UNIVERSITA DEGLI STUDI DI TORINO* | | *Italy* |
| 32. | ASSOCIATION SAINT YVES | UCO | France |

| | | | |
|---|---|---|---|
| 33. | NEDERLANDSE ORGANISATIE VOOR TOEGEPAST NATUURWETENSCHAPPELIJK ONDERZOEK TNO | TNO | Netherlands |
| 34. | INSTITUT NATIONAL DE L ENVIRONNEMENT ET DES RISQUES INERIS | INERIS | France |
| 35. | AGENCE NATIONALE DE LA SECURITE SANITAIRE DE L ALIMENTATION DE L ENVIRONNEMENT ET DU TRAVAIL | ANSES | France |
| 36. | BIONANONET FORSCHUNGSGESELLSCHAFT MBH | BioNanoNet | Austria |
| 37. | LUNDS UNIVERSITET | LTH | Sweden |
| 38. | GENOK - SENTER FOR BIOSIKKERHET | GenØk | Norway |
| 39. | UNIVERSITETET I BERGEN | UIB | Norway |
| 40. | EIDGENOESSISCHES DEPARTEMENT DES INNERN | FOPH | Switzerland |
| 41. | VENETO NANOTECH SOCIETA CONSORTILE PER AZIONI | VN | Italy |
| 42. | INSTITUTO TECNOLOGICO DEL EMBALAJE, TRANSPORTE Y LOGISTICA | ITENE | Spain |
| 43. | UNIVERSIDAD DE LLEIDA | UdL | Spain |
| 44. | STIFTELSEN SINTEF | SINTEF | Norway |
| 45. | UNIVERSITAET LEIPZIG | ULEI | Germany |
| 46. | NPL MANAGEMENT LIMITED | NPL | United Kingdom |
| 47. | LABORATOIRE NATIONAL DE METROLOGIE ET D'ESSAIS | LNE | France |
| 48. | LABORATORIO IBERICO INTERNACIONAL DE NANOTECNOLOGIA | INL | Portugal |
| 49. | TYOETERVEYSLAITOS | FIOH | Finland |
| 50. | NORGES MILJO-OG BIOVITENSKAPLIGE UNIVERSITET | UMB | Norway |
| 51. | FUNDACION TEKNIKER | TEKNIKER | Spain |
| 52. | CHALMERS TEKNISKA HOEGSKOLA AB | Chalmers | Sweden |
| 53. | INSTITUTO DE SOLDADURA E QUALIDADE | ISQ | Portugal |
| | *3RD PARTY FOR PARTNER 53: INSTITUTO NACIONAL DE SAUDE DR. RICARDO JORGE* | | *Portugal* |
| | *3RD PARTY FOR PARTNER 53: INSTITUTO PORTUGUES DA QUALIDADE I.P.* | | *Portugal* |
| | *3RD PARTY FOR PARTNER 53: MINISTERIO DA SAUDE - REPUBLICA PORTUGUESA* | | *Portugal* |
| 54. | TURVALLISUUS JA KEMIKAALIVIRASTO | TUKES | Finland |
| 55. | BAYER MATERIALSCIENCE AG | BMS | Germany |
| 56. | ARKEMA FRANCE | ARKEMA | France |
| 57. | STORA ENSO OYJ | Stora Enso | Finland |
| 58. | UPM-KYMMENE OYJ | UPM | Finland |
| 59. | SP SVERIGES TEKNISKA FORSKNINGSINSTITUT AB | SP | Sweden |
| 60. | UNIVERSITY OF LEEDS | UnivLeeds | United Kingdom |
| 61. | ENVICAT CONSULTING SPRL | Envicat | Belgium |
| 62. | COMET BIOTECH AS | CBT | Norway |
| 63. | INSTITUT PASTEUR DE LILLE FONDATION | IPL | France |
| 64. | HERIOT-WATT UNIVERSITY | HWU | United Kingdom |
| 65. | HEALTH PROTECTION AGENCY HPA (= PARTNER 25) | HPA | United Kingdom |
| 66. | USTAV EXPERIMENTALNI MEDICINY AKADEMIE VED CESKE REPUBLIKY VEREJNA VYZKUMNA INSTITUCE | IEM | Czech Republic |
| 67. | VYSOKA SKOLA BANSKA - TECHNICKA UNIVERZITA OSTRAVA | VSB | Czech Republic |
| 68. | INSTITUTE OF PUBLIC HEALTH OSTRAVA | ZUOVA | Czech Republic |
| 69. | FOUNDATION FOR RESEARCH AND TECHNOLOGY HELLAS | FORTH | Greece |
| 70. | NATIONAL CENTER FOR SCIENTIFIC RESEARCH "DEMOKRITOS" | NCSRD | Greece |
| 71. | ECAMRICERT SRL | ER | Italy |

**COLLABORATION AGREEMENTS WITH BRAZIL AND REPUBLIC OF KOREA:**

| | | |
|---|---|---|
| CENTRO DE TECNOLOGIAS ESTRATEGICAS DO NORDESTE | CETENE | BRAZIL |
| EMPRESA BRASILEIRA DE PESQUISA AGROPECUARIA | EMBRAPA | BRAZIL |
| INSTITUTO NACIONAL DE METROLOGIA, QUALIDADE E TECNOLOGIA | INMETRO | BRAZIL |
| UNIVERSIDADE FEDERAL DO RIO GRANDE DO SUL | UFRGS | BRAZIL |
| UNIVERSIDADE DE SÃO PAULO | Gnano IFSC | BRAZIL |
| DEPARTMENT: SISNANO-USP | USP | |
| UNIVERSIDADE FEDERAL DO RIO GRANDE ; | FURG | BRAZIL |
| DEPARTMENT: INSTITUTO DE CIENCIAS BIOLOGICAS (ICB) | | |
| UNIVERSIDADE FEDERAL DE MINAS GERAIS | UFMG | BRAZIL |
| DEPARTMENT: INSTITUTO DE CIENCIAS BIOLOGICAS (ICB) | | |
| UNICAMP – UNIVERSIDADE ESTADUAL DE CAMPINAS; | NanoBioss | BRAZIL |

| | | |
|---|---|---|
| DEPARTMENTO DE QUIMICA INORGANICA NANOBIOSS/INSTITUTO DE QUIMICA MINISTERIO DE CIENCIA, TECNOLOGIA E INOVAÇÃO | MCTI | BRAZIL |
| HANYANG UNIVERSITY | HYU | REPUBLIC OF KOREA |
| KOREA RESEARCH INSTITUTE OF STANDARDS AND SCIENCE | KRISS | REPUBLIC OF KOREA |
| SUNGKYUNKWAN UNIVERSITY | SKKU | REPUBLIC OF KOREA |
| HOSEO UNIVERSITY; INSTITUTE OF NANOPRODUCT SAFETY RESEARCH, | HSU | REPUBLIC OF KOREA |
| KWANGWOON UNIVERSITY | KWU | REPUBLIC OF KOREA |
| UNIVERSITY OF SEOUL | UOS | REPUBLIC OF KOREA |
| DONGDUK WOMEN'S UNIVERSITY | DWU | REPUBLIC OF KOREA |
| MINISTRY OF ENVIRONMENT/ NATIONAL INSTITUTE OF ENVIRONMENTAL RESEARCH | MOE/NIER | REPUBLIC OF KOREA |
| MINISTRY OF FOOD AND DRUG SAFETY | MFDS | REPUBLIC OF KOREA |

Note: beneficiaries listed in grey font, left the Consortium before the end of the project

# Contents



## 1 Summary

In NANoREG over 85 institutional partners from EU member states, associated states, the Republic of Korea and Brazil collaborated in developing reliable, reproducible and relevant methods for testing and assessing the effects of nanomaterials on human health and environment in a regulatory context.

The duration of the project was 48 months; the end date was 28 February 2017.

The budget was approximately fifty million euro. Ten million was provided by the EU FP7 programme and forty million by member states, regions, partners and other sources.

## 2 Background

Nanotechnology is one of the six "Key Enabling Technologies" (KETs), the European Commission identified in its 2012 Communication on this topic. These technologies enable the development of new goods and services and the restructuring of industrial processes needed to modernize EU industry. They are of paramount importance for the transition to a knowledge-based and low carbon resource-efficient economy. KETs are regarded as crucial for ensuring the competitiveness of European industries in the knowledge economy.

A serious threat to the capitalization of the innovative and economic potential of Nanotechnology is the limited understanding of the environmental, health and safety (EHS) aspects of nanomaterials (NMs); often labelled nanoEHS. This limited understanding leads to uncertainty on how to judge the EHS aspects of these materials in a regulatory context. This has a negative impact on the investment climate and on societal appreciation of products containing NMs.

## 3 Objectives

The NANoREG project aims to eliminate part of these uncertainties by:

1. Identifying what nanoEHS aspects are relevant from a regulatory point of view ("questions and needs of regulators").
2. Identifying what the gaps in our knowledge are: what aspects are sufficiently covered by existing knowledge; what aspects need further research.
3. Carrying out the research to fill in the gaps.
4. Developing a NANoREG framework and toolbox for testing the EHS aspects and for the assessment and management of the risks. This includes proposed forward looking strategies such as safe-by-design to prioritize those nanomaterial applications that may lead to high exposure or high toxic potential and ultimately high risks for human health.
1. Creating support for the results of the project in order to contribute to a quick and broad implementation of the results.

## 4 Major Outcomes from project

For access to the deliverables mentioned in this chapter, please check the NANoREG Results Repository.

### *4.1 General overview*

The R&D results of the project are impressive in terms of quantity and quality. Partners produced a large set of well-defined experimental nanoEHS data. This dataset is of great value in- and outside the project since the data on exposure and effects are linked to accurate physical-chemical data. These are required for meaningful QSAR approaches and in silico correlational studies regarding the toxicological mode of action (MoA) of nanomaterials.

Numerous Standard Operation Protocols (SOPs) have been developed and tested regarding their reliability, reproducibility and relevance. Their status varies from "proof of concept" to "validated" by inter laboratory comparison. New insights have been developed regarding the importance of a standardized way of preparing dispersions, the need to characterize test media before and during experiments, the applicability of *in vitro* tests, the use of high throughput screening (HTS) and the importance of harmonized data reporting formats (ISA-TAB based), just to name a few. The results and knowledge acquired has been condensed in overarching deliverables such as the NANoREG Framework and accompanying NANoREG Toolbox providing, among others, a risk assessment (RA) scheme that supports a more cost-efficient RA of nanomaterials. The results also have been translated into Answers on the Regulatory Questions that formed the demand side of the project.

Also "soft results" have been achieved. The NANoREG Consortium has proven that it is feasible to come to a concerted action regarding the materials to be tested, test methods and cell lines to be applied, quality checks, etc. Such concerted action is an absolute must for generating meaningful data. The project also has proven that the basic willingness of partners to collaborate can be used to come to an agreement (possibly for the first time) to make data and deliverables publically available. This makes it possible for other projects to build on the results of NANoREG. In this context, it can be noted that NanoReg² and caLIBRAte will further elaborate on the data generated in the project. It would be a major step forward if other nanosafety projects would copy the example NANoREG has set, with respect to opening up the results. All NANoREG results are available in the NANoREG Results Repository.

Those results are important building blocks for "the White Paper process" of the H2020 project ProSafe. This process aims at developing a White Paper with recommendations for regulators and innovators regarding cost efficient RA of nanomaterials now and in the future. In this context, a ProSafe Task Force of senior experts evaluated a great number of nanosafety projects, including NANoREG, concerning the regulatory relevance of their results and generated data sets. The results of this evaluation are condensed in the "the Joint Document". A draft of this document was discussed during a scientific conference that was organized by ProSafe together with the OECD from 29 November - 1 December 2016. This conference also was the final meeting of the NANoREG project. The final Joint Document together with the NANoREG Framework and Toolbox form the basis for the White Paper.

### *4.2 Basic conditions for the R&D work (WP 1&2):*

At the start of the project, an inventory was carried out to identify the main questions and needs of regulatory relevance that should be addressed by NANoREG. The result is a list of 16 questions that formed the "demand side" of the NANoREG project (D1.01). The list was used during the project to keep focus on "doing what is needed". In the final stage of the project, most of the questions were (partly) answered on the basis of the results of the scientific work packages (D1.09). Further, a Gap Analysis of the knowledge needed in the area of regulatory toxicology and RA was made (D1.02).

To be able to combine and compare experimental data, a set of core nanomaterials was selected that all partners had to use. A web ordering system (NIWO) was set up. It provided partners with the core nanomaterials from known selected suppliers (D2.01).

For all NANoREG materials, a state-of-the-art physico-chemical characterization was done with the aim to cover as many of the key endpoints of the OECD WPMN sponsorship program as technically and practically possible (D2.02). Dispersion SOPs and minimum requirements for characterization for toxicological studies were established and laid down in a Guidance Document, thus supporting reliability and comparability of data. It was mandatory for the partners to perform their experiments in accordance with the Guidance Document. During the course of the project, those fundamental requirements were further refined.

A policy for NANoREG data management was established comprising a standardized way of data logging, mandatory uploading of data to the NANoREG data platform and opening up the data at the end of the project. This dataset is now available to- and exploitable by the nanosafety community.

## 4.3 *NANoREG R&D work*

**Synthesis, supplying and characterization (WP2)**

To support the implementation of the EC recommended definition of a nanomaterial, a SOP for measuring size distribution of nanomaterials by means of Transmission Electron Microscopy (TEM) was established (D2.10). The procedure for gas-adsorption BET analysis and the t-plot method for data analysis were improved to enable discrimination between external surface and porosity in NMs to feed into an alternative number-based VSSA approach developed to assess whether a powder was to be considered a NM or not. Identification of NMs by BET or VSSA may be applied for monitoring purposes, but the use of VSSA may be of limited use for identification of NM in a regulatory context (D2.11).

To identify and quantify selected types of inorganic- and organic chemical surface modifications, including surface functionalization and coatings, a Technical Guideline with several analytical SOPs was developed. The Guideline also can be used to screen NMs for the presence of associated impurities (D2.04).

To address the need for a practical system to categorize NMs, a scheme for an advanced categorization was developed. The scheme takes into account REACH naming and identification guidance to fit the existing European chemicals regulation (D2.05).

Ten OECD Test Guidelines were evaluated regarding the applicability for NMs. It resulted in several proposals for modifications of existing Guidelines or proposals for new Test Guidelines (D2.03/2.09), which have been proposed as a starting point for revision of relevant OECD TGs as part of a new project within the OECD WPMN.

A set of protocols for dispersion of NMs for aquatic eco-toxicological testing was developed or approved followed by extensive documentation and validation through interlaboratory comparison (D2.06). To better interpret and compare the results of *in vitro* tests, procedures for accurate quantification of NM exposure concentrations and characteristics, including NM reactivity and dissolution (fate) in ecotoxicity and *in vitro* exposure media were also developed and demonstrated (D2.08). All these methods form a solid basis for the regulatory (eco)-toxicological testing. One of the methods for assessment of MNM reactivity and dissolution during *in vitro* testing is under development as a CEN technical specification as part of a CEN/TC352 project.

Based on a literature review and experimental work conducted on different aerosol generation devices and NMs, a strategy was defined to characterize test aerosols. The strategy contributes to a better comparability and interpretation of inhalation toxicology results and can be applied for future inhalation toxicology studies (D2.07).

The deliverable "Framework and procedures for characterization of NM for regulatory needs" integrates results of several WP2 deliverables. It comes forward with recommendations for the further harmonization and improvement of the materials identification and registration schemes and guidance in REACH (D2.12).

**Exposure through life cycle analysis (WP3)**

To identify the most critical exposure scenarios during the life cycle of a product (in terms of potential exposure and economic importance) a model was developed taking into account factors such as production volume, main applications and information on activities with NMs along the life cycle. Exposure scenarios have been rated and ranked (D3.01).

A testing strategy based on mesocosms was developed and applied to better mimic the effects and impact of exposure of ecosystems to nanomaterials at different stages of their life cycle (D3.05).

To quantify the dustiness of NMs, three test methods were evaluated and applied to CNTs. For two of these methods, the work performed within NANoREG contributed to the development of draft standards in the framework of the CEN / TC 137 (assessment of workplace exposure to chemical and biological agents) (D3.02). These drafts now circulate for comments.

Standardized methods were developed and tested to quantify the release of core NMs, for selected processes during their lifecycle (D.3.03). Several improved measurement instruments, tools and methods were developed to make the link between release and exposure (workers, consumers and environment) (D3.06). Some methods were applied during a campaign of field measurements aimed at filling in a part of the gap in exposure data needed for modelling exposure and for further assessing the risks associated with NMs (D3.07).

A series of comprehensively-monitored nanoparticle dispersion experiment were undertaken inside a large climate-controlled chamber (D3.04). The resulting data can be used to test quantitative aerosol dispersion models and enable an assessment of the accuracy and uncertainty of model-predicted concentrations. Such aerosol dispersion models form the foundation for human exposure assessments to MNM.

To cover the knowledge gap on the effectiveness of currently available Risk Management Measures (RMMs) during NMs production and handling processes, a reliable methodology to obtain quantitative data on the effectiveness of personal protective equipment (PPE) and engineering controls (ECs) was provided and validated (D3.09).

Four Control Banding Tools models have been evaluated with respect to their applicability domain, assumptions made, inputs required and outputs as well as performance. For five different tools an inter-user study has been performed. The I-Nano tool developed under the umbrella of NANoREG is described and the demonstrated (D3.08).

**Biokinetics and toxicity testing in vivo (WP4)**

A long term (two year) inhalation study with female rats was performed to identify effects of two well characterized granular nanomaterials to determine concentration-response relationships and to verify/falsify the assumed mode of threshold-like action for carcinogenicity. The further aim was to investigate lung carcinogenicity and putative systemic effects of low-dose exposures to biopersistent nanoparticles. After 12 months of inhalation exposure, $CeO_2$ exposure-related histopathological findings were exclusively observed in the respiratory tract but not systemically. Adverse effects in the lung included alveolar/interstitial inflammatory cell infiltration, granulomatous inflammation and interstitial fibrosis. Although statistically not significant, some adverse effects were already observed in the 0.1

mg/m³ low-dose $CeO_2$ exposure group. After 12 months of inhalation exposure neither neoplastic nor pre-neoplastic treatment-related findings were seen in the lungs of $CeO_2$-exposed animals. No macroscopically visible tumours were found after 24 and 30 months.

Also in concentrations below overload, the $CeO_2$ the lung burden increased in a linear manner with a factor of ~5-7 including the accumulation over exposure time. The $CeO_2$ burden of liver, kidney, spleen, brain, heart, lymph nodes, bone and olfactory bulb was generally very low. In brain, maximum $CeO_2$ levels were 0.005 µg/g tissue, which is a factor of 700.000 below the lung burden. There was no evidence for systemic toxicity in the interim section after 12 months including the lung-associated lymph nodes although the cerium levels were relatively high in this tissue (D4.01-D4.07).

Ten commercial short, non-rigid, high aspect ratio nanomaterials (HARN, average length < 5 µm) have been tested after deposition of three doses in the lungs. There was no evidence of genotoxic effects in livers and spleens, or acute phase response in plasma. There was no evidence of MWCNT fibrogenicity. Remarkably, nanofibrillated celluloses were rather inflammogenic and persistent in mouse lung. The inflammatory responses in mice and in rats were strongly correlated. Some HARN materials were more inflammogenic and genotoxic than others. A high specific surface area (BET) and a low diameter were identified as a predictor of increased pulmonary inflammation. In addition, length significantly predicted pulmonary inflammation, whereas surface oxidation (-OH and -COOH) was a predictor of lowered inflammation. BET surface area, and therefore diameter size, significantly predicted genotoxicity in bronchia alveolar lavage (BAL) fluid cells and lung tissue.

In inhalation experiments with two pristine MWCNT, "long and thick" NM-401 and "short and thin" NM-403, NM-403 was more inflammogenic than NM-401. Since NM-403 had a 10-fold higher specific surface area than NM-401, these results were in agreement with those obtained by pulmonary instillation. Despite the persistent presence of carbon nanotubes in lung tissues, no significant histopathological changes were observed. The results may be helpful for the development of safer HARN materials (D4.13).

A repeated-dose 90-day oral toxicity study in rat with amorphous silica (NM203) did not find marked and clearly dose-dependent effects after oral doses of maximally 50 mg/kg bw per day (D4.11).

In prenatal toxicity studies with cerium dioxide JRCNM02102a and multi-walled carbon nanotubes (JRCNM04001a) in mice, no overt toxicity in terms of miscarriage or malformations was found (D4.14).

An instillation study was performed in rats using different metallic oxide NMs (TiO2, CeO2). All NMs were detected in the tracheobronchial lymph nodes after 35 and 90 days. There was no significant systemic distribution in liver, kidneys and spleen. No marked effects were seen for all tested NMs regarding the production of oxidative stress. Considering overall pro-inflammatory effects, lung inflammation seemed somewhat more pronounced for $TiO_2$ NM-105, $TiO_2$ NM-101 and $CeO_2$ NM-212 than for $TiO_2$ NM-100. In animals exposed to NM-100 and NM-101, no significant histopathological changes were observed. Pulmonary instillation of all tested NMs did not induce the formation of micronuclei in blood polychromatic (immature) erythrocytes (D4.15).

PBPK models for inert NPs (polyacrylamide, gold, titanium dioxide) and a PBPK model for inhalation exposure to cerium dioxide NPs (self-generated material) were developed. Further development is needed; especially with respect to regulatory use (D4.17).

Existing OECD and ISO standard methods for ecotoxicity assessment have been adapted specifically for NM testing and developed into defined SOPs. They have been applied on three priority species representing different trophic levels. Silver NPs (JRCNM03000a) showed high toxic potency. A lower toxicity was generally observed in the test systems for MWCNTs (JRCNM04000a, JRCNM04001a, JRCNM04100a) and titanium, cerium, silica and zinc oxide NMs (JRCNM01000a, JRCNM01001a, JRCNM01003a, JRCNM02000a, JRCNM01100a, JRCNM02102a). Concrete guidance to design and conduct eco-toxicity experiments is given in the form of decision trees and hazard potency categories based on specific cut off values (D4.12).

Immunotoxic and genotoxic effects of biopersistent nanofibrillated celluloses differed among four materials studied. Effects were also seen with the bulk-sized cellulose studied. The outcome of the in vivo toxicity tests was not consistently predicted by in vitro toxicity studies performed with the same materials (D4.16).

A summary and evaluation of the results of work package 4 is presented in D4.18.

**Advancement of Regulatory Risk Assessment and Testing (WP5)**

To address the need for more efficient ways to evaluate potential adverse effects of a NM, a system for the grouping and read-across of NMs was developed. It is based on expected biological, ecological and/or toxicological effects (D5.01).

Since solubility is a crucial factor when predicting the effects and risks of NMs, test procedures for application in regulatory testing were investigated. It was concluded that dissolution in a complex matrix is highly challenging. It was not possible to devise one universal, robust and rapid test method for regulatory testing that is applicable for all types of NMs in all types of matrices (D5.02).

The potential internalization and crossing of several NANoREG NMs through different *in vitro* barrier models and their impact on tissue integrity was evaluated. Results indicate that *in vitro* models have currently a limited suitability to allow reliable evaluation of NMs crossing barriers (D5.03).

Several *in vitro* techniques mimicking inhalation exposure were evaluated. Results were linked to the results of *in vivo* tests for the same materials. Classical monolayer culturing produced similar results to cells grown in an air/liquid interface (ALI) model. Results indicate it is better to use co-culture cellular models, which were shown to be more sensitive under the conditions of the different studies. It was difficult to correlate between methodologies due to the low toxicity of the NM under study (D5.04, D5.05).

The suitability of *in vitro* assays in terms of reliability and predictive value was evaluated on the basis of a great number of experiments and a comparison with the result of *in vivo* experiments with the same materials. Due to very low toxicity of the NMs, it was difficult to correlate results with the *in vivo* situation. However it was found

that *in vitro* methodologies were able to rank NMs according to their toxicological outcomes in a fashion similar to the results obtained *in vivo*. For ease of data extrapolation to the *in vivo* situation, doses are better provided as µg/cm² (D5.06).

Based on a literature review and own experiments, an overview was made of the high throughput screening methods (HTS) and high content analysis (HCA) that can be applied nowadays. Several methods were standardized and applied for NM testing. They are promising to be used as robust methods for hazard assessment. Results of a preliminary evaluation show that standard test methods and the HTS/HCA approaches give similar results. The analysis of the generated data and the comparison, within and between different HTS/HCA tests as well as with standard *in vitro* assays is ongoing (D5.07).

A strategy was developed to prioritize those nanomaterial applications that may lead to high exposure or high toxic potential and ultimately high risks for human health. These aspects are summarized in six elements, which play a key role in the strategy: exposure potential, dissolution, NM transformation, accumulation, genotoxicity and immunotoxicity. With this approach it is possible to identify those situations where the use of nano-specific grouping, read-across and (Q)SAR tools is likely to become feasible in the future, and to point towards the generation of the type of data that is needed for scientific justification, which may lead to regulatory acceptance of nano-specific applications of these tools (D5.08).

**Keeping pace with innovation (WP6)**

A NANoREG Foresight system was developed aimed at monitoring innovation and evaluating the potential adverse impacts of NMs and their likely applications in a time horizon of 5 to 10 years. The Foresight system was applied to Graphene as a first test case (D6.01).

To get a better insight in the causes and remedies for the increasing gap between innovation and risk analysis regarding manufactured NMs, an analysis has been made of the social and technical issues currently inhibiting robust safety assessment of NMs and the key bottlenecks inhibiting the ability of researchers to deliver answers to regulatory questions (D6.02).

Taking into account lessons learnt from drug development testing, a NANoREG approach for Safe by Design has been elaborated building on the innovation Stage Gate Model. The concept of "risk potentials for nanomaterials" introduced in this deliverable, has been elaborated regarding relevance and availability of test methods for their applicability by innovators and for their relevance for later stages facing a regulatory context (D6.03, D6.04).

A data structure was developed as a basis for a database encompassing relevant physico-chemical characteristics in relation to toxicity endpoints. To that aim the database was filled with literature data screened for relevancy and quality (D6.05).

A literature review was performed aimed at identifying key physicochemical parameters of NMs that may influence the functionality in terms of cell uptake, optical properties, electronic properties and catalytic activity/biorecognition to physicochemical parameters (D6.06).

## 4.4 *Integrating results (WP1)*

Deliverable D1.09 "*NANoREG final report with (elements of) answers to selected issues/questions*" integrates the results of the R&D work packages. Several conclusions and elements of answers have been directly or indirectly produced by this FP7 project, as can be seen by reading through the "*Summary Of The Findings And Elements Of Answers*" (section A.3 of D1.09). In several cases, procedures (SOPs) to tackle part of an issue have been identified, developed and published, though for some SOPs the verification/validation process ('testing the tests') requires more time and resources than what the project had to offer. D1.09 findings fed directly into the development of the NANoREG Framework for the safety assessment of nanomaterials (D1.11) and the related NANoREG Toolbox (D1.12).

The NANoREG Framework provides a detailed overview of how the safety of NMs should be addressed / assessed in the context of the European REACH Regulation (Part I of the document). It also presents forward-looking strategies aiming at making safety assessment more practical and economically efficient (Part II) (D1.11). Its self-standing annex I – report on a harmonised terminology – is a rare effort in the nanoEHS community, which is attracting interest from parties in Europe and beyond.

The NANoREG Toolbox (D1.12) supports the implementation of the NANoREG Framework by providing an overview of test methods, datasets, models etc., applicable in a regulatory context. Just like the Framework, it will be a building block for the ProSafe White Paper mentioned above.

# 5 Impact beyond the project lifetime

***√ Relevance and quality of data in a regulatory context***

Working in a regulatory context is, when it comes to science, no "business as usual". It means that reliability, comparability, exchangeability and relevance of nanoEHS data are crucial for the usefulness of data. Contributing to the awareness regarding this statement is probably one of the main "soft" impacts of the NANoREG project. Almost all partners have been confronted with and discussed the necessity of using the same well-characterized materials to make it possible to combine their results with the results of other partners and projects. The same applies for the mandatory use of selected SOPs, the use of benchmark data and the testing with a limited number of agreed cell lines. It is a soft impact that actually goes well beyond the NANoREG project and that -nowadays- gets more and more attention in the EU NanoSafety Cluster (NSC) and at global level (for example in the US and in the US-EU Communities of Research (CoR)).

***√ Accessibility of results***

In the same category as "data quality", the awareness regarding the accessibility of the information generated under the umbrella of the project should be mentioned as an impact of the NANoREG project. Important in this context was the decision by the NANoREG General Assembly in June 2016 to open up all deliverables and experimental nanoEHS data at the end of the project. The decision was based on the conviction that open access to results of nanosafety projects is key to the effectiveness and

efficiency of nanosafety research. It is the only way to build on the results of previous projects.

To operationalize this decision, formal and practical hurdles had to be taken such as an amendment of the NANoREG Consortium Agreement, the development of data logging templates and data entry tool and creating a NANoREG Results Repository (including a database for the experimental data).

By making the NANoREG legacy available outside the project, an example has been set that hopefully will inspire other projects in the NSC and will lead to measures by EC and member states to make the opening up of project results common practice. Recommendations to this end are listed in section 4.1.

***√ Collaboration***

The NANoREG project has strongly contributed to the (science- and policy-oriented) dialogue regarding nanosafety at EU level and beyond. The National Coordinators have played -and will play- an important role in this field; acting as link between science, industry, regulators and funding agencies. The established collaboration with the Czech Republic, Greece, South Korea and Brazil also contributed to this dialogue. The start of the ProSafe project in early 2015 gave an additional boost to collaboration in- and outside the EU.

***√ Impact of specific activities***

The Result-Impact tables in section 3.2 summarize the impact of specific products and activities for each work package. Part 2 of the NANoREG Final Report will mirror expectations regarding impact to the actual situation on the basis of the impact chapter of the NANoREG Description of Work (DoW).

***√ Impact via White Paper process***

The ProSafe White Paper Process (2.2.1) is crucial for achieving impact of the NANoREG project, since it will integrate main NANoREG outcome (Framework and Toolbox) and the ProSafe Joint Document into the Policy Recommendations of the White Paper. The Final NANoREG meeting/Scientific Conference held at the end of 2016 in Paris contributed to the credibility of this document. The consultation procedure for the draft White Paper and the workshop with policymakers and innovators foreseen for autumn 2017 will also contribute to the support for the recommendations.

# 6 List of Publications from the project

For a complete overview of publications, please check the list of publications available in the NANoREG Results Repository.

# 7 Copyright

# NANOSOLUTIONS

## Biological Foundation for the Safety Classification of Engineered Nanomaterials (ENM): Systems Biology Approaches to Understand Interactions of ENM with Living Organisms and the Environment

Contract Agreement: Fp7 no: 309329 Website: www.nanosolutionsfp7.com
Coordinator: FIOH

*Table 1 Consortium List.*

| No. | Beneficiary name | Short name | Country |
|---|---|---|---|
| 1 | TYOETERVEYSLAITOS | FIOH | Finland |
| 2 | KAROLINSKA INSTITUTET | KI | Sweden |
| 3 | UNIVERSITY COLLEGE DUBLIN, NATIONAL UNIVERSITY OF IRELAND, DUBLIN | NUID UCD | Ireland |
| 4 | NEDERLANDSE ORGANISATIE VOOR TOEGEPAST NATUURWETENSCHAPPELIJK ONDERZOEK - TNO | TNO | Netherlands |
| 5 | UNIVERSITE BORDEAUX | UB | France |
| 6 | UNIVERSITY COLLEGE LONDON | UCL | United Kingdom |
| 7 | UNIVERSITY OF PLYMOUTH | UOP | United Kingdom |
| 8 | HERIOT-WATT UNIVERSITY | HWU | United Kingdom |
| 9 | ASOCIACION CENTRO DE INVESTIGACION COOPERATIVA EN BIOMATERIALES | CIC biomaGUNE | Spain |
| 10 | LUDWIG-MAXIMILIANS-UNIVERSITAET MUENCHEN | LMU MUENCHEN | Germany |
| 11 | INSTITUTE OF OCCUPATIONAL MEDICINE | IOM | United Kingdom |
| 12 | TURUN YLIOPISTO | U. TURKU | Finland |
| 13 | TEKNOLOGIAN TUTKIMUSKESKUS VTT | VTT | Finland |
| 14 | ACONDICIONAMIENTO TARRASENSE ASSOCIACION | LEITAT | Spain |
| 15 | DANMARKS TEKNISKE UNIVERSITET | DTU | Denmark |
| 16 | FONDAZIONE TELETHON | FTELE.IGM | Italy |
| 17 | UNIVERSITAET LEIPZIG | ULEI | Germany |
| 18 | EIDGENOESSISCHE MATERIALPRUEFUNGS- UND FORSCHUNGSANSTALT | EMPA | Switzerland |
| 19 | BIOBYTE SOLUTIONS GMBH | BIOBYTE | Germany |
| 20 | INSIGHT PUBLISHERS LIMITED | IPL | United Kingdom |
| 21 | PLASMACHEM PRODUKTIONS- UND HANDEL GMBH | PLASMACHEM | Germany |
| 22 | INKOA SISTEMAS SL | INKOA | Spain |
| 23 | BIOTESYS GMBH | BIOTESYS | Germany |
| 24 | Zhejiang University | ZJU | China (People's Republic of) |
| 25 | Fundação Universidade de Brasilia | UNB | Brazil |
| 26 | NATIONAL HEALTH LABORATORY SERVICES | NIOH | South Africa |
| 27 | NOORDWES-UNIVERSITEIT | NWU | South Africa |
| 28 | NATIONAL INSTITUTE FOR OCCUPATIONAL SAFETY AND HEALTH | NIOSH | United States |
| 29 | SAIC - FREDERICK INC CORPORATION SCIENCE APPLICATIONS INTERNATIONAL CORPORATION | NCL | United States |
| 30 | NANOCYL SA | NANOCYL | Belgium |
| 31 | NANOLOGICA AB | NANOLOGICA | Sweden |
| 32 | UNIVERSITA DEGLI STUDI DI SALERNO | NeuRoNe | Italy |
| 33 | SOLVAY SA | SOLVAY | Belgium |
| 34 | POLYMER FACTORY SWEDEN AB | POLYMERFACTORY | Sweden |
| 35 | POLIMEROS Y SISTEMAS DE APLICACION TECNICA SL | POLYSISTEC, S.L. | Spain |
| 36 | UNIVERSITY OF MANCHESTER | UNIMANT | UK * |
| 37 | MISVIK BIOLOGY | MISVIK | Finland** |

* replaced UCL , partner 6; ** replaced VTT, partner 13

# Contents



## 1 Summary

Project Duration: 1.4.2013-31.3.2017

Project Funding: Fp7 GA contract no 309329, € 10 000 000

The overarching aim of the NANOSOLUTIONS consortium is to provide a means to develop a safety classification for engineered nanomaterials (ENM) based on an understanding of their interactions with living organisms at molecular, cellular and organism levels. The objective is to determine the "biological identity" of ENM, and subsequently develop a proof of concept that can predict from the properties of ENM their ability to cause health or environmental hazards.

## 2 Background

Engineered nanomaterials (ENM) have attracted a great deal of interest over recent years and their potential for economic exploitation has risen exponentially. Some of their properties however have given rise to concern that they may be harmful to humans. Scientists, regulators and industry need an effective test of these properties in order to be sure they are safe to use. While testing of individual applications of ENM is possible, it is expensive and time-consuming and is a barrier to innovation. By identifying those characteristics of ENM that determine their biological hazard potential it will be possible to create a set of biomarkers of their toxicity that will assess and predict their safe use.

## 3 Scientific and technological challenges

The way ENM interacts with living organisms is complex and their biological effect is largely governed by their surface properties and the way different biomolecules bind to this surface. By understanding the fundamental characteristics of ENM underpinning these biological effects NANOSOLUTIONS will provide a sound foundation on which to classify the materials according to their safety. In other words, NANOSOLUTIONS will investigate how ENM interact with living organisms at a molecular, cellular and organism level based on their material characteristics. In determining the biological identity of ENM the project will develop a computational model that will predict from the properties of ENM their ability to cause harmful health or environmental hazards. This will give scientists the ability to predict these harmful effects rather than simply describe them once they have occurred.

## 4 Objectives

The main objective of NANOSOLUTIONS is to identify and elaborate those characteristics of ENM that determine their biological hazard potential. This potential includes the ability of ENM to induce damage at the cellular, tissue, or organism levels by interacting with cellular structures leading to impairment of key cellular functions. These adverse effects may be mediated by ENM-induced alterations in gene expression and translation, but may involve also epigenetic transformation of genetic functions. By determining the biological identity of ENM, the project will create a set of biomarkers of ENM toxicity that are relevant in assessing and predicting the safety and toxicity of ENM across species. This computational, predictive tool will become the global standard for ENM safety classification.

## 5 Progress and Outcomes to date

***The Nanosafety Classifier is taking shape***

The work on life-cycle analysis of nanomaterials has advanced greatly (WP4). Simulation of the engineered nanomaterials (ENMs) release and release characterization at laboratory scale have been set up for a variety of applications ranging from sportswear textiles to motor oil additives. Also, the basis for deriving the effect factors of nanomaterials and their application of the LCA case study for quantum dots has been established. These observations pave the way for a similar analysis of other types of nanomaterials.

One of the major objectives has been carrying out the experimental work to test the ENM and generate data on ENM interactions with living systems for the Classifier; this work has been performed in WP5-WP9 and further studies are still underway. For the first time, the relevance of the glycosylation on the bio-nano interactions has been shown. The work also highlighted the significance of a protein corona on nanoparticles in modulating particle properties and their biological interactions. The study shows that the post-translational modification of proteins can significantly impact nanoparticle–cell interactions by modulating the protein corona properties (WP5).

Results from WP6 (Cell models) have highlighted dose- and time-dependent effects of the tested ENMs, but also underscored that different surface functionalizations of ENMs have distinct effects on the toxicity in different cellular models including macrophages, lung cells, T cells, and mesenchymal stem cells. In WP7 (Cross-species models) a toxicity screen has been attempted with all materials for two model organisms – microbes (E. coli) and the water flea (D. magna) to determine low, medium and high toxicity materials. In the test conditions, the CdTeQDs were observed to be

the most toxic from all the tested nanomaterials to microbes. The used test models enabled identification of the most toxic nanomaterials over species.

In WP8 (Disease models) the data shows that the surface functionalization of quantum dots determines their association with atherosclerotic lesions in the carotid artery of ApoE-/- mice fed a high-cholesterol diet. Also, it has been discovered that in asthmatic mice, core CuO exposure activates innate immunity reactions while it diminishes T-cell mediated adaptive immunity response. In WP9, translocation studies have been conducted across different barriers at cellular, tissue, organ and organism level. It has been observed that the surface functionalization of nanomaterials determines their behaviour across endothelia barriers and cell membranes and influences on nanoparticle uptake.

OMICS methods have been addressed in detail. The omics assessment is progressing well, and for the transcriptomics part, the data production is advancing rapidly (WP10). In addition, the project has developed the computational framework for the Nanosafety Classifier and agreed on the data formats and data repository (WP11). The Classifier will highlight the most relevant features that, across the data layers, will predict the safety of the nanomaterials and classify against the effect. A novel computational method has been developed for feature selection and prioritization from omics data based on fuzzy logic and random forests approaches. The method is able to retrieve very robust sets of features. The WP12 (Safety classification) has carried out an option analysis of the Classifier. It will facilitate further the development of the Classifier concept and design through the prototyping and testing of the tool, and it will also develop a specification for a high throughput system for future testing and analysis. Thus, the computational infrastructure of the Classifier is in place, and the prototype is currently being tested. The promises and the potential of the NANOSOLUTIONS Project seem to be very encouraging now when the Project is starting its final year of work. The NANOSOLUTIONS project also provides by far largest OMICs data resource against highly characterized set (n=30) of carefully selected ENMs for further studies. The results of the project and ultimately the Classifier will enable a remarkable progress in the grouping of nanomaterials based on their hazard and other features.

In order to facilitate the dissemination of research results and promote topical discussion on nanosafety, the project has organized two large international Conferences, such as the International Congress on Safety of Engineered Nanoparticles and Nanotechnologies (www.ttl.fi/senn2015) in Helsinki 12-15 April 2015, and Systems Biology in Nanosafety Research Conference in Stockholm 9-10 November 2015.

## 6 Expected Impact

The main innovation of the NANOSOLUTIONS project has been envisioned to be the development of the engineered nanomaterial (ENM) safety classifier. This novel hazard profiling principle will provide a basis that enables us to understand and define the toxic potential of all types of ENM. It will be used by companies that manufacture ENM and by a regulatory community to manage, reduce uncertainty, and clarify the current debate, since it will provide the potential to effectively "de-classify" many types on ENM in many applications, in terms of safety risks. The NANOSOLUTIONS ENM safety classification model will benefit industry and enable innovation, since being able to effectively assess the safety characteristics of ENM will speed up the innovation cycle and the development of commercially viable products using ENM.

## 7 Directory

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

| First Name | Last Name | Affiliation | Address | e-mail |
|---|---|---|---|---|
| Kai | Savolainen | FIOH | Finland | kai.savolainen@ttl.fi |
| Bengt | Fadeel | KI | Sweden | bengt.fadeel@ki.se |
| Juha | Kere | KI | Sweden | juha.kere@ki.se |
| Roman | Zubarev | KI | Sweden | roman.zubarev@ki.se |
| Kenneth | Dawson | NUID UCD | Ireland | kenneth.a.dawson@cbni.ucd.ie |
| Ingeborg | Kooter | TNO | Netherlands | ingeborg.kooter@tno.nl |
| Didier | Astruc | ISM | France | d.astruc@ism.u-bordeaux1.fr |
| Kostas | Kostarelos | UOM | United Kingdom | kostas.kostarelos@manchester.ac.uk |
| Richard | Handy | UOP | United Kingdom | r.handy@plymouth.ac.uk |
| Teresa F | Fernandes | HWU | United Kingdom | t.fernandes@hw.ac.uk |
| Sergio E. | Moya | CIC Biomagune | Spain | smoya@cicbiomagune.es |
| Fritz | Krombach | LMU | Germany | krombach@med.uni-muenchen.de |
| Lang | Tran | IOM | United Kingdom | lang.tran@iom-world.org |
| Riitta | Lahesmaa | U. TURKU | Finland | riitta.lahesmaa@btk.fi |
| Roland | Grafström | KI | Sweden | roland.grafstrom@gmail.com |

| First Name | Last Name | Affiliation | Address | e-mail |
|---|---|---|---|---|
| Socorro | Vázquez | Leitat | Spain | svazquez@leitat.org |
| Katrin | Löschner | DTU | Denmark | kals@food.dtu.dk |
| Paola | Cormio | FTELE.IGM | Italy | p.cormio@tigem.it |
| Irina | Estrela-Lopis | ULEI | Germany | irina.estrela-lopis@medizin.uni-leipzig.de |
| Peter | Wick | EMPA | Switzerland | peter.wick@empa.ch |
| Ivica | Letunic | BIOBYTE | Germany | letunic@biobyte.de |
| William | Davis | IPL | Belgium | wdavis@ipl.eu.com |
| Alexei | Antipov | PLASMACHEM | Switzerland | antipov@plasmachem.com |
| María | Blázquez | INKOA | Spain | maria@inkoa.com |
| Jürgen | Bernhardt | BIOTESYS | Germany | bts@biotesys.de |
| Changyou | Gao | Prof. | | cygao@zju.edu.cn |
| Paulo | Cesar De Morais | UNB | Brazil | pcmor@unb.br |
| Mary | Gulumian | ZJU | South Africa | mary.gulumian@nioh.nhls.ac.za |
| Victor | Wepener | ZJU | South Africa | victor.wepener@nwu.ac.za |
| Scott E. | McNeil | NIOSH / NCL | USA | ncl@mail.nih.gov |
| Julie | Muller | NANOCYL | Belgium | julie.muller@nanocyl.com |
| Roberto | Hanoi Labrador | NANOLOGICA | Sweden | hanoi@nanologica.com |
| Roberto | Tagliaferri | UNIMANT | Italy | rtagliaferri@unisa.it |
| Jacques-Aurélien | Sergent | SOLVAY | Belgium | jacques-aurelien.sergent@solvay.com |
| Andreas | Nyström | POLYMERFACTORY | Sweden | andreas.nystrom@polymerfactory.com |
| Jordi | Férnandez | POLYSISTEC | Spain | export@polysistec.com |

# 8 Copyright

# SUN

## Sustainable Nanotechnologies

Contract Agreement: 604305 Website: http://www.sun-fp7.eu
Coordinator: Danail Hristozov, Antonio Marcomini

*Table 1 Consortium List.*

| No. | Beneficiary name | Country |
|---|---|---|
| 1 | University Ca' Foscari Venice | Italy |
| 2 | European Research Services | Germany |
| 3 | BASF | Germany |
| 4 | Nanocyl | Belgium |
| 5 | Colorobbia | Italy |
| 6 | Plastic Components and Modules Automotive | Italy |
| 7 | Veneto Nanotech | Italy |
| 8 | MBN Nanomaterialia | Italy |
| 9 | Die Innovationsgesellschaft | Switzerland |
| 10 | Plasmachem | Germany |
| 11 | Malsch Techno Valuation | Portugal |
| 12 | The REACH Centre | UK |
| 13 | ETSS Gottschalk and Co | Switzerland |
| 14 | Institute for Occupational Medicine | UK |
| 15 | Spanish National Institute for Agriculture and Food Research and Technology | Spain |
| 16 | Rijksinsituut voor Volksgezondheid en Milieu | The Netherlands |
| 17 | Toegepast Natuurwetenschappelijk Onderzoek | The Netherlands |
| 18 | National Research Centre for the Working Environment | Denmark |
| 19 | Swiss Federal Laboratories for Materials Science and Technology | Switzerland |
| 20 | Fraunhofer-Institut für Molekularbiologie und Angewandte Oekologie | Germany |
| 21 | Centre Européen de Recherche et d'Enseignement | France |
| 22 | Consiglio Nazionale Delle Ricerche | Italy |
| 23 | RIKILT Institute of Food Safety | The Netherlands |
| 24 | Technical University of Denmark | Denmark |
| 25 | Heriot-Watt University | UK |
| 26 | Karolinska Institute | Sweden |
| 27 | University of Aveiro | Portugal |
| 28 | University of Plymouth | UK |
| 29 | Rheinisch-Westfaelische Technische Hochschule Aachen | Germany |
| 30 | Aarhus University | Denmark |
| 31 | University of Vienna | Austria |
| 32 | Vrie Universitet Amsterdam | The Netherlands |
| 33 | University of Leeds | UK |
| 34 | University of Bremen | Germany |
| 35 | University of Limerick | IE |

# Contents



## 1 Summary

Project Duration: October 2013 - March 2017

Project Funding: 13.5 million Euro

Nanotechnology is one of the Key Emerging Technologies identified in the European Union (EU) 2020 Strategy. Its enormous potential for innovation has fostered large investments in developing new industrial applications and consumer products. However, the outlooks for a rapid growth in the sector have raised not only hopes and high expectations, but also societal concerns about the adequacy of nanotechnology regulation. Indeed, despite their clear benefits, engineered nanomaterials may pose environmental and health risks.

The main reason to launch the project SUN was to investigate these risks and find ways to prevent or reduced them. With a budget of over 13.5 million EUR, its ambitious work program involved more than 100 scientists from 35 research and industrial organisations across 12 European member states and became one of the first EU-funded projects to address risks along the entire lifecycles of real industrial products.

The implementation of SUN has been a challenging task due to the overwhelming uncertainties that marked each step of both risk assessments and innovation activities. The challenges that we encountered provoked the need to develop reliable methods for characterization of nanoparticles released from various product matrices into complex biological, environmental and food media, and for the assessment of their human and environmental exposure, hazard and risk. These tools and the newly developed safety by design procedures have become the highlights of SUN. Their integration into a Decision Support System (SUNDS) and practical risk management guidelines provided industries and regulators with the means to streamline effective decision making about safer products and processes.

SUNDS is one of the "flagship" results of SUN. This user-friendly software system can be used by stakeholders from industry, academia and regulatory bodies to assess environmental impacts and/or to identify and manage possible occupational, consumer and ecological risks arising from the manufacturing, handling, use and end-of-life treatment of nanotechnology products. In situations where the risks are not controlled the tool proposes suitable measures to reduce them. In doing this SUNDS also provides information about the costs of risk reduction as compared to the anticipated benefits from the products. This is particularly useful for industries and SMEs for checking supplier risks, competing products, market opportunities, or for performing an internal risk and benefit analysis.

The industrial partners in the SUN Consortium "reality-checked" and evaluated the new methods and tools developed in the project against real nanotechnology applications. The nanotechnology applications were represented by supply chains of real products containing nanoscale Tungsten Carbide (sintered, wear-resistant ceramics), Copper Oxide (antimicrobial/fungal wood preservatives), Silica (food), Titanium Dioxide (self-cleaning ceramic tiles and air purification systems), organic pigment (the red colour of the Ferrari cars), and multi-walled carbon nanotubes (anti-fouling coatings, lightweight plastics). The extensive development and testing of methods and tools for nanomaterials risk assessment and management did not only generate an enormous amount of new scientific data and knowledge on the release, exposure potential and hazard potency of diverse material types, but new insights into key nano-bio/eco interactions, release pathways, modes of action, and adverse outcome pathways. This validation of the SUN approach culminated in guidelines for safer product and process design, which are publically available on the project website: www.sun-fp7.eu.

## 2 Background

SUN was based on the hypothesis that the available knowledge on the environmental and health risks of manufactured nanomaterials (MN), while limited, can nevertheless guide the nanotechnology industry to avoid future liabilities provided that an integrated approach to their risk assessment and management is applied that addresses the entire lifecycle of nano-enabled products (NEP). To facilitate this, SUN generated environmental, health and safety (EHS) data and methods and integrated them into a Decision Support System for risk management of MN and NEP. The goal was that this approach would enable safer manufacturing, use and end-of-life treatment of nanotechnology products; it would result in more solid risk prevention and mitigation strategies, and would be easily applicable to different materials and industrial settings.

This goal was achieved: the project significantly improved the current approaches to risk assessment and lifecycle assessment of MN and successfully combined them into the user-friendly software SUN Decision Support System (SUNDS) for practical use by industries and regulators. The industrial partners in the SUN Consortium evaluated and "reality-checked" SUNDS against real products in terms of costs and benefits. This validation culminated in guidelines for risk management, including safe nanoscale product and process design. In addition, SUN significantly contributed to scientific progress in the areas of human and environmental exposure, hazard and risk, identified needs for future research and assigned priorities for current regulation. We have involved major international stakeholders in implementing the project results into practice and regulation.

## 3 Objectives

SUN achieved the following integrated set of objectives within three central themes of nanotechnology innovation:

**THEME I. MATERIALS, PRODUCTS AND PROCESSES**

- Perform a data gap analysis to prioritise the generation of new information in the project
- Map hot spots release of MN at different stages of NEP supply

chains to guide cost-effective strategies for release and exposure estimation

- Assess the environmental impacts arising from each lifecycle stage of the SUN case studies and compare the results to conventional products with similar uses and functionality
- Develop and validate criteria and guiding principles for green nanomanufacturing (low energy consumption, eco-friendly materials) and for setting environmental quality targets

**THEME II. RISK ASSESSMENT**

- Collect and characterize MN released from NEP in different lifecycle stages for use in (eco)toxicological and behaviour/fate studies
- Model the behaviour/fate of MN and assess their exposure concentrations in the environment (i.e. air, water, sediment and soil compartments)
- Develop and validate methods (incl. high-throughput and content tools) for prediction of long-term effects on humans and ecosystem services in environments subjected to multiple stressors
- Develop and validate a tiered approach for qualitative to quantitative assessment of inhalation and dermal to gastro-intestinal occupational and consumer exposure to MN, based on high-quality collated and project-generated emission rates, exposure measurements and contextual information
- Use the exposure and effects data acquired from other projects and the data newly produced in SUN for quantitative lifecycle-oriented ecological and human health risk assessment

**THEME III. SAFE PRODUCT AND PROCESS DESIGN**

- Describe best available technologies/practices for reduction of exposure and effects of MN in different lifecycle stages
- Develop the following innovative risk reduction methods and practices and include them in guidelines for safe nanoscale product and process design:
  - safety by design (SbD) elimination/substitution and waste isolation practices to reduce the release of MN from products/composites or to induce their accelerated alteration/degradation in order to reduce their environmental persistence and bioaccumulation
  - methods to analyse the evolution of the product quality parameters, process conditions and interactions, in real-time, to subsequently exercise control over them, increasing both product safety and quality
  - best practices to minimise release and exposure of MN during handling of waste flows containing MN
- Develop and test the user-friendly SUNDS for estimating MN risk for different targets (e.g. workers, consumers, ecosystems) in each lifecycle stage and evaluating to which extent the available risk management measures could reduce this risk (incl. cost-effectiveness analysis)

To succeed in achieving the above objectives, the project adopted an innovative approach following four guiding principles:

I. Study the longer-term effects of MN on both human health and the environment

*SUN substantially advanced the area of environmental nanotoxicology and had an important contribution to the field of human nanotoxicology. The project achieved this through designing longer-term (eco)toxicity tests with lower exposure concentrations and focussing on issues such as biodistribution, inflammation, histopathology, genotoxicity and epigenetic effects as well as transformation, bioaccumulation, bio-transfer, pool/diversity loss and ecosystem services damage/loss.*

II. Understand the release, fate and exposure to MN and their risks from lifecycle perspective

*SUN adopted a lifecycle analysis approach, focusing on identification of likely MN emissions and exposures arising from each lifecycle stage of real products, represented by seven overarching case studies.*

III. Prevent and control risks from exposure to MN in occupational, consumer and environmental settings

*SUN collated and developed high-quality product and process-specific MN release and exposure data libraries and a three-tier inhalation and dermal-to-oral exposure modelling framework. Moreover, SUN introduced innovative SbD elimination/substitution and waste isolation practices to reduce the release of MN from products or to induce their accelerated alteration/degradation in order to diminish their environmental exposure and the resulting risks.*

IV. Achieve safe/sustainable nanomanufacturing

*SUN developed safety by molecular design strategies and a nano-specific Process Analytical Technology (nanoPAT) useful to define Process Control Strategies that ensure scale up of safe high-quality products.*

*SUN implemented a Decision Support System for risk control and sustainability assessment of MN and NEP.*

# 4 Major Outcomes from project

**THEME I. MATERIALS, PRODUCTS AND PROCESSES**

***Portfolio of Data on Real Industrial Materials and Products***

SUN developed and maintained an impressive data portfolio, consisting of data generated internally or collected from other sources in regard to seven case studies. These case studies correspond to supply chains of real industrial products (coatings and composites for the energy, transportation and construction industries as well as nanomaterials used in food). The products embed the following MN: Tungsten Carbide-Cobalt (WC-Co), Copper Oxide (CuO), Silica ($SiO_2$) Titanium Dioxide ($TiO_2$), organic and inorganic ($Fe_2O_3$) pigments, and Multi-Walled Carbon Nanotubes (MWCNT). Data on the release, exposure and (eco)toxicity of these materials have been collected for different lifecycle stages of these products in a data inventory: Sun.iom-world.co.uk. The physicochemical properties of the MN have been characterised both as powders and in relevant biological media for the purpose of (eco)toxicological testing and fate experiments, activities that ultimately generated results useful for risk assessment.

The SUN case studies represent a balanced portfolio of both legacy and novel NEP. In early 2014, when SUN had just started, the first mandatory reporting requirement for MN was introduced in France. In this context, the project chose to balance its case studies in three categories:

1. Highly studied benchmark nanomaterials, for which the project would generate no or limited experimental data:
   - Nanoscale Silver used in textiles.

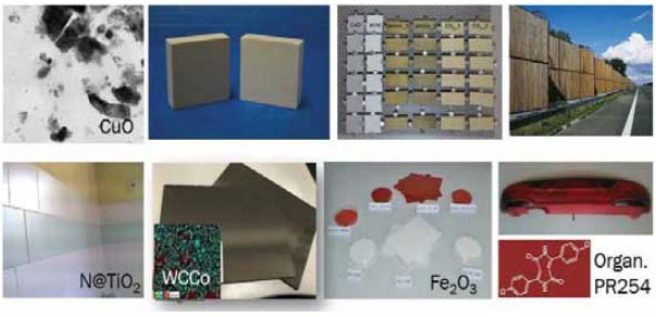
- MWCNT used in marine coatings and automotive parts.

2. Less well-known nanomaterials of high societal relevance. These were chosen from particulates with a history of use, which are now identified as "nanomaterials" in regulatory terms. These case studies had significant data gaps that SUN needed to fill:
   - Organic pigments for automotive parts.
   - Inorganic pigments for automotive parts.
   - SiO2 anticaking agent for use in foods.
3. Innovative nanomaterials of potentially high commercial relevance: SUN essentially had to generate all nanosafety relevant data from scratch:
   - Nitrogen doped Titanium Dioxide for air purification will became a new product enabled by SUN and exploited by the large company Colorobbia.
   - Copper based coating and/or impregnation for wood protection: a product development was re-oriented based on SUN safety assessment, to optimize the balance of performance, costs, safety and sustainability.
   - Tungsten Carbide based coatings on steel for paper mills: This product is marketed based on our results.

Very specific to SUN, for all materials the complete value chain was covered by experiments and modelling. Materials representing each lifecycle stage were provided to partners for testing. In doing so academic and industrial partners collaborated closely together to assess properties, release, exposure, hazards and risks. Of note, all these products were of industrial (product-ready) quality and were derived from pilot lines, actual production lines or batch control labs:

1. SYNTHESIS of nanomaterials (at the premises of the industrial and SME partners Nanocyl, Colorobbia, PlasmaChem, BASF and MBN).
2. FORMULATION into nanotechnology products (by the industrial/SME partners PCMA, Nanocyl, Colorobbia, BASF, MBN).
3. USE in realistic industrial and consumer settings.
4. DISPOSAL / END of LIFE treatment under realistic industrial conditions.

As anticipated back in 2014 when the project started, the "less well studied nanomaterials of high societal relevance" indeed are now registered with large volumes of production in nanoforms (from 100 tons/year to above 100,000 tons/year) according to French reporting. This validates the choices of SUN, in the sense that the project captured many of the nanomaterial application segments, product matrices, material classes that are highly relevant for European consumers. The tools developed by SUN are thus applicable to both established and novel nanomaterials and nanotechnology products.




***Environmental Impacts***

The environmental impacts of the selected MN and the associated NEP have been investigated by means of the established Life Cycle Assessment (LCA) methodology. To do so SUN developed specific life cycle models and collected/generated Life Cycle Inventory data. These data were used to perform Life Cycle Impact Assessment based on LCA midpoints combined with shadow prices. Since the investigated MNs and their applications were very diverse, this resulted in interesting and informative variety in the details of the LCA case studies. In some case studies the environmental impacts were very low, while in others they were more significant, with the impacts strongly depending on the type of the involved manufacturing process (energy demand, operating supplies, yield, purification rate). It is important to note that the potential for reducing environmental load by nano-enabled products and processes depends on the type and level of innovation (e.g. incremental vs. radical, end-of-pipe vs. integrated). Today most nanotechnology applications are incremental innovations (i.e. improved conventional products), which limits the possibility for redesigning them to meet high environmental standards. To contribute to the future development of "greener" nanotechnologies, SUN developed design principles for entire product portfolios represented by the project's case studies. One of the key conclusions is that to benefit the environment the future nanotechnology applications should have a combination of following characteristics:

- Use MN as additives leading to better functionality of the NEP.
- Environmental benefit in the use phase (higher resource and/or energy efficiency).
- Long-life (persistent) product.
- Nanomaterials integrated in the product matrix (low release).

**THEME II. RISK ASSESSMENT**

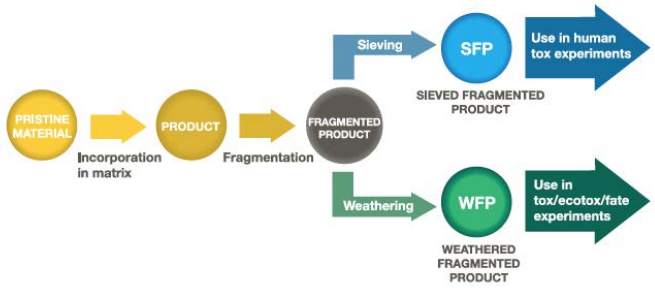
***Lifecycle Release and Environmental Exposure***

The analysis of the potential risks of nanomaterials has so far been almost exclusively focused on the pristine, as-produced particles. However, when considering a life-cycle perspective, the nanomaterials released from genuine products are far more relevant. The properties of the materials released during the manufacturing, use or end-of-life phases depend on the nature of the matrix and the way the particles are incorporated in it (i.e. surface-bound or internally embedded). Research on release of nanomaterials from products has been growing and the next necessary steps have been to investigate the behaviour and effects of the released materials in the environment and on humans. SUN has been one of the first projects to achieve a considerable progress in these research areas. To do this it was necessary to collect and characterize nanoparticles released from the selected SUN nanotechnology products in different life cycle stages for use in hazard and behaviour/fate studies.

The key requirements identified by our partners for producing such fragments of nano-enabled products have been:

- Use of formulated materials instead of just aging pristine particles.
- The process is reliable and quick.
- The use of samples close to real-world exposure scenarios, such that assays can be prepared for "released" materials.
- They should be available in a sufficient amount (hundreds of grams to kilograms) for testing in hazard studies and with a relevant size distribution.
- A nano-free formulated material is available as a reference.

Based on these requirements, SUN developed an approach to provide materials in hundreds of grams quantities mimicking actual released materials from coatings and polymer nanocomposites by producing what is called "Fragmented Products". These released fragments can further be exposed to environmental conditions (e.g. humidity, light) to produce "Weathered Fragmented Products" or can be subjected to a further size fractionation to isolate "Sieved Fragmented Products" that are representative for *in vivo* inhalation studies.

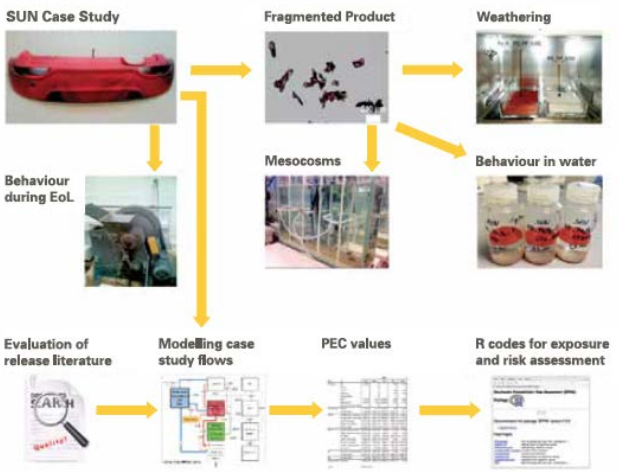



The SUN approach to produce fragmented materials.

SUN significantly advanced the environmental exposure field by contributing methods in a variety of areas. For instance, the project developed tools to analyse MN releases and characterise the released materials in complex matrices. Some examples are the following: 1) sp-ICPMS with reaction cell technology for detection of nanoscale $Fe_2O_3$ and CuO; 2) combination of AF4 and ICPMS for detection of nanoscale $SiO_2$ in food; 4) protocols for release of $Fe_2O_3$ from plastics and $SiO_2$ from food; 5) climate chamber weathering protocols for: a) $Fe_2O_3$ in plastics, and b) CuO painted on wood; 6) wet weathering protocols for $Fe_2O_3$ and organic pigments in plastics; 7) a protocol for studying the release of MWCNT from polymers (based on isotopic labelling - $^{14}C$-CNTs); 8) protocols for studying the release of MN from waste during incineration, recycling and landfilling. Moreover, the project has made significant progress in optimizing the above methods so that they can better discriminate MN from background materials. To generate released MN for experimental purposes, manual and mechanical grinding and cryomilling methods were developed. One important focus of SUN was the measurement of MN release during end-of-life treatment (e.g. recycling, incineration) and disposal. In this regard the project 1) developed analytical capabilities for measuring the content of MN in different end-of-life products as well as by-products from waste processes (e.g. leachate); 2) completed tests to assess the recovery of MN during preparation of samples prior to lab analysis; 3) established procedures for leaching tests aiming at assessing the release of MN when NEP come in contact with water in end-of-life scenarios; and 4) performed measurement campaigns targeting the release of MN during pre-treatment of waste prior to recycling processes. The obtained results demonstrated that: 1) the current procedures for sample preparation may be inadequate for dealing with nanowaste; 2) losses of MN during recycling processes are significant and may induce workplace and environmental exposure; 3) leaching tests aiming at assessing release of MN need adjustments compared with the standard protocols and additional tests (e.g. TEM) may be required. Moreover, the environmental transformation of the released particles was investigated using bench scale and mesocosm scale studies in collaboration with our U.S. Advisory Board member Duke University.

The overall concept is visualized in the figure below: starting with the SUN case studies, fragmented products were produced and characterized. These materials were further weathered under environmental conditions and their behaviour in water and mesocosms was studied using methods and approaches developed during the project. The release of nanosized particles from the SUN case study materials was also tested under conditions relevant for the end-of-life treatment. Another line of research used modelling to follow the flows of nanomaterials within the products and after release. This also included assessments of the release literature and development of codes to model environmental exposure, hazard and risk. Using these tools, the nanomaterial flows for the SUN case studies and the resulting environmental concentrations were predicted. Moreover, SUN partners performed modelling of the environmental fate of MN released from solid waste due to recycling and incineration.

Overall, the work performed in SUN presents the most realistic assessment of the environmental exposure of nanomaterials so far because it is based on real-world materials and incorporates release processes. For some case studies such as automotive parts the SUN partners found no significant releases of the nanomaterials, whereas for other case studies such as wood protected with Copper the release was linked to transformation of the nanomaterials and depended a lot on the product formulation.




Overview of the environmental exposure research performed in SUN: Starting with the SUN case studies, fragmented products were produced that were investigated with respect to their weathering and fate. Modeling studies complemented the work by quantifying environmental exposure.

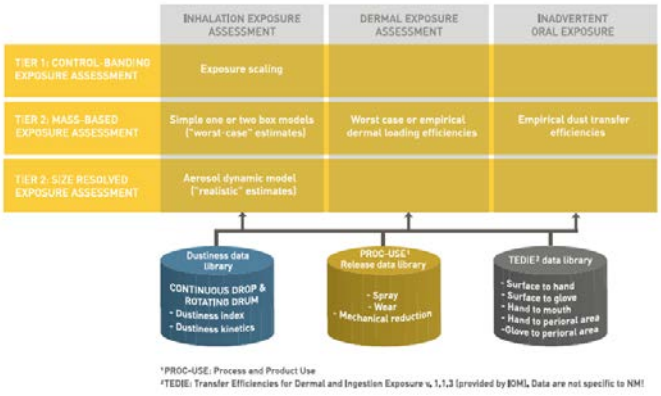
***Occupational Exposure***

The above release work has relevance not only to estimating environmental exposure, but also exposure in occupational settings. In this regard, SUN has significantly progressed in developing a versatile tiered modelling framework for assessing workers' inhalation, dermal and inadvertent oral exposure to MN. The highlight of this framework is a new aerosol dynamic inhalation model developed in the project. To support the modelling approach, release rate libraries were developed for powder respirable dustiness and different processes based on comprehensive reviews and additional data generated as part of SUN. For assessment of inadvertent oral exposure, dermal to perioral transfer efficiencies were proposed.

The Exposure Control Efficacy Library (ECEL) library on engineered and personal protection equipment was also further developed to

include efficiencies against nanomaterial exposure based on data generated in the project.

Five workplace exposure measurement campaigns were completed to establish values for comparison with modelled exposure levels. The measurements were specifically conducted for synthesis of nanoscale CuO, production and handling of WC, production of car bumpers with organic and inorganic pigments, and application of $TiO_2$ coatings. The results of this work and especially the establishment of the dustiness and release rate data libraries will have a significant impact on the capability and quality of future nano-specific exposure assessments. These libraries will become publically accessible and subscribing laboratories will be able to contribute with further data in the future.




The conceptual SUN occupational exposure assessment framework for nanomaterials.

***Consumer Exposure***

SUN has contributed to the field of nanomaterials consumer exposure assessment through developing realistic scenarios of nanomaterial release from various products. Data from several NEP inventories were analysed to gain information regarding nanotechnology products and their availability, distribution across product groups, and the use of different nanomaterial types. Consumer exposure data libraries were established. Several exposure models were applied to the release data to quantify potential consumer exposure that may arise from using these products. The release of nanomaterials from commercially available articles was experimentally tested, considering conditions relevant for consumer exposure such as leaching from food contact materials, textiles and personal care items as well as dermal transfer from product surfaces. These experiments were designed to allow close to realistic exposure potential estimation.

Overall, the work conducted within SUN regarding consumer exposure provides insight and novel data for both measurements and modelling, with focus on relevant real-world consumer articles and likely exposure scenarios.

***Environmental Hazard Studies***

SUN took environmental hazard assessment of nanomaterials an immense step forward as it developed a vast array of testing methods that allow us to predict longer-term ecotoxicity of nanomaterials. It is possible to test both pristine MN and such released from products at different lifecycle stages. All environmental media were covered, i.e. sewage sludge treatment plants, soil, sediment and water, with a focus on ecosystem services and key environmental species. The tools include 1) short-term high throughput studies, e.g. *in vitro*, *ex vivo* and *in vivo* omics-related methods, and 2) long-term *in vivo* studies, e.g. longevity, full life cycles, multi-generation and multi-species test methods. The biological endpoints range from omics to population interactions. This includes cell viability, various omics-responses (gene, protein and metabolites expression), individual life stage endpoints, species interactions, and trans-generation effects, including epigenetic effects.

SUN developed new nano-specific testing methods for assessing the effects of MN on ecosystem services, including sewage treatment plant (STP) function and crop production. This has resulted in an array of results for MN effects in sewage treatment plants (e.g. ammonium oxidizing bacteria), terrestrial environments (e.g. *Enchytraeus crypticus*, *Eisenia andrei*), aquatic sediments (e.g. *Lymnaea stagnalis*, *Daphnia magna*), and pelagic parts (e.g. zebrafish embryos). Moreover, longer-term ecotoxicity tests with lower exposure concentrations were performed focusing on transformation, bioaccumulation, biotransfer, gene-pool/diversity loss and ecosystem service damage/loss. In this regard, many long-term *in vitro* and *in vivo* test systems were evaluated or developed *de novo*.

The *in vitro* methods for the terrestrial ecosystem span over more than five species, and the aquatic *in vitro* methods include both single- and multi-generation cell systems. The developed *ex vivo* methods are highly effective tools to study uptake mechanisms in fish-gut. Uptake studies were important for identifying dietary exposure and bioaccumulation. The tools were integrated so that the results are mutually supportive; this enables the risk assessor to develop a better risk mitigation strategy. The implementation of high throughput tools allowed for Adverse Outcome Pathways (AOP) to be developed. AOP enhanced the understanding of the mechanisms of toxicity and enabled designing materials according to SbD approaches. Pristine MN, modified pristine MN (SbD), and fragmented materials were tested. Reference materials were also tested, e.g. soluble salts of the respective metal MN and fragmented products without embedded MN.

Overall, the work performed by SUN presents the most advanced and realistic assessment of the environmental hazard of nanomaterials so far. It includes real-world materials and deals with long-term highly relevant ecological processes.

The STP tests have shown that the passage through a STP may increase the ecotoxicity of the CuO MN. There is no evidence that microbial toxicity differs following single or repeated exposures to MN, provided the same final concentration. In the terrestrial environment, advanced single species and multispecies long-term tests have shown pronounced CuO toxicity in contrast to lower toxicity of the rest of the MN investigated in SUN. In the aquatic sediment, all MN have been screened in two species (i.e. *Lymnaea stagnalis* and *Daphnia magna*) using short-term tests, while long-term tests have been performed with *Lymnaea stagnalis* only. The results from these tests demonstrate that the toxicity of the CuO MN is dependent on the water pH. Some epigenetic tests have shown low CuO methylation in collembolan and enchytraeids. Methylation of specific genes was also measured. *In vitro* tests with MN on fish and worm cells have shown decreased viability and disturbance of cellular stability. For earthworms, the cells of five species were tested. Repeated exposures have been performed showing effects different from non-repeated long-term exposure. The relevant results were used to perform ecological risk assessment of NEP along their lifecycles.

***Human hazard studies***

The SUN project began with an ambitious list of nanomaterials for risk assessment, but due to budget constraints and ethical concerns not all were tested for human hazard using animal models. Instead an intelligent testing strategy was implemented that prioritized a set of nanomaterials for which data did not exist in the published literature or existing projects for *in vitro* and *in vivo* toxicity testing. The *in vitro* models included immune (macrophage) and liver (hepatocyte) cell lines. Macrophages were chosen as these cells are responsible for clearing particles from the body, as well as eliciting an inflammatory response that could be indicative of potential toxicity. Hepatocytes were chosen as the liver is a major site of nanomaterial accumulation in the body following exposure via either inhalation or ingestion. Dose-response relationships were generated for each cell type using the SUN panel of nanomaterials, and both identified pristine CuO nanoparticles as being more toxic than the others. The lack of existing data on this material along with this *in vitro* hazard data and potential widespread use in wood treatment products resulted in its prioritization for *in vivo* hazard testing. The protocols used for the *in vitro* toxicity testing were taken from previous projects (e.g. ENPRA) to allow comparison of data across studies. In the ENPRA project both *in vitro* and *in vivo* instillation studies had been conducted. There was therefore a pre-existing understanding regarding the relationship between the *in vitro* dose response relationship and the doses likely to induce inflammation in the lung *in vivo*. This relationship was used to predict the most suitable deposited dose and hence airborne mass concentration ranges to use for short-term inhalation studies (STIS; 5-day exposure, followed by sacrifice on day 6 or day 28). The dose range chosen generated a dose-dependent inflammation (e.g. neutrophil accumulation and cytokine up-regulation) at day 6 which was largely resolved at day 28. The inflammation was confirmed by quantification of inflammatory cell influx into the lung as well as gene, protein and genomic analysis of the lung tissue. The data generated contributed both to risk assessment and the further modification of the pristine CuO using a SbD approach. A panel of modified CuO nanomaterials were tested *in vitro*, again using the macrophage and hepatocyte cell lines in order to prioritise coatings for further testing *in vivo*. Coating with ascorbate decreased *in vitro* toxicity in both models, and was associated with a lower inflammatory response *in vivo* both at day 6 and day 28. It also did not prevent the antimicrobial function of the CuO. This approach therefore demonstrates the usefulness of alternative models in refining animal studies and reducing the number of animals used.

To investigate the effects of the CuO nanomaterials following ingestion, a new short term oral study (STOS) protocol, based upon the STIS protocol was devised. Similarly to the STIS study, treatment with CuO nanomaterials for 5 days resulted in a measurable inflammatory response at day 6 which was largely resolved at day 28. This study demonstrates similarities between impacts regardless of the route of exposure for CuO nanomaterials, and furthermore provides a useful protocol for investigating the consequences of oral exposure to potentially toxic substances. The STIS and STOS generated kinetic data suitable for pharmacokinetic (PBPK) modelling and a methodology was developed on this basis to perform *in vitro-in vivo* and animal to human extrapolations for human health risk assessment.

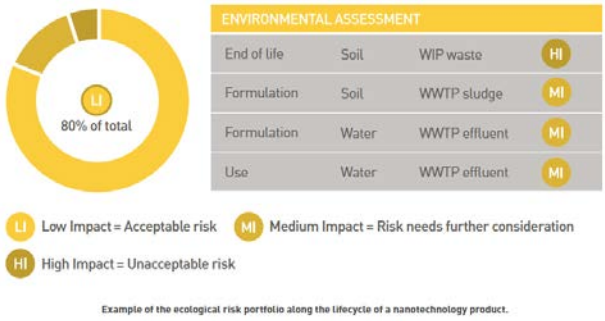
Moreover, *in vivo* epigenetics and transcriptomics tests were performed with the CuO case study in collaboration with Health Canada. In the epigenetic study the levels of gene methylation were found to be low (<1%) overall for all genes, and highly variable. No significant differences between any experimental treatments were observed. The transcriptomics study showed that exposure to the higher dose for 5 days yielded the most significant changes in gene expression, accounting for significantly changed mRNA of about 1000 genes, most of which were up-regulated. In contrast, animals collected after recovery yielded < 20 dysregulated genes, thus indicating return to levels similar to the controls. Most of the deregulated genes related to inflammation and cell proliferation in a dose-response manner, which was reversible after the recovery period.

***Health and Environmental Risks***

SUN advanced in the field of human health risk assessment of nanomaterials by developing a probabilistic risk assessment methodology, which was implemented as a software module in SUNDS. Hazard and exposure data can be estimated deterministically or probabilistically, depending on data availability. Traditional (deterministic) risk assessment relies on single point estimates of hazard, exposure and risk, and often fails to explicitly report the uncertainties that are needed for robust risk management decision making. In this context, a considerable strength of the developed probabilistic approach is that the estimated risk distributions explicitly communicate these uncertainties and support the identification of the parameters (e.g. exposure conditions, selection and use of assessment factors) that most strongly affect the estimated risks.

Specifically, health risk assessment in SUN was performed considering the entire value chains of the SUN priority nanomaterials and exposure of both workers and consumers via inhalation and ingestion. The estimated probabilistic health risks were then classified as acceptable or non-acceptable. Once risk is estimated for individual targets, activities and routes of exposure, an aggregation step produces a single risk value for each lifecycle stage (synthesis, formulation, use, end of life) as well as for the entire lifecycle of the investigated NEP.

SUN also advanced in the field of ecological risk assessment of nanomaterials by developing the first methodology and tool for the estimation of risks along the lifecycle of nanotechnology products covering key environmental compartments (e.g. surface water, soil, sediments). Specifically, a probabilistic material flow environmental exposure model developed in the project predicted environmental concentrations (PEC) resulting from flows of nanomaterials released from products in each lifecycle stage. Moreover, Predicted No Effect Concentrations (PNEC) were derived by means of both deterministic and probabilistic (i.e. Species Sensitivity Distributions) procedures compliant with the REACH guidelines on Chemical Safety Assessment. Thus, an ecological risk portfolio along the lifecycle is calculated by choosing the maximum risk for each lifecycle stage:




Example of the ecological risk portfolio along the lifecycle of a nanotechnology product.

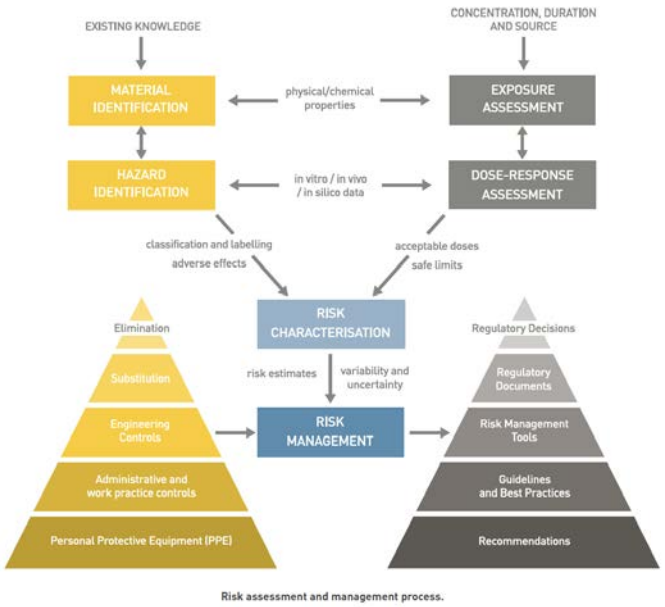
Both the ecological and human health methodologies were tested in the SUN case studies and were implemented as software modules in SUNDS for practical use by industry and regulators.

## THEME III. SAFE PRODUCT AND PROCESS DESIGN

### *Risk Management*

The risk management of nanotechnologies has received much attention over the last years and significant data on the efficacy of risk management measures applied to nanomaterials (e.g. local exhaust ventilation, fume hoods, glove boxes) have been generated. To collect these data and information on SbD methods and make them easily available to our industrial stakeholders SUN developed an inventory of Technological Alternatives and Risk Management Measures (TARMM), which was hosted in the ECEL online library. The compiled information was then summarised in easy-to-read guidelines. The analysis of the data showed that engineering controls and protective clothing are more commonly used to reduce risks of MN as compared to SbD practices targeting the elimination, substitution and modification of NEP. This is mainly due to the unknown or unacceptable effects of manipulating the characteristics of the nanomaterials on their desired functionality. Some of the key conclusions drawn by SUN partners for selecting the appropriate risk management measures for nanomaterials are the following:

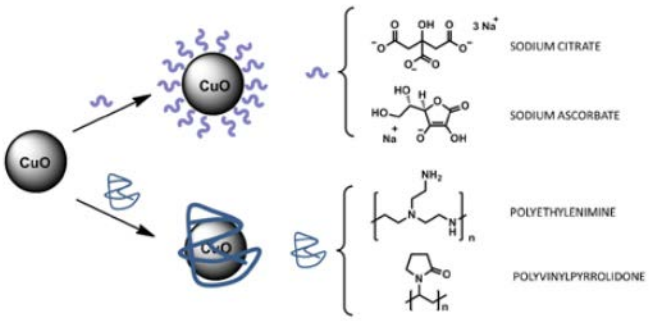
- When the intrinsic safety measures (e.g. elimination and substitution) are not viable, the second-best option for safe handling of MN is to implement engineering control measures.
- The selection of engineering controls at workplaces should be made based on the state of the nanomaterial (e.g. physical form and properties), the level of concern about a hazard (e.g. low, medium, high), the exposure potential (e.g. low, medium, high) and the primary routes of exposure (e.g. inhalation, dermal absorption or ingestion).
- When there is no/low potential for airborne release (e.g. nanoparticles bound in solid matrix), advance engineering controls are usually not needed. This applies also to nanomaterials suspended in liquids, except for the combination of substances of elevated hazard potential (such as CNTs) with processes of high energy input (such as sonication). In any case, drying of suspensions is to be prevented.
- Working with dry nanoparticles of low hazard potential (such as the pigments investigated in SUN) can generate a measurable emission, but the risk can stay in the acceptable limits without enclosure. In general, removing the airborne emissions through local exhaust ventilation is advised nonetheless.
- Working with dry nanoparticles of elevated hazard potential requires very careful attention. When there is high probability of airborne emissions leading to exposure (e.g. nanoparticles in powder form or pellets), the work should be performed in fume hoods or an enclosed system such as glove box or glove bags. To assess the probability of airborne emissions, SUN generated tools and libraries via measurements of the dustiness of powders.




Risk assessment and management process.

### *Safe Product Design*

The design of safer nanotechnology products and processes can prevent risks. However, the selection of SbD solutions is complex and often requires data-intensive validation. SUN developed SbD strategies for the WCCo and CuO case studies. Specifically, for the WCCo micronization techniques (i.e. spray and freeze drying) were developed. In the case of CuO four surface modifying agents were chosen: positively charged (branched polyethylenimine-PEI); neutral (polyvinylpyrrolidone-PVP); negatively charged (sodium citrate-CIT); negatively charged with strong anti-oxidant capacity (sodium ascorbate-ASC). Once the surface was modified we tested the effects arising from the different surface chemistries and charges to identify the most promising design alternatives. The aim was to control surface charge and its direct electrostatic interaction with cell membranes and to test some antioxidant molecules (citrate and ascorbate) for their protective action against free radicals.




Schematic representation of SbyD strategy applied: introduction of surface modifying agents (i.e. CIT, ASC, PEI and PVP) by self assembling.

To validate the above strategies, we adopted a stepwise approach that aimed at providing answers to the following questions:

1) Do the introduced modifications affect the design properties (chemical composition, crystallinity, surface area/chemistry/charge, primary size) that define synthetic identity?

2) Do the modifications affect risk determinant properties (structural alerts) such as properties that define exposure identity (e.g. evolution of synthetic properties in testing and life cycle

media, particle size distribution (PSD), ZETA potential, colloidal stability, MN release/mobility and bioavailability, dustiness) and properties relevant for estimating hazard potential because they are driving some of the most established modes of actions (e.g. ROS production; IONS dissolution/speciation/ distribution)?

3) If no, then hazard assessment through (eco)toxicity testing was performed and different scenarios occurred:

- The tested (eco)toxicity endpoints did not show differences or the differences were not consistent (some results showed a reduction of toxic potential others showed an increase); in this case, further mechanistic investigation was necessary and new design modifications had to be introduced.
- The tested (eco)toxicity endpoints showed a consistent response as the results of the selected modification showed either an increase or a decrease of toxic potency. In this case, we looked for a physically possible compromise between toxicity and requested functionality.

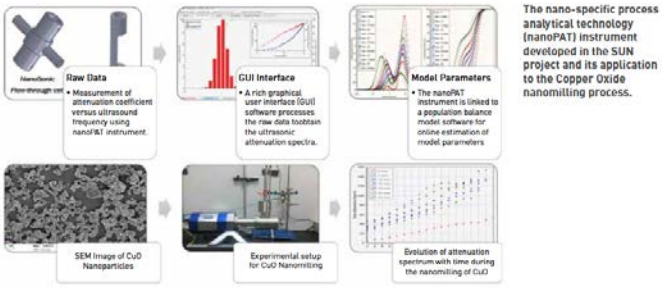
4) If the results from (eco)toxicity testing were useful to consistently predict a reduction of *in vitro* or *in vivo* toxicity along the established modes of action, performance evaluation was necessary to prove the sustainability of the proposed solutions and a cost-effectiveness evaluation also had to be performed.

The results from the performed activities provided useful data to support the assessment of nano-bio interactions and make hypothesis on mechanisms of toxicity with the real possibility to act on molecular design and drive adverse biological effects.

***Safe Process Design***

Safe nanomanufacturing is of crucial importance for sustainable nanotechnology, but the dynamic properties of nanomaterials require new analytical and assessment techniques. SUN partners developed a nano-specific Process Analytical Technology (nanoPAT) to measure the real-time evolution of particle size distribution. It is an online particle sizing system based on acoustic spectroscopy. Two different hardware configurations, a flow through cell and a probe, were developed and optimised. The flow through cell operating in transmission mode was designed in a way to ensure easy connection to a flow system while the probe was designed to be inserted into a crystalliser or a reactor. Rich graphical user interface software was developed using Windows Presentation Foundation graphical subsystem to automatically process the raw particle sizing data and to convert the attenuation spectra into the particle size distribution. SUN also developed a new population balance modelling software for online estimation of the process parameters. The nanoPAT instrument was linked to the population balance model software to allow online comparison of the measured and estimated particle size distributions during nano-processing operations.

The nanoPAT system was applied to the processing of α-alumina and CuO. The evolution of particle size distributions during the processing was measured online using the instrument and validated by two other particle sizing techniques, i.e. Dynamic Light Scattering, and Laser Diffraction. The instrument and the software packages have shown great promise in predicting and measuring the evolution of particle size distribution online during nano-processing. Undoubtedly, the ability to dynamically monitor the changes in the particle size distribution of nanoparticle solutions in real time is very significant in the establishment of an effective process control methodology to achieve and maintain desired quality parameters and process specifications.




The nano-specific process analytical technology (nanoPAT) instrument developed in the SUN project and its application to the Copper Oxide nanomilling process.

***End of Life Treatment and Waste Management Practices***

The SUN project specifically addressed the presence of MN in waste streams. The activities focused on three major aspects:

1. Identifying major waste materials and the waste treatment processes.
2. Analysing the factors influencing the release of nanomaterials during waste handling procedures.
3. Developing guidelines for safe handling of waste streams containing nanomaterials.

Using independently maintained websites such as nanodb.dk as centre of evidence, we identified waste plastic as the waste stream where nanomaterials could be found more frequently, while Silver is the nanomaterial most frequently present in this website. It is interesting to note that the legally required reporting of nanomaterial production or import in France prioritizes other nanomaterials much higher (as reflected by the SUN case studies).

The apparent contradiction is evidence of the challenges to understand and regulate nanomaterials. For the three scenarios analysed (i.e. Denmark, United Kingdom, and Europe), we estimated that recycling would be the end-of-life treatment option mostly involved in handling of MN, followed by either incineration (e.g. Denmark) or landfilling (e.g. United Kingdom). When analysing factors determining the release of nanoparticles during waste handling, we found that a range of different aspects should be considered in assessing the potential release, which depend on both the process under consideration (e.g. recycling, incineration, landfilling) and the specific waste material (e.g. plastic, paper, glass, metals). For recycling processes, we identified the following aspects: 1) hardness of the matrix, 2) temperature reached during the process, 3) the affinity of nanoparticles towards the air, solid, or liquid phase. For incineration processes, we assessed that release to the environment would be affected by the 1) combustibility of the matrix, 2) the melting/boiling/degradation points of nanomaterials in relation to the combustion temperature, and 3) the overall performance of the flue gas cleaning system as well as 4) the treatment of the solid residues (e.g. bottom ash, fly ash) from the system. With respect to landfilling, important aspects are: the degradability of the matrix, the affinity of the nanomaterials for the solid/liquid/air phases, mobility/aggregation of the nanomaterials, and finally the presence of a treatment system for landfill leachate.

Based on the identified factors, we developed waste treatment recommendations, which can be used for development of SbD products. These recommendations come along with the general need to better understand the streams of nanomaterials in products.

| RECYCLING | INCINERATION | LANDFILLING |
|---|---|---|
| • High melting/boiling points of the ENMs<br>• Low affinity for the liquid phase<br>• Limit the use of persistent ENMs<br>• Limit the use of ENMs in construction materials | • Low combustibility of the matrix<br>• High melting/boiling points of the ENMs<br>• State-of-the-art flue gas cleaning systems | • Non-degradable matrix<br>• Low affinity for the liquid phase<br>• ENMs not inhibiting aerobic and anaerobic processes<br>• State-of-the-art landfills with leachate treatment |

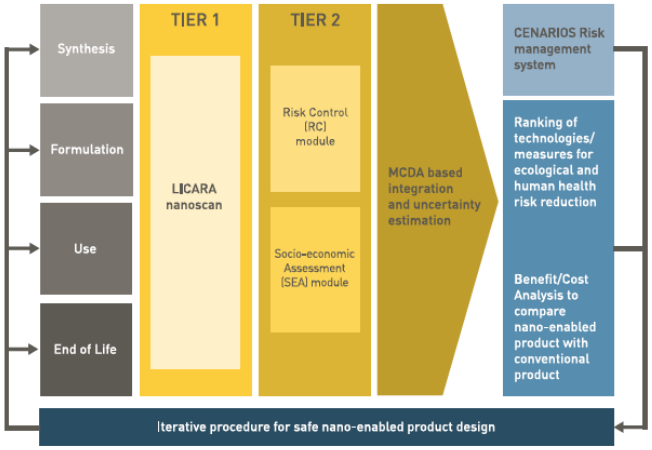
Overview of safe-by-design recommendations in relation to major waste treatment processes.

***Decision Support System for Risk Assessment and Management of Nanotechnology Products***

SUNDS is one of the highlights of the project. It is a user-friendly software system that estimates occupational, consumer and environmental risks from nanomaterials in real industrial products along their lifecycles. In situations when the risks are not acceptable SUNDS proposes suitable risk management measures, including information about their costs compared to the benefits of the nanotechnologies. SUNDS has been tailored to the needs of key stakeholders from e.g. industry, regulation and insurance who were engaged in a series of interviews and workshops to discuss its design and functionality.

SUNDS comprises Risk Control (RC) and Socioeconomic Analysis (SEA) modules. RC can be demonstrated by reducing risk to below threshold levels or by investigating feasible alternatives to the substance. If risks are not adequately controlled and no feasible alternatives to a substance are found, SEA is used to demonstrate that benefits of using a certain MN significantly outweigh the risks/costs.

SEA analyses all environmental, economic and social impacts, at both micro and macro levels. Integrating RC and SEA within the SUNDS allows its users to be guided on the technical and economic performance of risk management along the lifecycles of NEP.




SUNDS Conceptual Framework

In addition to the two tiers shown in the figure above a stand-alone module based on CENARIOS (Certifiable Nano-specific Risk Management and Monitoring System) standard was developed and included in SUNDS.

Tier 1 of the decision support system comprises of the NanoSCAN developed within the FP7 LICARA project specifically for SME that often do not have the resources and expertise to apply complex decision support systems. Therefore, NanoSCAN is a very user-friendly screening-level tool with relatively low data requirements that provides a semi-quantitative evaluation of the environmental, social and economic benefits and the ecological, occupational and consumer health risks of MN in products from lifecycle perspective. In addition, NanoSCAN can assist SMEs in checking supplier risks, competing products, market opportunities or making an internal risk and benefit analysis.

SUNDS Tier 2 implements an integrated RC and SEA module, consisting of the following sub-modules:

The Ecological Risk Assessment (ERA) sub-module derives ecological risk quantitatively by integrating outputs from: a) an environmental exposure model that estimates PEC in different environmental compartments (e.g. water, soil), and b) deterministic procedures or Species Sensitivity Distributions that estimates PNEC for various species in these compartments. Both models were developed in SUN based on work in previous projects.

The Public Health Risk Assessment sub-module estimates the risks for humans exposed to nanomaterials via the environment by integrating outputs from: a) the environmental exposure model described above, and b) deterministic and probabilistic procedures for dose-response assessment and intra/inter-species extrapolations (developed in SUN). The resulting estimation of human health risk will be always quantitative, but either deterministic (Exposure dose/Derived No-Effect Level (DNEL) >1) or probabilistic (i.e. 5% of the population has at least a 10% response with 95% confidence) depending on the nature, quantity and quality of the input exposure and effects data.

Occupational and Consumer Human Health Risk Assessment (HHRA), which derives occupational and consumer health risk by integrating outputs from: a) Human health exposure model (developed in SUN), that assess relevant occupational and consumer exposure scenarios according to three tiers (i.e. qualitative, semi-quantitative and quantitative) and taking into account the effect of applied risk management measures, and b) the above deterministic and probabilistic procedures for dose-response assessment and intra/inter-species extrapolations.

Environmental Impact Assessment (EIA) sub-module, which accepts LCA midpoints calculated as per explicitly specified LCIA methodology (e.g. ReCIPe, CML, etc.). The conduction and interpretation of LCA requires specific expertise; therefore in order to protect the user-friendliness of SUNDS it was decided not to program an LCA software and database within its platform, but to link it as external software to estimate impacts such as climate change, ozone depletion, terrestrial acidification, eutrophication, photochemical oxidant formation, particulate matter formation, ionising radiation, land use, water resource depletion, resource depletion, human toxicity and ecotoxicity.

Economic Assessment (EA) sub-module, which assesses microeconomic impacts due to NEP. Microeconomic impacts are at the individual company level, and implement a cost evaluation methodology for nanomanufacturing. BASF contributed to developing this module based on their SEEBALANCE tool, which is based on lifecycle costs from a consumer perspective.

Social Impact Assessment (SIA) sub-module, which assesses social impacts due to NEP. It focusses upon impacts due to workplaces, products and regional contexts. Social indicators are normalized to suitable impact classes and expressed in absolute terms. The company BASF contributed also to developing this module based on their SEEBALANCE tool.

In the Risk Control (RC) Module outputs of the ERA and HHRA sub-modules are integrated with the TARMM inventory by means of Multi-Criteria Decision Analysis (MCDA).

The Socioeconomic Assessment (SEA) module in Tier 2 which integrated outputs of ERA, HHRA, EIA, EA and SIA sub-modules,

each classified by the user as benefit or cost, to compare NEP to conventional products.

Integrating RC and SEA within SUNDS allows its users to be guided on the technical and economic performance of risk management along the lifecycle of NEP. This is particularly interesting for industry and SME as it will enable them to easily perform regulatory safety assessment and to make decisions concerned with product innovation. This can reduce their R&D&I costs and can enable them to compete more effectively.

To explore SUNDS go to http://sunds.dais.unive.it.

## 5 Impact beyond the project lifetime

The large-scale production and commercialization of nanotechnologies require an understanding of their environmental, health and safety impacts, and must develop strategies for their safe production, use and disposal. Today we still face challenges to understand and mitigate the potential risks from nanotechnologies. One of the main reasons for this is the fact that MN undergo complex transformations when incorporated in products and when released from them in occupational, consumer and environmental settings. The overall impact of SUN is to provide industries and regulators with data and tools to address these challenges. The project achieved this through development and application of methods for: 1) prediction of release of MN from industrial processes, consumer products, end-of-life processing and waste; 2) estimation of the longer-term effects of released and weathered MN in ecosystems and in humans; 3) occupational and consumer exposure and risk assessment; and 4) risk prevention and control. The project has covered the entire lifecycles of MN and has developed safer by molecular and process design strategies.

We carefully scoped the data generation in SUN to achieve high impact by addressing key concerns of industries and regulators. The markets covered by the SUN case studies $TiO_2$, $SiO_2$ and organic pigments used in plastics and fillers are large: 235,000,000 tons/year of plastics worldwide, thereof € 295 billion worth sales and 1,450,000 jobs in Europe; pigments: 317,000 tons, worth €4 billion; and nano-fillers: 242,000 tons. Because the highest profit margins for material producers are in the formulation and synthesis of compounds we have focused the SUN activities on these steps of the supply chains. The sintering ceramic material WC was selected to ensure the applicability of the methods developed in the project also to the impressive portfolios of the cement/concrete and fillers industries. The rest of the materials were selected less for their commercial impact, but because of their very considerable consumer and environmental safety impact: CuO, Ag: fewer than 1,000 tons/year, but of high ecological concern; MWCNT: less than 300 tons, but of high human health concern.

In addressing the risks from some of these materials (i.e. CuO and WC), SUN has shifted the research focus from risk assessment to risk prevention by developing safer by molecular and process design strategies. To increase the safety of these materials without compromising their successful scale-up we also assessed their performance and tried to keep it in commercially viable ranges. In addition, we developed approaches for Inherently Safe Process Design based on a nano-specific Process Analytical Technology, which was improved in SUN and applied to case studies to analyse the evolution of key product quality parameters and process conditions in real time. The obtained results were useful to establish mechanisms to control these parameters in order to increase the safety of production.

To ensure that the above risk prevention and management solutions will effectively guarantee the MN fate, we studied the release of MN from industrial and waste products. The obtained results helped us to partially answer the question of regulators whether the processes studied in the laboratory have relevance to the real-world situations, which has had impact in the implementation of the REACH regulation for MN. The release and exposure data produced in SUN have been also very important for industries to analyse the overall risk and environmental impacts of their products in order to understand where they stand in terms of safety and environmental performance and therefore refine their R&D investment and marketing strategies. Specifically, our industrial partners have used these results to benchmark the environmental performance of their nanotechnology products against conventional alternatives. This has already had a huge impact on their product development, resulting not only in reduction of environmental burdens but also on developing innovative technological solutions. These results are also valuable for regulators to help them estimate the risk-benefit ratios of these technologies.

To base the above mentioned innovative solutions for risk prevention and control on robust experimental data, SUN has completed the development of tools to analyse the long-term effects of pristine, released and aged MN on humans and ecosystems. In doing this SUN has targeted some of our most vulnerable ecosystems and extended environmental risk assessment to cover longer-term realistic scenarios of ecosystems subjected to multiple stressors, including accumulation and contaminant transfer in the food chain up to humans. SUN also studied several important ecosystem services that are essential to public health and society, including the effects of MN on sewage sludge treatments (e.g. aerobic and anaerobic treatment before agricultural use), which provided data of great benefit for utility companies and regulators. These tools enable a fast and direct estimation of risks as well as ability to alter production in time to minimise them. The coverage of the three main environmental media (water, sediment and soil) deals both with the media receiving MN and with vital ecosystem services such as wastewater treatment, food production and genetic pool/variation. The obtained results have had a significant impact on developing the field of ecological risk assessment in general and on the implementation of the REACH Chemical Safety Assessment guidance for MN.

The (eco)toxicity testing in SUN has been to a great extend based on existing standards (e.g. OECD, ISO), often aiming at improving them and contributing to the development of new standards. Specifically, regarding the newly developed in vivo test protocols (e.g. STOS, tests for enchytraeids and fish) we have looked for compliance with the existing standards that industry and regulatory agencies are already familiar with (e.g. OECD 407: Repeated Dose 28-day Oral Toxicity Study in Rodents; OECD 220: Enchytraeids test; OECD 210: Fish, Early-Life Stage Toxicity Test). Similar approaches were adopted for the terrestrial multispecies test systems to support the development of OECD guidance also for them. Our partners (e.g. INIA, UNIVIE and IME) have been involved in the OECD WPMN and have contributed to the adaptation of various OECD test guidelines based on results from SUN. Specifically SUN contributed to the OECD Technical Guidance (TG) document on multigenerational tests with Enchtraeids, forming the basis for long-term toxicity tests covering epigenetic

effects. In this regard, a Standard Operating Procedure (SOP) enabling validated markers for methylation status (correlated with phenotypic/reproductive toxic consequences) and high-throughput tools for gene expression were developed. Moreover, the SUN results were used to develop the OECD TG on testing dispersion stability and dissolution (kinetics) as well as the OECD environmental fate decision tree and the corresponding guidance document.

In addition to contributing to standardisation activities SUN generated high-impact results in the areas of human health hazard and risk assessment. For instance, SUN focused on investigating the epigenetic effects of MN both in vitro and in vivo. In this regard, we established a strong collaboration with Health Canada, who dedicated their own resources to testing an array of samples from SUN. This provided an excellent opportunity to gain epigenetic information and guidance on how to use this information for risk assessment. This and the newly developed in vivo protocols (e.g. STOS, tests for Enchytraeids and fish) will have a significant impact on the regulatory risk assessment of MN.

SUN significantly progressed beyond the state of the art of workers' and consumers' inhalation, dermal and dermal-to-oral exposure assessment by developing an exposure assessment and modelling framework and toolbox. This toolbox will have major impact on the risk assessment of MN for regulatory purposes and the implementation of the ECHA guidance on Chemical Safety Assessment for MN.

The knowledge acquired in the project and derived from other sources has served as the basis for developing SUNDS. This decision support system will be of significant practical value for both industries and regulators since it would make it possible to integrate technical data about the risks, benefits and costs of MN into a sustainability portfolio to make informed decisions about how to address their safer production, handling and end of life treatment. It can also aid industries in making decisions whether to invest in developing new nanotechnology products. In addition, SUNDS will have practical impact on the work of regulators as it will enable them to prioritize MN based on their risk profiles and select the most adequate risk mitigation measures. In cases when this is impossible, SUNDS will help regulators to compare the risks of MN to their potential benefits. The decision support system is also particularly relevant for SME as it will enable them to easily perform regulatory safety assessment and to make decisions concerned with risk management and product innovation. This will reduce their R&D&I costs and will enable them to more effectively compete with larger industries. Moreover, the application of SUNDS and its underlying tools will reduce uncertainty in the early stages of innovation and will improve risk communication. This will lead to a more positive market interpretation and the perception of safe/responsible innovation will result in better business cases. The application of the decision support system and the risk prevention strategies developed in the project will help industries by providing input into the design of safer products and processes. This will lead to safer workplaces and products, and will facilitate compliance with regulations.

The pressure to assist companies in making technically challenging decisions about safety of their products has increased proportionally to the evolution of regulations. While there are a small number of consultancies providing safety assessment support to businesses, those are limited in terms of the analytical tools they can use, and in terms of the scope and the materials for which they can provide advice. The SUN strategies, methods and the SUNDS could be used by these consultants or directly by the businesses for risk analysis and/or innovation decision making. The same also applies to researchers working in academia who design, develop or use MN or NEP. In addition, regulators in a variety of sectors (e.g. consumer products, cosmetics, food, medicines, chemicals and substances) can use the SUN tools to do their own safety assessments and decision-making.

One barrier to nanotechnology innovation is the relatively slow but constant evolution of relevant regulations and standardisation activities. These processes require input/agreement from multiple stakeholders across national borders. Involvement of key players from ECHA OECD, U.S. EPA and Health Canada in our Advisory Board and the organisation of several workshops with regulators has ensured that the SUN results contribute to regulation. By working closely with representatives of regulation, standardisation, industry and research communities we transparently integrated their input into our research processes, and worked to develop trust in our research products. In addition, the respective skills needed to implement these scientific approaches were developed through training activities targeting these communities. Intensive dissemination of the project's results raised safety awareness and promoted a debate on how the SUN results can help companies in bringing their products faster to the market while complying with regulatory requirements. This will eventually speed up the evolution of the nanosafety regulations and will increase the market confidence in these technologies and their acceptance by businesses and consumers.

SUN has had a significant impact on research cohesion and integration with other nanosafety projects. For instance, intense collaboration has taken place with NANoREG, eNanoMapper, GUIDEnano, NanoSolutions, NanoMILE and caLIBRAte to avoid overlaps, strengthen complementarities and create synergies among the projects. This has led to the organisation of major international conferences, workshops and initiatives in the areas of nanosafety data management, risk assessment and governance of nanotechnologies. Moreover, SUN partners are leading and/or have been actively engaged within all working groups of the European Nanosafety Cluster and the EU-U.S. Communities of Research. SUN has also considerably contributed to strategic research roadmaps in the nanosafety area.

## 6 List of Publications from the project

The following figure provides an overview of the SUN publications already published (96) or submitted in the last six months of the project (+55).


Selected publications:

Amorim, M.J.B. The Daunting Challenge of Ensuring Sustainable Development of Nanomaterials. Int. J. Environ. Res. Public Health 2016, 13, 245.

Baldisserri, C.; Costa, A. L., Electrochemical detection of copper ions leached from CuO nanoparticles in saline buffers and biological media using a gold wire working electrode. Journal of Nanoparticle Research 2016, 18 (4), 96.

Bicho, R. C.; Ribeiro, T.; Rodrigues, N. P.; Scott-Fordsmand, J. J.; Amorim, M. J. B., Effects of Ag nanomaterials (NM300K) and Ag salt (AgNO3) can be discriminated in a full life cycle long term test

with Enchytraeus crypticus. Journal of Hazardous Materials 2016, 318, 608-614.

Bicho, R. C.; Santos, F. C. F.; Scott-Fordsmand, J. J.; Amorim, M. J. B., Effects of copper oxide nanomaterials (CuONMs) are life stage dependent – full life cycle in Enchytraeus crypticus. Environmental Pollution 2017, 224, 117-124.

Roelofs, D.; Bicho, R. C.; de Boer, T. E.; Castro-Ferreira, M. P.; Montagne-Wajer, K.; van Gestel, C. A. M.; Soares, A. M. V. M.; van Straalen, N. M.; Amorim, M. J. B., Mechanisms of phenanthrene toxicity in the soil invertebrate, Enchytraeus crypticus. Environmental Toxicology and Chemistry 2016, 35 (11), 2713-2720.

Boldrin, A.; Hansen, S. F.; Baun, A.; Hartmann, N. I. B.; Astrup, T. F., Environmental exposure assessment framework for nanoparticles in solid waste. Journal of Nanoparticle Research 2014, 16 (6), 2394.

Nowack, B.; Boldrin, A.; Caballero, A.; Hansen, S. F.; Gottschalk, F.; Heggelund, L.; Hennig, M.; Mackevica, A.; Maes, H.; Navratilova, J.; Neubauer, N.; Peters, R.; Rose, J.; Schäffer, A.; Scifo, L.; Leeuwen, S. v.; von der Kammer, F.; Wohlleben, W.; Wyrwoll, A.; Hristozov, D., Meeting the Needs for Released Nanomaterials Required for Further Testing—The SUN Approach. Environmental Science & Technology 2016, 50 (6), 2747-2753.

Brunelli, A.; Zabeo, A.; Semenzin, E.; Hristozov, D.; Marcomini, A., Extrapolated long-term stability of titanium dioxide nanoparticles and multi-walled carbon nanotubes in artificial freshwater. Journal of Nanoparticle Research 2016, 18 (5), 1-13.

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# 7 Copyright

# Introduction of new Nanosafety Cluster structure

# and

# Updates from EU NanoSafety cluster Working Groups (WGs)

# Introduction of revised Nanosafety Cluster Working groups

The Nanosafety Cluster (NSC) has acquired a strong identity over the past 10 years and, moving forward under a new leadership (Eva Valsami-Jones, University of Birmingham (UoB) coordination of the Horizon2020 ACEnano project, supported by a coordination team consisting of Flemming Casee (RIVM), Andreas Falk (BioNanoNet) and Iseult Lynch (UoB), it must capitalise on this identity by becoming more ambitious and outreaching, with clear structures for allocating tasks to projects and greater responsibility for ensuring delivery of the allocated tasks by projects.

The NanoSafety Cluster Working Groups (WGs) have, from the outset of the cluster, provided a focus for the bottom-up activities of the NSC and a means for collaboration between the running projects. The original 7 WGs were expanded as new topics / projects emerged, leading to 11 WGs by 2016, with varying degrees of activity and overlap. As part of the refreshing of the NSC, it was agreed as to reduce the number of WGs to 6, by merging related topics, removing areas that were inactive or no-longer relevant, but retain aspects that are working well (such as the exposure and databases WG). The communication and dissemination activities, formerly undertaken within WG7 – dissemination are retained by via the Communications Group rather than as a WG, given the importance of these activities (such as the newsletter, the compendium etc.) as **Core Elements** of the NSC. The new WGs, and their proposed scopes, are presented in in Figure 1 below.

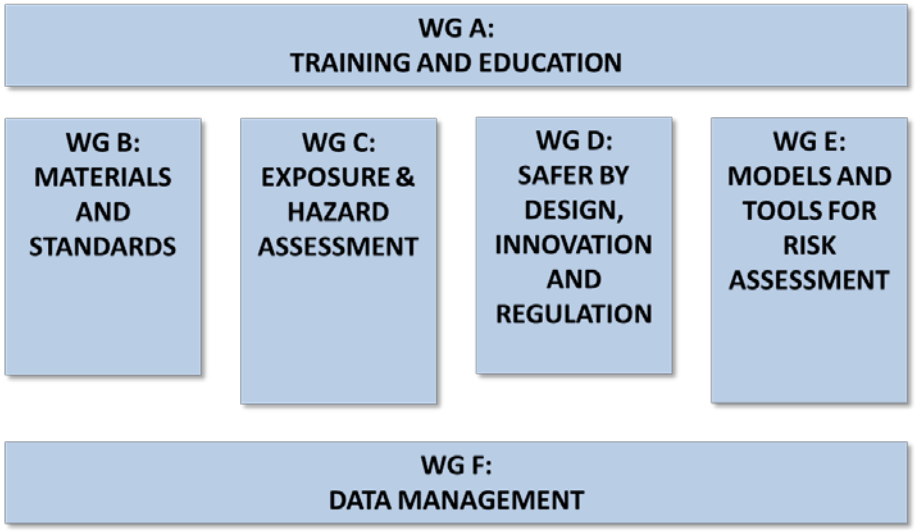


**Figure 1:** Proposal for merging and reformation of NSC Working groups

As the new WGs have not yet started, the previous WGs provide an update on their activities over the last year and some suggestions for activities that the new WGs can take forward.

## WG A: TRAINING AND EDUCATION

WG **Training & Education** was formerly a sub-group of WG7 Communication, but is now a stand-alone WG. Its focus will be on developing strategies for harmonisation of training provision across NSC projects, facilitating training secondments for young researchers, organisation of the Young Nanosafety researchers biannual events, as well as beginning a focus on development of Continuous Personal Development (CPD ) and stakeholder-focused events (e.g. for SMEs, for regulators etc.). Liaison and interaction with EC4SafeNano training activities also needed.

## WGB: MATERIALS AND STANDARDS

WG **Materials and Standards** will include the old WG1 materials, and the Standardisation sub-group from the old WG7 (which is no longer a working group but is replaced by the Communication cross-cutting core activity). Activities could focus on provision and

upscaling of nanomaterials libraries, support for pilot lines projects, as well as supporting applications of nanomaterials in related areas such as counterfeiting, traceability etc.

### WG C: EXPOSURE & HAZARD ASSESSMENT

WG **Exposure & Hazard Assessment** merges the old WG 2 (Hazard), WG3 (Exposure), and WG8 (Systems Biology). Given the breadth of the topic, it is suggested that retaining a set of focus groups here (but with clear tasks and timlines) and should ensure cross-talk where appropriate. Focus here should be on consensus building, agreement on protocols, validation of new and alternative (*in vitro*) methodologies, and translation to regulation, etc.

### WG D: MODELS AND TOOLS FOR RISK ASSESSMENT

WG **Models and Tools for Risk Assessment** integrates the old WG5 and WG6. A focus should be on integrating existing tools, and developing strategies for community evaluation of the tools to build (regulatory) acceptance.

### WG E: SAFE BY DESIGN, INNOVATION AND REGULATION

**Safe by Design, Innovation and Regulation** working group integrates WG9 and WG10, with a focus on regulatory aspects in due course as they develop sufficient critical mass of activities. The proposal is to have one sub WG "Innovation" and the other subgroup on "Regulation". Safety-by-design (SbD) is a "tool" that underpins both of these activities.

### WG F: DATA MANAGEMENT

**Data management** is the old WG4 (Databases), which is functioning well, but this change enhances its status as a cross-cutting WG that all other WGs need to feed into / cooperate with and highlights its important role in securing longevity of access to NSC datasets. This working group would also take up the responsibility of securing and sharing data produced by finished projects.

Decision are needed from the Steering Committee as to whether some of these activities should become "NSC *Core Activities*" and thus be prioritised for support by specific NSC projects and as topics for dedicated NSC funding applications (call topics).

- Maintain and expand the nanosafety ontology (follow-up eNanoMapper).
- Data Mining of data from ongoing or completed projects, and their storage
- Data curation: monitoring the quality of the available incoming data
- Ensure continuity of data storage and accessibility of data
- Control of the formal aspects of data management; include: commitment to providing data, what type of license; removal of existing formal barriers to data sharing. Note also that interaction with EC4SafeNano is essential, as they are planning a route from research to regulation also.

## Role of the NSC WGs

The WGs function to:

- Address environmental, health, and safety questions about nanomaterials and to collaboratively advance the field
- Identify gaps in knowledge and research priorities for future funding
- Address collaboratively an agreed programme of activities, which can be linked with activities of the EU-US CoRs or can be bespoke activities designed to bridge the activities planned in the collaborating projects to enhance their interconnectivity.
- Agree protocols, templates, methodologies, etc., including for data harmonisation.

The work done in WGs will continue to be funded by the running FP7/Horizon2020 projects (but with a clear nominal (effort per project) budget determined at the start of each project / activity in order to ensure they are realistic, and to allow identification of a suitable delivery date).

The WGs will be largely self-run with the NSC Steering group providing administrative support. Future funding will be sought to strengthen the inter-WG-collaboration (e.g. via an EU coordination support action). A call for Chairs for the new WGs will be issues via the NSC website and agreed via the NSC Steering Committee, for announcement in late summer 2017.

# Working Group Number: 1

## Working Group Name: Materials

Website: http://www.nanosafetycluster.eu/working-groups/materials-wg.html
Chair: Sergio Moya Co-Chair: Rune Karlsson

*Table 1 Working Group Contributing projects List.*

| No. | Project Acronym | Role in Working Group | Representative |
|---|---|---|---|
| 1 | NanoSolutions | Safety classification | Sergio Moya |
| 2 | NanoDefine | Methods development | Rune Karlsson |
| 3 | NanoDefine | Nanomaterial definitions | Rudolf Reuther |
| 4 | NanoMILE | Nanomaterials libraries | Douglas Gilliland |
| 5 | FutureNanoNeeds | Nanomaterials libraries | Wolfgang Parak |
| 6 | NanoDefine | Batch Variability | Hans Marvin |

# Contents



## 1 Scientific and technological challenges

Existing test methods are not likely to enable safety assessments of the numerous novel nanomaterials (NMs) and products based or entailing NMs that are emerging at an ever increasing pace. The international dialogue and collaboration activities have helped to understand the complexity of EHS aspects, further highlighting the need for joint international efforts in developing protocols and test methods to assess the health and safety impact of NMs and to provide proper characterization methods and protocols that are relevant for the NMs description in view of the toxicological tests.

The necessity for an improved characterisation of NMs with a minimum of physico-chemical properties necessary to describe the NMs for toxicological testing has recently emerged as a crucial aspect for the reliable assessment of the risks associated with ENP-handling and potential emission of NMs from products and devices including nanomaterials in their formulations through their life cycle . However, to date there is no clear understanding about what minimum requirements are necessary to establish a reference material (RMs)and the best set of properties necessary for the NM description which can be used to validate and compare toxicology methods. Actual approaches to RMs for nanotoxicology are based on the certification of one parameter, either size or composition, and frequently ignore other important characteristics such as particle size distribution in real testing media, nanomaterial stability, degradation, aging, protein corona formation, etc. One-parametric RMs are probably insufficient for the realisation of reliable and comparable toxicological studies.

## 2 Objectives (short, medium & long term)

Short term: Worldwide, various groups e.g., governments, treaty-based organisations, standards development organisations, and research consortia, have more or less independently generated priority lists of NMs for the potential development of RMs, and created lists of characterisation requirements for the RMs to better understand the results of exposure and toxicity assessments. It is generally recognised that one of the principal obstacles to obtaining adequate characterization of NMs and their potential risk is the scarcity of reliable RMs (that is, RMs produced in a scientifically valid way) for the development and validation of exposure assessment tools (instruments, protocols, methods) and toxicological evaluations (materials, protocols). RMs can provide researchers with suitable materials (including positive and negative controls) to develop harmonised protocols for in vitro and in vivo toxicity testing and elucidate mechanisms of toxicity resulting from nanoscale properties. Also, materials to verify instrument or method performance and operator or laboratory proficiency can be made available.

Medium term: There have been multiple efforts to define NMs, including a focus on defining them for regulatory purposes which enables products containing NMs to be identified and regulated with limited success to date. Most of these definitions focus solely on size aspect at the nanoscale with some also including surface, area and shape. There have also been several suggestions for approaches to classify and prioritise NMs for safety assessments,

including the OECD Sponsorship Programme approach based on commercial importance and volume of production.

Long term: Some of the longer term required research priorities identified for material characteristics include: development of systematic sets of ENMs with physical-chemical properties varied in a stepwise manner allowing the assessment of the significance of each property for toxicity; descriptions of "reference" states and media compositions to enable the identification of significant biomarkers and facilitate a move towards a predictive toxicity assessment; development of reference analytical methods that enable the studying of the longer term fate of particles following their interaction with living systems, i.e. complex matrices and developing risk assessment procedures that include the changes of ENM during their life cycle in a targeted manner. The emphasis in the EU Commission's definition on NMs is on external dimensions, which may result in the exclusion from the definition of materials with an internal structure (e.g. porous materials with a relatively large internal surface area) or materials with a surface structure at the nanoscale. Therefore, further information is necessary on the interpretation of information on NMs in products and the impact of porosity (internal surface area) on the hazard of NMs. Closely related to the problems associated with the definition of NMs is the choice of a proper metrics for NMs. Particularly complex is how to define the metrics to measure nanomaterial concentration for toxicological testing: particle number, surface area, element concentration, etc. The proper detection, quantification and characterization of NMs are critical prerequisite tests for the safety assessment of the materials under analysis.

1. *Classification by dimensionality / shape / morphology:*

Shape-based classification is related to defining NMs, and has been synopsised in the ISO terminology. The dimensionality relevance is high-lighted with the recent development of graphene, which can be considered a two dimensional material. Shape and morphology are associated concepts. Nanomaterial shape if round, elongated, and morphology, if the NMs entails peaks, defects or display a rough surfaces, can impact on the toxicological response.

2. *Classification by composition / chemistry:*

This approach groups NMs based on their chemical properties. NMs are made upon organic molecules, polymers, metal/metal oxides and carbon in SP2/SP3 hybridisation or by a combination of some of these elements. NMs often offer a core shell structure. NMs are often engineered with a coating to modify their surface properties. Changing the surface chemistry for the same core may results on very different toxicological end points.

3. *Classification by complexity / functionality:*

Currently, the NMs that are in routine use in products are likely to be displaced by NMs designed to have multiple functionalities or resulting from hierarchical fabrication, displaying supramolecular organization, etc. ; the so called 2nd-4th generation NMs.

4. *Classification by biointerface:*

There is also the hypothesis that NMs acquire a biological identity upon contact with biofluids and living entities, formed by proteins, lipids and sugars.

Multiple reports have identified sets of physical-chemical parameters that should be reported for NMs. However, not all properties are relevant for all NMs in regards to their toxicological evaluation, and many are not easily measured on a routine basis. An additional challenge is the fact that many of the physical-chemical properties of NMs are dependent on the environment and, as such, will be subject to change, depending on the surroundings in which the ENM are presented. The distinction between the synthetic and biological identities of NMs is therefore suggested. The synthetic identity describes the chemical, structural and compositional nature of the nanoparticles, including any surface coatings, ligands or labelling molecules; the *biological identity* describes the bio molecules that absorb to the nanomaterials under specific conditions and the impact of these on their dispersion properties

-Long term: A full understanding of the key descriptors for characterising ENM along with validated methods to identify and quantify ENMs in complex matrices is vital in order to identify crucial parameters relevant for risk assessment. This is also important for the measurement of the relevant ENM properties that correlate exposure with biological impacts. This will require agreed reference states for NM characterization, libraries of reference materials, and a framework for understanding later generation NMs.

The required research priorities to achieve this are to:

1. Develop systematic sets of ENMs with properties varied in a stepwise manner that will allow assessment of the significance of each property for toxicity.

2. Describe "reference" states and agreed media compositions to enable identification of significant biomarkers and enable a move towards a predictive toxicity assessment.

## 3 Progress and Outcomes to date

NanoDefine hosted the 2nd NSC Synergy Workshop in Brussels on 2nd February 2016. The event was attended by experts from a number of EU projects in addition to NanoDefine, including NANoREG, NanoSolutions, SUN, GUIDEnano, NanoMILE, eNanoMapper, NanoMag, NanoDetector and NanoValid. The aim was to identify overlaps and synergies existing between different projects that could develop into cooperation opportunities. Among the topics discussed were the exchange of test and reference materials, protocols and SOPs for characterisation method testing, validation and standardisation, and planning of joint ILC studies. The number one interest of NanoDefine is to get as much as possible reliable information related to sizing techniques, e.g. DLS and EM. The particular field of BET is also of importance due to evaluation of size from BET measurements in NanoDefine. Another point as critical as the sizing techniques themselves are sample preparation SOPs. Uniform protocols for at least some classes of materials are clearly needed.

In the framework of the NanoMILE-NanoSOLUTIONS meeting in Stockholm, Sweden, issues on material selection, characterization and SOPs for dispersion and characterization in both projects were discussed among the participants. The different criteria for material selection among the projects were analyzed.

Dr. S.E. Moya participated in the scientific committee of the 2nd Nanosafety Forum for Young Scientists in Li, Sweden 15-16 september 2016. Materials was one of the topics adressed by the presentation.

There has been a continuous discussion on materials issues related to nanotoxicology among the member of the working group in the different meetings of the involved projects and in the nanosafety cluster meetings.

## 4 Final status report

WG 1 Materials has aimed at contributing to a harmonised terminology regarding nanomaterials definition, metrics, etc., since at the moment this hinders accurate description of many nanomaterial properties. There has been a continuous discussion within the cluster and with different stakeholders to build consensus within the nanotechnology, and environmental, health and safety communities to prioritise reference methods needs and better define the required properties and physical-chemical characteristics of possible materials for the description of the NMs in a toxicological context.

The WG has also aimed to further, discuss and clarify that where RMs are not available if "representative test materials" that lack reference or certified values may be useful for toxicology testing towards establishing validated methods for the characterisation of nanomaterials as well as for their delivery in relevant media and administration to toxicological models.

## 5 Suggestions actions for new WGs

Activities from the WG materials that can be transferred to future WPs include:

- Continue with the compilation of materials list that are under consideration/development as RMs from various groups;

- Continue with the reviewing of literature to identify which physical chemistry properties are priorities for risk assessment of ENPs; search on new developments on material science that could be of interest for the nanosafety community.

- Continue discussion on the physico chemical characterization issues related to 2$^{nd}$ to 4$^{th}$ generation nanomaterials in a nanosafety context.

- Promote discussion among the participants in the WG and with other WGs within the cluster on issues related to the materials in the perspective of nanosafety evaluation in cluster meetings, project meetings and scientific/innovation venues.

- Foster discussion on materials issues in the nanotoxicological field within the broader scientific community.

## 6 Copyright

# Working Group Number 3

## Working Group Name: Exposure

Website: https://www.nanosafetycluster.eu/working-groups/3-exposure-wg.html
Chair: Wouter Fransman Co-Chair: Socorro Vázquez-Campos

*Table 1 Working Group Contributing projects List.*

| No. | Project Acronym | Role in Working Group | Representative |
|---|---|---|---|
| 1 | GUIDEnano | Chair/Co-chair | Wouter Fransman<br>Socorro Vázquez-Campos<br>Claus Svendsen<br>Jean-Francois Damlencourt |
| 2 | SUN | Contributor | Keld Alstrup Jensen |
| 3 | FutureNanoNeeds | Contributor | Wouter Fransman |
| 4 | NanoSolutions | Contributor | Socorro Vázquez-Campos |
| 5 | NanoReg | Contributor | Martie van Tongeren<br>Jérôme Rose |
| 6 | nanoFase | Contributor | Claus Svendsen |
| 7 | CaLIBRAte | Chair/Contributor | Keld Alstrup Jensen<br>Wouter Fransman<br>Socorro Vázquez-Campos |
| 8 | NanoMile | Contributor | Iseult Lynch |
| 9 | EC4SafeNano | Chair | Wouter Fransman |
| 10 | NanoSTREEM | Chair | Wouter Fransman |
| 11 | NanoValid | Contributor | Pilar Lobera |
| 12 | Smart-Nano | Contributor | Stefano Cattaneo |

# Contents



## 1 Summary

The Working Group has a common main objective, i.e. promoting of harmonization and validation of methodologies. It has three subgroups dealing with release, human exposure and environmental exposure, respectively. All three subgroups are currently well coordinated between projects and overarching efforts are made to achieve this objective. Databases for harmonized storage of exposure data have been developed and sharing of data has been initiated within and between EU funded projects.

## 2 Scientific and technological challenges

Key for development of predictive exposure modelling is the harmonization of (human) exposure assessment methods and sharing of exposure data. Joint efforts with regard to data generation have encouraged sharing of state-of-the art methodologies and data, which facilitates the future of exposure assessment (modelling).

## 3 Scope and Objectives

To promote harmonization by encouraging sharing of project methods, techniques/results/data and by coordination through various means and activities.

To encourage cross project technical best practice to extract, prepare and analyse samples, to characterise and quantify NM exposure properties in real product lifecycle, exposure and environmental samples.

To encourage sharing of project results/data by coordination of transfer and hosting of libraries/databases.

Design and validate tests to monitor release from nano-enabled products at the different stages of their life cycle. Work with the European and International scientific community to harmonize these tests.

Evaluate and minimize exposure driven risks associated to nanotechnologies of adverse or unexpected effects towards public health and the environment (risk management strategies). Propose safer-by-design solutions to reduce exposure driven human and/or environmental health risks

Provide industry and legislators with validated methodologies for exposure assessment to be integrated in future regulations as an RTD requirement in the pre-market testing for novel nanoproducts and applications.

## 4 Progress and Outcomes to date

*Human Exposure*
Started from developed techniques and concepts for particulate exposure and control banding – now has 10 years of Nano relevant research with datasets of human exposure – in the phase of harmonizing and consolidate data collection, databases and sharing (NanoREG, SUN, GUIDEnano, Prosafe, Enanomapper, CaLIBRAte) using NECID (Nano Exposure and Contextual Information Database) as a shared structure to collate and share exposure data as well as a range of specific quantitative exposure modelling tools for NMs. Currently, between-project coordination and tuning with respect to human exposure assessment efforts has been achieved.

Recently, a joint NanoReg, SUN and GUIDEnano measurement campaign was concluded and a peer reviewed publication has been written. Review of risk management measures (RMM) along the lifecycle of nano-enabled products within the SUN and GUIDEnano projects has been performed.

*Release*
Common efforts in several European projects in the direction of developing novel methodologies to monitor releases from nano-enabled products (GUIDEnano, SUN, Nanosolutions, NanoREG)

Compilation of all the existing measurement methodologies and instrumentation used for human and environmental exposure monitoring, including methods to identify nanomaterial-based releases from complex matrices (NanoREG)

Refinement of current production volumes for nanomaterials at country level (NanoFase). Release factors refinement for environmental exposure models (NanoFase)

Revision and compilation of exposure data generated in different case studies in all the previous Nanosafety projects and level of data availability (NanoFase, caLIBRAte). Collection of data in a harmonized way for validation of air dispersion models via indoor and outdoor combined field campaigns (NanoFase, caLIBRAte, NanoREG 2).

*Environmental Fate and Exposure*
Progressing in technical developments to extract, prepare and analyse samples, to characterise and quantify NM exposure properties (especially those related to transformations) in real environmental samples – time to share experiences and develop common best methods, techniques and practice.

*Dissemination*
Frequent communication with other NSC WGs and US-EU COR "Exposure through Product Life" and collaborative workshops and training events for outreach outside the NSC such as:

- Joint NanoFASE, Innanopart and COST action – SP-ICPMS, Course RIKILT, Jan 2017;
- Joint conference SUN / GUIDEnano, Feb 2017, Malaga;
- Joint SUN/CaLIBRAte stakeholder workshop, March 2017, Venice;
- 1st Open NanoFASE stakeholder workshop and training course - Sept/Oct 2017;
- Open NanoFASE workshop/conference on airborne ENM: Measurements, Implications and Modelling - Nov/Dec 2017;
- Joint GUIDEnano, NanoREG and SUN peer reviewed publication on joint measurement campaign on release, exposure, and dermal deposition;
- CEN TC 137 WG3 standardization of methodology Initiative on "Exposure driven RA"
- review/position paper with US-EU COR.

## 5 Final status report

Human exposure assessment has evolved over the last decade from qualitative exposure estimation in control banding tools to quantitative exposure assessment models, which are underpinned with measured exposure data. The harmonized collection and storage of these exposure data (with multiple measurement instruments measuring various exposure metrics) has supported this process and will further facilitate the future sharing, use and comparing of exposure measurement data, for multiple purposes (compliance testing, exposure assessment modelling, epidemiology, risk assessment and risk management).

## 6 Suggestions actions for new WGs

We foresee the further harmonization and consolidation of data collection, databases and (open access) sharing using NECID (Nano Exposure and Contextual Information Database) as a shared structure to collate and share exposure data as well as databases for release factors and fate characteristics for model/tool validation purposes. This will facilitate the development and validation of exposure assessment models that will support industry and SME in their risk assessment strategies. The future merging of Working Groups on exposure and hazard will further enhance this collaboration between exposure and hazard assessment, which will derive quantitative risk assessment of nanomaterials.

## 7 Copyright

# Working Group Number 4

## Working Group Name: Database

Website: https://www.nanosafetycluster.eu/working-groups/4-database-wg.html
Chair: Nina Jelaizkova

*Table 1 Working Group Contributing projects List.*

| No. | Project Acronym | Role in Working Group | Representative |
|---|---|---|---|
| 1 | eNanoMapper, NanoReg2 | Chair since 2016-10 | Nina Jeliazkova (IDEA) |
| 2 | EnanoMapper, OpenRiskNet | Chair until 2016-10 | Egon Willighagen (Maastricht University) |
| 3 | NanoSolutions | | Peter Ritchie (IOM) |
| 4 | SUN | | Shahzad Rashid (IOM) |
| 5 | NECID | | Johannes Pelzer (IFA) |
| 6 | MARINA, NANoREG, eNanoMapper | | Axel P. Mustad (NQCG) |
| 7 | NANoREG, ProSafe | | Hugues Crutzen (JRC) |
| 8 | NANoREG, ProSafe | | Camille de Garidel-Thoron (CEREGE) |
| 9 | CERASAFE | | Mar Viana (IDAEA-CSIC) |
| 10 | caLIBRAte | | Martine Bakker (RIVM) |
| 11 | GUIDEnano, SUN, MARINA, CaLIBRAte | | Wouter Fransman (TNO) |
| 12 | NanoMILE | | Jan Kirrbach (Biomax) |

*WG4 mailing list has in total 96 participants. That list is available to list members only.*

## Contents



## 1 Summary

The Databases WG is tasked with agreeing on data interoperability and a common language (one or more ontologies) for European databases for project results and data, that will ensure longevity of data (beyond individual project lifetimes) and enable wide uptake and accessibility of the data to modellers, risk assessors, life and health scientists and other researchers, as well as being able to communicate with other international databases. WG4 organized in the past year a series of monthly conference calls, exchanging ideas and updates from various FP7 and H2020 projects, and collaborated on various tasks. Its main communication channel, the mailing list, features a total of 96 participants. The monthly conference calls are attended by, on average, 10 participants. Two new tasks were initiated, with the objectives to continue the work on community agreed data templates and eNanoMapper ontology.

## 2 Scientific and technological challenges

The goal of data interoperability requires overcoming scientific, technological and social challenges. The past year of NanoSafety Cluster is characterized with high activity in data management related tasks, with several online resources with ontology, templates, databases and related NSC project output became available. The following paragraph is a non-exhaustive summary:

The ProSafe (Promoting the Implementation of Safe by Design) project (02/2015-04/2017) includes proposals for further harmonisation of test methods by the OECD and proposals for a more efficient use of the results of nano safety projects by improving the data management infrastructure. A white paper « Toward a more effective and efficient governance and regulation

of nanomaterials » for the OECD and the EU Commission has been established. The SUN project carried out an extensive exercise with project partners to develop data collection templates, procurement, completeness, quality-checked, collation and storage of the scientific project data into a flexible and user friendly operational database. SUN project has successfully accomplished the design, implementation and population of a web-based SUN data repository, a searchable operational project database to store and maintain the data generated by the project. The GuideNano project developed web-based Exposure Scenario Library to enable read-across the exposure scenarios http://guidenano.iom-world.co.uk/.

NECID (Nano Exposure and Contextual Information Database) supports the user to collect and store data of exposure measurements against NOAA (Nano Objects and their Agglomerates and Aggregates). NECID WWW.NECID.eu is developed under the leadership of IFA (Institute for Occupational Safety and Health of the German Social Accident Insurance) and TNO (TNO – innovation for life) a working group of PEROSH (Partnership for European Research in Occupational Safety and Health) institutes.

The data and ontology platform developed by the eNanoMapper project (01/2014-01/2017) can now be used can now be used to capture data and knowledge of ENMs. Several NanoSafety Cluster projects have shown strong interest in the eNanoMapper data management and integration solutions, manifested by the NANoREG decision to transfer all data to the eNanoMapper database, https://search.data.enanomapper.net. The results (data, SOPs, exposure field measurement, material aging data) of the NANoREG project (3/2013-02/2017) are available in the NANoREG Results Repository[1], the e-NanoMapper and the NECID databases. Subsequently the new H2020 projects NanoReg2 and caLIBRAte decided to adopt the same solution and are currently collaborating in integration of results of closed projects into the database. The new ACEnano and OpenRiskNet projects plan to pursue alignment and interoperability with the nano-specific characterisation extensions of the eNanoMapper ontology, protocols and templates and deliver knowledge infrastructure addressing the need of NSC community.

The interoperability of existing data management systems and resources, streamlining and facilitating data collection/management, harmonisation of reporting and consolidating a common language within the nanosafety community are major challenges which can only be addressed through collaboration and exchanging ideas and resources between different projects.

A common thread between all requirements is the support of standardized templates for data entry, based on minimum information checklists and ISA-TAB and ISA-TAB-Nano specifications. The usage of user friendly templates for data logging is only capturing one of the potential data sources. While challenges for nanomaterial data curation workflows for extracting and compiling literature data, are extensively described in the literature, another common task is the integration of existing structured databases and output of past research projects. Here a common language and a common data model are also needed, along with user friendly presentation of the data and curation or validation facilities.

Finally, the WG4 identified the need to collaborate on release and exposure data management in order to harmonize existing efforts and establish links with eNanoMapper database and ontology.

[1] http://rivm.nl/en/About_RIVM/International_Affairs/International_Projects/Completed/NANoREG

## 3 Scope and Objectives

The working group has as aim to act as a communication platform to discuss anything around databases and ontologies in the context of work performed in the NanoSafety Cluster. A key objective is to provide information of with current activities, and encourage project collaboration around mutual benefit. The list of projects changes over time and an overview is given here. A wish list is kept for possibly future projects.

Task 2013-1: Provide an updated list of (NSC) databases

Task 2016-1: Linked Data, about connecting databases

Task 2016-2: Community Spreadsheet Templates

Task 2017-1: Ontology

## 4 Progress and Outcomes to date

WG4 has held monthly conference calls and three extra meetings on exposure data (two conference calls and one face-to-face meeting) during the reporting period (until June 2017). The typical participation is ten people. Minutes are available from the WG4 web site. Egon Willighagen (UM) was a WG4 chair until autumn 2016, when he decided to step down. Nina Jeliazkova (Ideaconsult) was elected as a chair during the 11-10-2016 meeting.

**Database survey.** The NSC Working Group on Databases together with the caLIBRAte project, distributed a database survey in December 2016. Thirty-two responses were received, from the following projects: Cerasafe, Cosmo, DaNA, eNanoMapper, Handbook of Chemistry and Physics, HSE Nano, Keele University (several projects), Mercury, NanoFate, NanoImpactNet, NanoMILE, NanoPUZZLES, NANoREG, Nanosolutions, Nanovalid, NECID, Neptune, S2NANO, Sanowork, Scaffold, Serenade, SIRENA, SUN, TINE, UK NanoRegister, and VieilleNanos. According to the responses, the majority of types of data and information on nanomaterials collected by the responding projects (multiple answers possible) were on physicochemical characterization (24), in vitro toxicity (17), in vivo toxicity (17), ecotoxicology (14), human exposure (12), or environmental release/fate (10). Other questions of the survey addressed the main objective(s) of the database, database design and implementation, database availability/accessibility, the use of semantics technology methods, the data collection and curation, the copyright and licensing aspects. The results of the survey will be published on NSC web site.

**Data templates.** Within NANoREG, under the leadership of JRC, has developed Excel-based "ISA-Tab-logic" templates that are intended to be used for harmonised data logging of processed experimental results produced by the project and released them under open license (CC-BY-SA 4.0)[2]. eNanoMapper was collaborating closely with JRC/NANoREG during the templates development have further cleaned, annotated and created configuration files for these templates enabling direct import into the database through a web browser or programmatically. The templates are used or considered for adoption by several NSC projects, such as caLIBRAte and NanoReg2. A documentation site, (including templates download) is available at https://github.com/enanomapper/tutorials/tree/master/DataTemplates. Additionally, eNanoMapper developed tools to read the Excel spreadsheets and convert into the ISA-TAB compliant files as well as into semantic formats. The review of the content of the templates was initiated by several efforts and meetings on NanoSafety Cluster level as well as Task 2016-2.

**Exposure data collaboration initiated.** The release and exposure data management was identified as of high priority for several projects (e.g. SUN, NANoREG, NanoReg II and caLIBRAte). Three additional meetings were held to discuss various aspects of this collaboration. A special Task Force on exposure data is proposed. The activities include mapping the ES Library variables with those already available in the eNanoMapper database and to add new terms if necessary with the aim of constructing an exposure ontology and ultimately to make all the exposure data available to eNanoMapper. SUN anticipates making its data available to eNanoMapper database (data sharing permissions, embargos etc. needs to be formalised with SUN project partners). NANOREG2 and CaLIBRAte projects are also involved in activities of integration of exposure data. Linking between NECID and enanoMapper database is projected. During the construction of NECID cooperation and exchange of information to other projects like NANoREG, Marina, caLIBRAte, GUIDEnano were and are important parts of the work. The recent availability of NECID database license free of charge (request to NECID@DGUV.de) will likely facilitate the integration.

**Linked data**. While most activities above are related to ontology use and linked data (Task 2016-1), one illustrative example of eNanoMapper ontology use is the annotations of the abstracts for the NMSA meeting at Malaga (02-2017) abstracts and the related presentation at https://www.slideshare.net/egonw/answering-scientific-questions-with-linked-european-nanosafety-data. The annotated abstracts themselves are available at https://egonw.github.io/nmsa/.

**Literature collection.** A NSC publication collection was compiled by E. Willighagen, Stephanie Dawson and Iseult Lynch and available at https://www.scienceopen.com/collection/EUNanoSafety [1].

**Activities in the context of U.S.-EU Communities of Research.** The activities of WG4 are aligned with the U.S.-EU: Bridging NanoEHS Research Efforts initiative and its Databases and Computational Modeling CoR co-chaired by Dr Frederick Klaessig in US and Dr. Barry Hardy in Europe. The community serves as a platform for American and European scientists to share information on nanoEHS research. Dr. F. Klaessig presented his talk "Informatics for nanoEHS and the Materials Genome Initiative" during the Jan 2017 monthly meeting of WG4. The 5th annual face to face workshop (http://us-eu.org/2016-us-eu-nanoehs-workshop/) organised June 6–7, 2016, in Arlington, Virginia (USA) included an interactive event (scrimmage) structured to spark collaborations, sanity checks, and new ideas. The workshop brought together the US-EU Communities of Research (COR), which are a platform for scientists to develop a shared repertoire of protocols and methods to overcome research gaps and barriers and to address environmental, health, and safety questions about nanomaterials. A workshop titled "Enabling a sustainable harmonised knowledge infrastructure supporting nano environmental and health safety assessment" was organised on 24 October 2016 in Rheinfelden (Germany) (http://nanoehs-workshop.eu/). The event served also to support the ongoing US-EU dialogue in the area of NanoEHS and engage US, EU and Asia experts in active discussions, encourage joint programs of work and support communities of research. CEREGE-Labex SERENADE is the primary contact in Europe for the US database efforts led by CEINT– Duke University with ongoing effort on data management, curation and with the US-nanoinformatics program as to determine a strategic planning for data standardization, templates and guidance documents for data harmonization between Europe and USA. Discussions were also active during the ProSafe –OECD conference in Paris (end of 2016) to link EU and US databases (interoperability, ontology, data exchange formats). The CEINT group works in close collaboration with the EU NanosafetyCluster Database Group and the EU-US Database CORs (Community of Research) on templates harmonization and especially on the NanoReg templates and format. All partners agreed to be associated to share expertise for products stability assessment (simulation of products use), environmental fate study, ecotoxicology, end of life. and develop common set up, protocols in order to compare data and implement exposure models.

## 5 Final status report

The NSC Working Group on Databases together with the caLIBRAte project, distributed a database survey in December 2016 (Task 2013-1). The results of the survey will be published on NSC web site. Tasks 2016-2 and 2017-1 are new, and serve to continue the activities of data templates and ontology developed by NANoREG and eNanoMapper project. Several activities on exposure data management and collaboration were initiated and Task Force on exposure data was suggested. The WG4 served as a platform to exchange information, ideas, etc, between projects and harmonize the efforts towards nanosafety research data management, the H2020 Open Data pilot, and various European initiatives around data sharing at large. Collaborations are established to link EU and US databases.

[2] http://www.nanoreg.eu/media-and-downloads/templates/269-templates-for-experimental-data-logging

## 6 Suggestions actions for new WGs

Besides serving as communication channel the WG on data management, the WG F data management may need to work on specific problems, collaboratively identified of importance to the NSC community. For improved efficiency such activities have to be specific, instead of generic "harmonization". Setting examples with pursuing specific goals, e.g. linking eNanoMapper database or the scenario library of GUIDEnano or curation / annotation of data from previous projects will help to illustrate what is possible with existing tools and resources and what needs to be defined as core NSC activity. The example of NANoREG project that has proven the basic willingness of partners to collaborate can be used to come to the first agreement making data and deliverables publically available. It would be a major step forward if other nanosafety projects would follow the example of opening up the results and facilitate data sharing.

## 7 References

1. Willighagen, E., Lynch, I., Dawson, S. 2017 A ScienceOpen collection of NanoSafety Cluster publications. *figshare*. doi:10.6084/m9.figshare.4725424.v1

## 8 Copyright

# Working Group Number: 7

## Working Group Name: Dissemination

Website: http://www.nanosafetycluster.eu/working-groups/7-dissemination-wg
Co-Chair: Claire Mays (NanoFASE) Co-Chair: Claire Skentlebery (NIA)

*Table 1 Working Group Contributing projects List.*

| No. | Project Acronym | Role in Working Group | Representative |
|---|---|---|---|
| 1 | NanoFASE | Chair of WG | Claire Mays |
| 2 | ProSafe | Co-Chair of WG | Claire Skentlebery |
| 3 | FutureNanoNeeds, NanoFASE, NanoMILE | Representative of WG 7 to NSC Steering Group; NSC Compendium | Iseult Lynch |
| 4 | NanoDefine | NSC Newsletter, Events calendar, Social and digital media | Lesley Tobin |
| 5 | FutureNanoNeeds, NanoReg, SUN | Liaison with US-EU CoRs | Teresa Fernandes |
| 6 | NanoFASE, NanoMILE, NanoRegII | Chair, Standardization subgroup | Benoît Hazebrouck |
| 7 | SUN | Chair, Training subgroup | Judith Friesl |
| 8 | FutureNanoNeeds | Aide, Surveys | Georgina Kaklamani |
| 9 | eNanoMapper | Aide, Newsletter | Gözde Kilic |
| 10 | FutureNanoNeeds | Webmaster | Andrzej Fima |

## Contents



## 1 Summary

The Working Group for Dissemination develops distinct platforms of communication to facilitate the multi-directional information flow between all the Working Groups, Project Partners of all NanoSafety Cluster Projects, and the wider community of nanosafety interest groups and stakeholders.

## 2 Objectives (short, medium & long term)

The main objectives are:

1. Serve as a communication platform for the NSC as an EU hub of information on nanomaterial safety, characterisation, and measurement knowledge

2. Using appropriate channels and media, make the wider community aware of the resources and exploitable outcomes being developed within the projects.

3. Maintain, develop and identify new tools and channels for pan-directional targeted dissemination and communication between members of the NSC and the wider community.

## 3 Workplan for 12-month horizon

For 2016-2017, Working Group 7 aims to continue to develop and maintain communication tools and platforms for the NSC as an EU hub of information on nanomaterial safety, characterisation, and measurement knowledge. It will assess the Cluster members' perceptions and needs regarding these tools, and engage in the following activities:

- Aggregate relevant updates, output, results and information from NSC project partners, advisory bodies and complementary initiatives.
- Identify, develop and maintain tools and channels to receive information.
- Identify, develop and maintain tools and channels to deliver information

In addition, Working Group 7 aims to make the NSC and wider communities aware of the resources and exploitable outcomes being developed within the projects (see figure 1), and to this end it will:

- Develop existing subscriber and dissemination databases of stakeholders to extend the reach of the NSC.
- Seek active exchange with stakeholders from different industries as well as interaction and exchange with all relevant end-user groups concerning their needs and experiences.
- Ensure targeted delivery to different stakeholder groups via a combination of electronic platforms and channels, and physical engagement for cross fertilisation of information, updates and news.

| Stakeholders | Push-pull platforms & channels | Tools |
|---|---|---|
| Standardization bodies<br>Regulatory authorities<br>Academia<br>Industry<br>RTD providers<br>Instrument suppliers<br>Metrology<br>Policy-makers<br>NGOs<br>Wider public | NSC website<br>Partners'/EAB websites<br>Industry websites<br>Industry newsletters<br>Community websites<br>Professional networks<br>News aggregators<br>Committees - eg CEN<br>YouTube / Vimeo<br>Industry / Trade fairs<br>Conferences, workshops<br>Meetings/ dissemination events<br>Polls & surveys<br>Peer reviewed journals<br>Network memberships<br>Popular press | News items<br>Bulletins<br>Press releases<br>Fact sheets<br>Case studies<br>Online reports<br>Word-of-mouth<br>Flyers Posters<br>Presentations<br>Webinars<br>Podcasts<br>Manuals<br>Articles<br>Blogs<br>Webcasts |

*Figure 1. Target stakeholders, platforms and tools for dissemination*

Working Group 7 aims to achieve these objectives using the following tools and platforms:

**a. The NSC Website: www.nanosafetycluster.eu**

The Nanosafety cluster website was established by the project administration team at University College Dublin, with Dr. Andrzej Fima as webmaster, under the remit of the FP7 NeuroNano project, and continues to be maintained by UCD via the Future NanoNeeds project (and before that, QualityNano) with support from NanoValid for WG7. WG page updates are designed and sometimes executed by WG members. In 2016-17 we will seek to broaden the basis of contributions, enrolling assistants to update and develop site components.

The Nanosafety cluster website is intended to serve several functions for the community, including:

- as a first point of contact with EU-funded nanosafety research, e.g. via the Nanosafety cluster compendium and the links to the websites of all past and running nanosafety projects;
- as a platform to facilitate the exchange of information and data between H2020- and FP7-funded projects thereby reducing duplication, mainly driven by the Working Groups (WGs) and discussion fora; and, in the longer term
- as a means of centralising and hosting the many scientific and technical outputs from FP6 &7 and H2020 projects, thereby acting as an archive.

Responsibility for developing content for the NSC website, including news updates, RTD developments, project newsletters and output, event promotions, surveys, etc. lies with the community.

**b. The NSC Newsletter**

As the numbers of NSC projects, partners and stakeholders, and even working groups are increasing, the NanoSafety Cluster Newsletter was launched in 2013 to provide an outreach channel for news dissemination and communication for and by the NSC and wider community. Issued on a quarterly basis, targeting the entire NanoSafety Cluster network, including all partners of every NanoSafety Cluster project, researchers, industrialists, policy-makers, regulatory and standardisation bodies, SMEs and NGOs. Moreover, links are sent to news aggregators such as NanoWerk, networks such as TINC, initiatives like NanoFutures, and authorities - including CORDIS. Back copies of the newsletter are available on the website.

The Newsletter predominantly consists of items produced by NSC project partners and stakeholders and designed to inform readers about project activities, latest RTD initiatives,

events and conferences, summer schools and workshops, new publications, job and partnering opportunities, and research breakthroughs. There are profiles of featured NSC projects along with the partners involved and updates from the 8 NSC Working Groups. There are also sections for publications, events (workshops, conferences and project meetings), job opportunities, calls for tender, and special guest features, including opinion articles, and features on issues of interest to the community. New subscribers can register via the website:
http://www.nanosafetycluster.eu/newsletter/subscribe.html

**c. The EU Compendium of NanoSafety Cluster Projects**

The NSC Compendium, first published in 2013, is the central resource for information about NSC projects, including descriptions of workpackages, outcomes, results, comprehensive contacts lists and much more. It is compiled on an annual basis to ensure that information remains current. As were former editions, the 2016 edition has been compiled and produced by WG7 Steering Group representative Iseult Lynch at the University of Birmingham.

**d. The NSC Events Calendar**

The NSC Events Calendar is designed to facilitate collaborative events, prevent clashing schedules and aid planning among and between NSC project partners. It is hosted on the home page of the NSC website and users can search by month and date as well as event type: NSC Meetings, Project meetings, conferences, summer schools, workshops webinars and forums.

The new Training subgroup WG7b established in Fall 2015 also maintains an internal training calendar for planning.

**e. Social and Professional Networks**

The NSC has its own LinkedIn group at http://www.linkedin.com/groups/EU-NanoSafety-Cluster-7471509. This group provides a discussion and networking platform for NSC project partners and stakeholders as well as a forum for problem solving and planning R&D activities among the wider community. The platform can be used to start discussions, contribute to current ones, expand networks, share output and expertise, or announce events, training and job opportunities and publications. Members are welcome to invite other colleagues and contacts to join too. The NSC also has a Twitter account with 1279 followers at @EUNanosafety.

**f. NSC YouTube Channel**

In October 2014 the NanoSafety Cluster launched its Youtube Channel at www.tinyurl.com/youtubeNSC (Figure 2). The channel hosts a montage of images and short interviews captured at the Nanosafety Forum for Young Scientists held in Syracuse, Sicily, 8-9 October 2014 and attended by over 80 participants. The upcoming Forum in September 2016 will provide the occasion for more uploads.

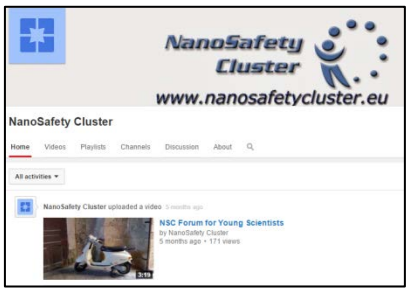


*Figure 2. NSC YouTube Channel*

**g. Other**

Flyers and PPT decks that provide a brief description of the NSC, its objectives and partners may be developed, listing the tools, platforms and channels that users can exploit in order to engage with the wider community. Members scientists could use these at events in order to increase visibility of the NSC and expand the network of contacts.

## 4 Progress and Outcomes to date

**a. The NSC Website**

The website's features include regular updates of news and developments within the NSC community from the Working Groups and the NSC projects, a Wiki for WG2 Hazard, a Newsletter subscription page, a news submission form complete with image upload, a button for direct registration and access to the LinkedIn group and a direct link to the Twitter account.

Collection of links to publications has greatly advanced, giving access to some 500 peer-reviewed articles output by NSC projects.

Since its establishment in early 2010, website consultations constitute 70,979 total sessions and 280,484 page views, as shown in the Google Analytics report from 11 April 2016. The site has attracted more than 50 % new (first-time) visitors in the year since the 2015 Compendium was published.

**b. The NSC Newsletter**

Six issues of the NSC have been published with a seventh in press. Numerous bulletins have been issued. The web-based news submission form is well-known to and used by the whole community. In total, over 2,000 stakeholders are direct recipients of the newsletter. The geographic distribution of the targets, based on the former IoN database, comprises approximately 50% Europe, 25% US and 25% the rest of the world. Their listed organisation types include: research entities; industrial bodies; policy-making, regulatory and standardisation bodies; small and medium-sized enterprises (SMEs) and non-governmental organisations (NGOs).

The NSC Newsletter subscription form implemented on the NSC website and is well used. All targeted stakeholders are regularly encouraged via the NSC website and Newsletter bulletins to subscribe to the Newsletter and their details are stored in a dissemination database. Since it was implemented, the number of voluntary subscribers has risen from 0 to over 390 and continues to increase. Data for 220 subscribers provides accurate demographic information for this sample of the readership, showing that readers are currently based in more than 41 countries.

Links. *Subscribe to the newsletter:*
http://www.nanosafetycluster.eu/home/subscribe-to-newsletter.html
*Submit news:*
http://www.nanosafetycluster.eu/news/submit-news.html

### c. The EU Compendium of NanoSafety Cluster Projects

Since its first publication in 2013 Compendium, the webpage on which it is presented is in the top four of pages visited on the NWC site. Acknowledgement of its value as an information tool has led to the augmentation of contents to include roadmaps and updates from the 8 Working Groups, and dedicated sections for the EU-US Communities of Research (CORs), thereby adding to the body of information available to the EC and international entities.

### d. The NSC Events Calendar

The Calendar is placed on the NSC website home page ensuring its visibility. It is regularly updated with key events, and the community submits events for inclusion via a user-friendly form at:
http://www.nanosafetycluster.eu/news/calendar-add-event.html

### e. Social and Professional Networks

A LinkedIn group was established in March 2013 and by 2016 members totalled 305. In April 2014 the Nanosafety cluster Twitter account had 595 followers. There were 1051 followers in April 2015 and the total has grown to 1279 followers in April 2016. Tweeters can either follow @EUnanosafety and the NSC will re-tweet the message, or they tweet content to the NSC for tweeting on their behalf. The Cluster follows 791 Twitter accounts.

## 5 Expected Impact

WG7 will continue to devise and adopt dissemination and communication strategies as well as maintain and develop a range of tools and platforms to maximise the synergies between existing and forthcoming projects addressing all aspects of nanosafety including toxicology, ecotoxicology, exposure assessment, mechanisms of interaction, risk assessment and standardisation.

While project partnerships are essentially Eurocentric, WG7 bringsnews, output and results to the global nanosafety community and beyond, enlarging the geographic spread in each of the stakeholder groups featured in figure 3.

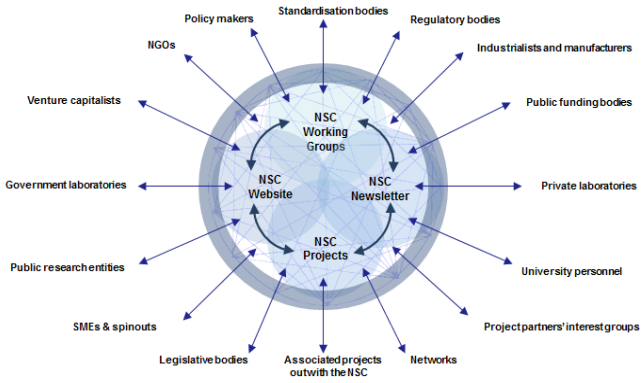


*Figure 3. The NSC platforms, the targeted stakeholder groups. and multidirectional flow of information and communication between them.*

The effectiveness of different aspects of the dissemination strategy can be assessed by assessing the richness of medium, the extent of its reach and the time and effort involved in implementing or auctioning it (see figure 4).

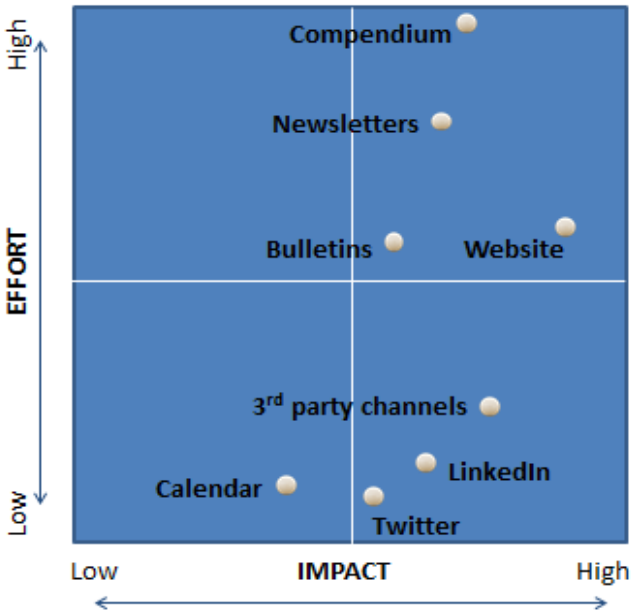


*Figure 4. Gauging activity efficacy by measuring impact against effort*

Ultimately, WG7 expects to raise the profile and prestige of the NanoSafety Cluster Working Groups and individual projects within the wider community of international stakeholders by sharing knowledge, encouraging shared innovative practice, fostering collaborations and promoting research excellence.

# 6 Directory

*Table 2 : Directory of people involved in this Working Group.*

| First Name | Last Name | Affiliation | Address | e-mail |
|---|---|---|---|---|
| Claire | Mays (Chair) | SYMLOG | France | clairemaysnanofase@gmail.com |
| Claire | Skentlebery(Co-Chair) | NIA | Belgium | Claire.skentlebery@nanotechia.org |
| Iseult | Lynch (Steering Group) | UB | UK | eunanosafetycluster@gmail.com, I.Lynch@bham.ac.uk |
| Benoît | Hazebrouck (Chair WG7a Standardization) | Eu-VRI | Germany | bh@eu-vri.eu |
| Maxence | Viallon (Co-Chair WG7a) | LEITAT | Spain | mviallon@leitat.org |
| Judith | Friesl (Chair Training WG7b Training) | TRC | UK | j.friesl@thereachcentre.com |
| Danail | Hristozov (Co-chair WG7b) | UNIVE | Italy | danail.hristozov@unive.it |
| Claire | Auplat | Novancia Business School | France | cauplat@novancia.fr |
| Paola | Bazzoni | Pro&Do | Italy | paola.bazzoni@abich.it |
| David | Carlander | NIA | Belgium | david.carlander@nanotechia.org |
| Valentina | Castagnola | University College Dublin | Ireland | Valentina.castagnola@cbni.ucd.ie |
| Regis | Coeurderoy | ESCP | Paris | rcoeurderoy@escpeurope.eu |
| Camille | de Garidel-Thoron | CEREGE | Marseille | cgaridel@cerege.fr |
| Katarzyna | Dembowska | Innobaltica | Poland | k.dembowska@innobaltica.eu |
| Elina | Drakvik | TTL | Finland | Elina.Drakvik@ttl.fi |
| Albert | Duschl | PLUS | Austria | albert.duschl@sbg.ac.at |
| Andreas | Falk | BioNanoNet | Austria | andreas.falk@bionanonet.at |
| Lucian | Farcal | Douglas Connect | Switzerland | lucian.farcal@douglasconnect.com |
| Teresa | Fernandes | Heriot-Watt University | UK | t.fernandes@hw.ac.uk |
| Andrzej | Fima | University College Dublin | Ireland | andrzej.fima@cbni.ucd.ie |
| Judith | Friesl (Chair) | TRC | UK | j.friesl@thereachcentre.com |
| Karen | Griffin | University College Dublin | Ireland | Karen.Griffin@cbni.ucd.ie |
| Arno | Gutleb | LIST | Luxembourg | arno.gutleb@list.lu |
| Markus | Hank | Aerosol Akademie | Germany | mh@aerosol-akademie.de |
| Steve | Hankin | IOM | UK | steve@safenano.org |
| Karl | Höhener | TEMAS | Switzerland | karl.hoehener@temas.ch |
| Sinead | Jackson | UCD | Ireland | sinead.jackson@cbni.ucd.ie |
| Georgina | Kaklamani | FORTH | Greece | georgina@iesl.forth.gr |
| Georgina | Kaklamani | FORTH | Greece | georgina@iesl.forth.gr |
| Rune | Karlsson | NOMI | Sweden | rune.karlsson@enas-online.com |
| Jérôme | Labille | CEREGE | France | labille@cerege.fr |
| Hender | Lopez Silva | UCD | Ireland | hender.lopezsilva@cbni.ucd.ie |
| Marco | Monopoli | RCSI | Ireland | marcomonopoli@rcsi.ie |
| Catherine | Mouneyrac | UCO | France | cmouneyr@uco.fr |
| Lea | Pylkkänen | FIOH | Finland | lea.pylkkanen@ttl.fi |
| Rudolf | Reuther | NOMI | Sweden | rudolf.reuther@enas-online.com |
| Olivier | Salvi | INERIS | France | olivier.salvi@ineris-developpement.com |
| Michael | Schmitt | DIN | Germany | Michael.Schmitt@din.de |
| Philipee | Steiert | CSEM | Switzerland | Philipee.steiert@csem.ch |
| Stella | Stoycheva | TRC | UK | s.stoycheva@sun-fp7.eu |
| Emmanuel | Stratakis | FORTH | Greece | Stratak@iesl.forth.gr |
| Claus | Svendsen | NERC | UK | csv@ceh.ac.uk |
| Chantal | Tardif | CEA | France | chantal.tardif@cea.fr |
| Lesley | Tobin | NHS | UK | Lesleytobin@hotmail.com |
| Anna | Undas | RIKILT | The Netherlands | anna.undas@wur.nl |
| Eugenia | Valsami-Jones | UB | UK | e.valsamijones@bham.ac.uk |
| Stefan | Weigel | RIKILT | The Netherlands | stefan.weigel@wur.nl |

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# Working Group 7b

## Training

Chair: Judith Friesl Co-Chair : Danail Hristozov

*Table 1 Working Group Contributing projects List.*

| No. | Project Acronym | Role in Working Group | Representative |
|---|---|---|---|
| 1 | SUN | Leadership of training subgroup | Judith Friesl, Danail Hristozov |
| 2 | COST | | Arno Gutleb |
| 3 | eNanoMapper | | Lucian Farcal |
| 4 | FibralSpec | | Costas Charitidis |
| 5 | FutureNanoNeeds | | Marco Monopoli, Georgina Kaklamani |
| 6 | GuideNANO | | Maxence Viallon |
| 7 | NanoDefine | | Rune Karlsson |
| 8 | NanoFASE | | Claire Mays, Anna Undas |
| 9 | NanoMILE | | Eugenia (Eva) Valsami-Jones |
| 10 | Nanoreg II | | Alex Rinkus |
| 11 | NanoValid | | Rune Karlsson |
| 12 | SIINN project NANOHETER | | Jérôme Labille |
| 13 | SMART-NANO | | Philipee Steiert |

# Contents



## 1 Summary

The Training subgroup of WP7 was initiated to support the need for coordination of nanosafety training offerings at NSC level. Although not all NSC projects have specific work packages dedicated to training, most of them have budgets to perform either internal or external training in order to ensure knowledge transfer among consortium members and external stakeholders. Such training workshops constitute a very effective form of dissemination. The NSC Training subgroup provides a forum for coordination of co-hosted and/or open project workshops and also aims at ensuring the efficient transfer of the generated knowledge to external stakeholders.

## 2 Objectives (short, medium & long term)

The objective of NSC Training subgroup is to coordinate the training agendas of the NSC projects in order to:

- ensure a consistently high level of skill across the projects;
- transfer the generated knowledge to external stakeholders;
- enhance the training offerings through collaboration and sharing of experience.

The following short, medium and long term goals have been recognized:

**Short-term goals:**

- Ensure the formalization and the visibility of the subgroup – **completed.**
- Create a strategy for engaging external support for the NSC training projects such as the Marie Curie Actions and the Research Initiative for Scientific Enhancement (RISE) programs – **to be implemented along with the newly set targets aimed at expanding the outreach of the group.**

**Mid-term goals:**

- Organize training sessions within the NSC cluster on tools developed by the projects in order to foster knowledge exchange and dissemination – **completed and ongoing**
- Serve as an information platform for Transnational Access activities – **to be implemented.**

**Long-term goals:**

- Coordinate the training agendas of the NSC projects – **ongoing.**

## 3 Workplan for 12 month horizon

- Involvement of external support for the NSC projects to join the group's activities.
- Expand the group membership beyond the NSC community to include other relevant communities (i.e. nanomedicine) by establishing relationship with the The European Technology Platform for Nanomedicine and other relevant platforms.
- Develop constant summaries of the existing training supported by the NSC projects delivered in the form of PhD dissertations and disseminate those among the NSC community and other relevant communities (i.e. nanomedicine).
- Serve as an effectives information platform aimed at supporting and promoting young researchers.
- Serve as an information platform for Transnational Access Activity.
- Identify areas of training which are not currently covered in order to develop training provisions for future projects.
- Coordination of training activities in order to avoid duplication of training offerings and foster collaboration among projects and promotion of events.

## 4 Progress and Outcomes to date

While most of the short- and mid-term goals of the group were completed in the first 12 months of the group functioning, in the period April 2016- June 2017 the group has focused on discussing and exploring opportunities on how to expand its membership base and be able to serve as an effective information and coordination platform of training offerings.

Within this second period the actual coordination of the group's activities was taking place in the form of documentation of projects' planned events in the formal shared database and through discussions of potential partnerships during the group's teleconferences. The following joint events and trainings were realized:

- 2nd Nanosafety Forum for young scientists, held on 15-16 September, 2016 in Visby, on Gotland, Sweden, jointly organized by the NSC and eNanoMapper.

- EU-US nano-EHS workshop on the Enabling a Sustainable Harmonised Knowledge Infrastructure supporting Nano Environmental and Health Safety Assessment, held on 24 October, 2016 in Rheinfelden, Germany, jointly organized by eNanoMapper and BILAT USA 4.0.

- spICP-MS Hands-On Workshop (training to conduct analysis using single particle inductively coupled plasma mass spectrometry (SP- ICP-MS), held on 10-12 January 2017 in Wageningen, The Netherlands, hosted by NanoFASE.

- New tools and approaches for nanomaterial safety assessment, held on 7-9 February in Malaga, Spain - a joint nanosafety event representing the final conferences of the projects SUN, GUIDEnano, NANOSOLUTIONS, NanoMILE and eNanoMapper.

- SRA Policy Forum: Risk Governance for Key Enabling Technologies, held on 1- 3 March in Venice, Italy, jointly organized by SUN, Calibrate and the Society for Risk Analysis.

- SUN-CaLIBRAte Stakeholders workshop: From nano risk management to innovation governance: Developing state of the art, reliable and trustable, governance models and tools for nanomaterials, held on 2-3 March 2017 in Venice, Italy, jointly organized by SUN and Calibrate.

- Nanomaterials: Industrial Workshop on Safe-by-Design, jointly organized by NanoFase, NanoMILE, NanoReg2, forSafeNano, caLIBRAte and NANOGENTOOLS, held on 24-25 April 2017 in Bilbao, Spain.

- NanoDefine Summer School 2017 (training on the tiered approach and practical experience and use of NanoDefiner eTool for the classification of nanomaterials including practical training on sp-ICPMS and Electronic Microscopy), to be held on 21-23 June 2017 in Wageningen, The Netherlands.

Apart from supporting the coordination of joint dissemination activities the group was exploring other possibilities for coordinating the training agendas of the NSC projects, namely discussing the opportunity that the group serves as an information platform giving a holistic overview on the training landscape within the NSC community. Therefore, a new activity was suggested aimed at boosting the promotion of performed training among the NSC projects. Although one of the most important training supported by the NSC projects is delivered in the form of PhD dissertations, these efforts have not been widely promoted within the NSC. In order to give visibility to this work the group members discussed possible ways of documenting such activity which ranged from creating a new page on the NSC website dedicated to the graduated and current PhD Students containing brief

summary of their PhD theses and short biography to compiling this information into a Training Compendium and a press release for this activity. A template for the Training compendium was created and circulated among group members for discussion. The suggested compendium should list students (mostly PhD candidates) funded by each project, providing an overview of their background and work. In addition, it would provide information on the training events (e.g. schools, workshops) organized by the projects. A pilot study aimed at evaluating the usefulness of such initiative was conducted by the SUN project which resulted in the publication of the following informative webpage hosted by the project: http://www.sun-fp7.eu/suns-phd-students/. Most of the enquired students found the initiative useful and expressed their willingness to include the link of the subpage dedicated to them in their CVs. The main challenges in the process were the fact that some students are at a different stage in their PhD studies and cannot give a more detailed description of their activities.

During the last teleconference of the group (on 6 June, 2017), a final decision was made that this initiative should become a core activity of the group and thus resulted in the redefinition of the purpose of the group, i.e. to serve as an effective information platform aimed to:

- promote and support young researchers,

- identify areas of training which are not currently covered thus ultimately resulting in developing training provisions for future projects.

In order to address these needs the group will:

- Develop constant summaries of the existing training supported by the NSC projects delivered in the form of PhD dissertations and disseminate those among the NSC community and other relevant communities (i.e. nanomedicine).

- Inform students about Transnational Access Activities and exchange opportunities.

- Organize targeted workshops for young researchers aimed at presenting exchnage opportunitites, the benefits from being part of the platform and practical training on how to write proposals.

## 5 Expected Impact

The ambition of WG7 training subgroup is to foster a consistently high level of skill across Cluster projects through effective collaboration and sharing of experience, as well as transfer of knowledge beyond the Cluster.

## 6 Directory

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

| First Name | Last Name | Affiliation | Address | e-mail |
|---|---|---|---|---|
| Judith | Friesl (Chair) | TRC | UK | j.friesl@thereachcentre.com |
| Danail | Hristozov (Co-chair) | UNIVE | Italy | danail.hristozov@unive.it |
| Daniel | Breitenstein | Tascon | Germany | breitenstein@pta-db.eu |
| David | Carlander | NIA | Belgium | david.carlander@nanotechia.org |
| Costas | Charitidis | NTUA | Greece | charitidis@chemeng.ntua.gr |
| Christine | Connolly | UCD | Ireland | christine.connolly@cbni.ucd.ie |
| Kenneth | Dawson | UCD | Ireland | kenneth.a.dawson@cbni.ucd.ie |
| Aart | Dijkzeul | Min I&M | The Netherlands | aart.dijkzeul@minienm.nl |
| Albert | Duschl | PLUS | Austria | albert.duschl@sbg.ac.at |
| Maria | Dusinska | NILU | Norway | maria.dusinska@nilu.no |
| Lucian | Farcal | Douglas Connect | Switzerland | lucian.farcal@douglasconnect.com |
| Emeric | Frejafon | INERIS | France | emeric.frejafon@ineris.fr |
| Arno | Gutleb | LIST | Luxembourg | arno.gutleb@list.lu |
| Macrus | Hank | Aerosol Akademie | Germany | mh@aerosol-akademie.de |
| Karl | Höhener | TEMAS | Switzerland | karl.hoehener@temas.ch |
| Sinead | Jackson | UCD | Ireland | sinead.jackson@cbni.ucd.ie |
| Georgina | Kaklamani | FORTH | Greece | georgina@iesl.forth.gr |
| Rune | Karlsson | NOMI | Sweden | rune.karlsson@enas-online.com |
| Jérôme | Labille | CEREGE | France | labille@cerege.fr |
| Hender | Lopez Silva | UCD | Ireland | hender.lopezsilva@cbni.ucd.ie |
| Iseult | Lynch | UB | UK | eunanosafetycluster@gmail.com |
| Claire | Mays | SYMLOG | France | clairemaysnanofase@gmail.com |
| Marco | Monopoli | RCSI | Ireland | marcomonopoli@rcsi.ie |

| First Name | Last Name | Affiliation | Address | e-mail |
|---|---|---|---|---|
| Catherine | Mouneyrac | UCO | France | cmouneyr@uco.fr |
| Lea | Pylkkänen | FIOH | Finland | lea.pylkkanen@ttl.fi |
| Rudolf | Reuther | NOMI | Sweden | rudolf.reuther@enas-online.com |
| Alex | Rinkus | NIA | Belgium | alex.rinkus@nanotechia.org |
| Philipee | Steiert | CSEM | Switzerland | Philipee.steiert@csem.ch |
| Stella | Stoycheva | TRC | UK | s.stoycheva@sun-fp7.eu |
| Claus | Svendensen | NERC | UK | csv@ceh.ac.uk |
| Chantal | Tardif | CEA | France | chantal.tardif@cea.fr |
| Anna | Undas | RIKILT | The Netherlands | anna.undas@wur.nl |
| Eugenia (Eva) | Valsami-Jones | UB | UK | e.valsamijones@bham.ac.uk |
| Maxence | Viallon | LEITAT | Spain | mviallon@leitat.org |
| Stefan | Weigel | RIKILT | The Netherlands | stefan.weigel@wur.nl |

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# Working Group 9

## Safety by Design

Website: https://www.nanosafetycluster.eu/working-groups/Safe-by-Design-and-Industrial-Innovation-WG9.html
Chair: Éva Valsami-Jones, University of Birmingham
Co-Chair : Emeric Frejafon, INERIS Co-Chair : Andreas Falk, BioNanoNet

*Table 1 Working Group Contributing projects List.*

| No. | Project Acronym | Role in Working Group | Representative |
|---|---|---|---|
| 1 | NanoMILE | Chair | Éva Valsami-Jones |
| 2 | NANoREG II | Co-chair, regulation & standardisation liaison | Emeric Frejafon |
| 3 | European Pilot Projects | Co-chair, industrial innovation liaison | Andreas Falk |
| 4 | ProSafe | Contributors, data & processes harmonisation | Juergen Hoeck |
| 5 | | | Karl Hoehener |
| 6 | HISENTS | Contributor | Andrew Nelson |
| 7 | GUIDEnano | Contributor | Camilla Delpivo |
| 8 | GUIDEnano & SERENADE | Contributor | Victor Puntes |
| 9 | CALIBRATE | Contributor | Keld Alstrup Jensen |
| 10 | | Contributor | Marina Moser-Johansen |
| 11 | NIA | Contributor | Sean Kelly |
| 12 | NIA | Contributor | Claire Skentelbery |

# Contents



## 1 Summary

A number of recent EU funded projects have a dedicated "safety by design" component, either explicitly in their approach, or implicitly resulting from the data generated, which can be used to inform future nanomaterial designs.

This new WG aims to facilitate discussion and integration of ideas, approaches and data from these projects, and represent the "go to" group when information and advice on designing safer nanomaterials is required. It has two focus areas, one around regulation and standardisation and a second around industrial innovation, where the Industrial Innovation Liaison (i2L) group will serve as a cross linking body with the European Pilot Production Network.

## 2 Scientific and technological challenges

**Safety by design**, as a concept, implies that safety is addressed at the design stage of a process or product rather than tested after their development. Central to this is the idea that safety aspects should inform the design of a product or process right at the inception point and along with other key concepts such as function, quality and cost. Safety by design is well embedded in many current manufacturing processes, but it has yet to be part of products and processes related to nanotechnology. This is primarily because nanosafety research is not yet robust enough to operate in a predictive manner.

More specifically, in terms of scientific challenges, a critical mass of data has been reached in nanosafety research, but problems remain in transforming understanding of toxicity into precise

mechanistic models that would support grouping and read-across. Technological challenges are described in Figure 1 and involve a major shift in the approach of manufacturing new materials. Currently any safety assessment happens after product manufacturing and even commercialisation. The anticipated REACH legislation for nanomaterials will shift safety assessment to a stage before manufacturing. However, optimally, and to enable industry and SEMs to avoid developing a product all the way to manufacturing, only to discover that it fails safety testing, it is important to bring the safety assessment to the design stage and, for certain products where sufficient knowledge is available, even before.

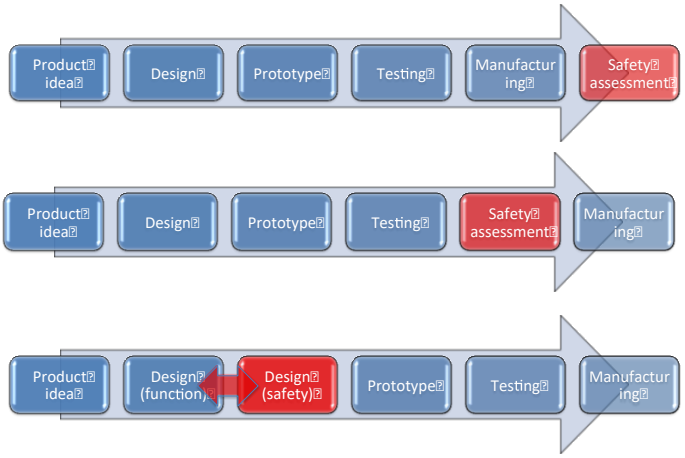


**Figure 1:** Stages in nanomaterial manufacturing showing current (top), anticipated, following nano-REACH legislation (middle), and future, following implementation of safety by design (bottom) flow in material designs. The red box indicates the stage where material safety considerations influence product development.

## 3 Objectives (short, medium & long term)

The overall objective of WG9 is to show leadership from the Nanosafety Cluster in embedding safety by design concepts in the future approaches of industry and regulation. Specific objectives will be:

- In the first two years of NG9 activities to gather, group and digest the current state of the art in safety by design research relevant to nanotechnology and to digest it in a form that will help and support industry and regulation.
- Throughout its operations, to assemble Europe's experts on nanosafety who can expertly advise on safety by design concpets.
- To create forums and task forces in collaboration with equivalent bodies outside Europe.

## 4 Progress and Outcomes to date

**Malaga**

As part of the joint 5-projects meeting in Malaga, there was a dedicated session, sponsored by NanoMILE, on safe-by-design.

The programme is listed below and recordings of the talks can be found at: http://nmsaconferencetalks.eu/lectures?tag=13

DAY 3 – THURSDAY, 9TH FEBRUARY 2017

Keynote 4: NanoMILE

Keynote: Towards mechanistic understanding of nanomaterials interactions: a hazard assessment framework (Andalucia 1)

Presenter: Eva Valsami-Jones (University of Birmingham)

**Parallel Session 5: Safety by Design**

Chairs: Eva Valsami-Jones and Andreas Falk

Jesús López de Ipiña (Tecnalia): A methodological approach for the safe deisgn and putting into service of new processes for manufacturing cnt nano-enabled products

Zahraa Al-Ahmady (University of Manchester): Size dependent internalisation profile of PEGylated multi-walled carbon nanotubes and the impact of protein adsorption

Changyou Gao (Zhejiang University): Influences of gold nanoparticles with surface anchored chiral poly(acryloyl-l(D)-Valine) on protein adsorption, cellular uptake and cytotoxicity

Sophie Marie Briffa (University of Birmingham): Importance of multi-method characterisation approach in the development and behavioural understanding of nanomaterial libraries for nanosafety studies: polyvinylpyrrolidone (PVP) -capped metal oxide Nanoparticles (NanoMILE highlight)

Carolin Merker (University of Leipzig): Translocation and quantification of nanomaterials as single cell level by means of label-Free imaging and dosimetric techniques

Vicki Stone (Heriot-Watt University): An in vitro and in vivo strategy to prioritise nanomaterials for toxicity testing and to inform safety-by-design (SUN highlight)

Magda Blosi (Institute of Science and Technology for Ceramics): Justification of safer-by-design solutions for CuO-NPs: a case study

Anna Luisa Costa (National Research Council of Italy): Safer nano-product and processes by design (SUN highlight)

Sandra Verstraelen (VITO): Safety-by-design in practice: metallic nanoinks for printed electronics and nano-modified alloys for additive Manufacturing

**Bilbao**

Over 40 delegates came together in Bilbao in April 2017, hosted by Gaiker, under the umbrella of the NANOGENTOOLS project with the participation of several EU NanoSafey Cluster projects, to understand the role and potential for Safe-by Design (SbD) in commercial development. The concept of SbD was established to help the development of nanoparticles and materials with risk minimisation at every stage of the design process to achieve long term commercial potential and consumer confidence. Projects across Europe are developing tools that help industry and regulators to understand risks minimisation and they came together in Bilbao to share their knowledge for industry feedback and evolution.

Expert speakers from projects across the spectrum of risk assessment and SbD, including ProSafe, NanoReg2, CaLIBRAte, EC4safenano, nanoFASE, nanoMILE and guideNANO. The full agenda and presentations are available for download here (to be linked to agenda with slide links embedded).

The workshop also hosted discussion groups on Safe by Design, on how the projects refine and focus the SbD is perceived and critically, what the bottlenecks are to its implementation within industry.

The meeting closed with the agreement to finalise a widely-accepted, industry friendly definition of Safe by Design within nanotechnology and explore its practical implementation into industry, with actions including:

- Identification of potential benefits from embedding SbD into companies, including reduced insurance costs and enhanced recognition by regulators, investors, downstream partners and clients
- Development of a potential 'label' or other recognition that allows SbD users to confirm and promote their use of SbD and platforms through which industry can be audited and supported for SbD development with the tools coming to maturity that were showcased at the workshop

Workshop outcomes will be developed through multiple sources, including the NanoSafetyCluster and shared activities through events such as EuroNanoForum, with a dedicated online conference for industry planned for October 2017.

## 5 Expected Impact

By establishing a Safety by Design workgroup, the Nanosafety Cluster has indicated a vision to move beyond passive support to industry and regulation and proactively underpin future activities towards resolution of safety issues in nanotechnology. Expected impacts will materialise through dedicated work by the WG membership and will be in the form of documentation (white papers and guidance documents) and activities (meetings and workshops) within the Nanosafety Cluster and beyond. A measure of success for WG9 would be the implementation of guidance produced and recognition of the group's activities outside NSC.

## 6 Suggested actions for new work groups

Activities to date have demonstrated the value of safe-by-design approaches, but there are clearly major needs by industry in receiving guidance for implementation and operationalization of the safe-by-design process. Going forward a recommendation would be to work towards clarifying a definition for safe-by-design and developing case studies of how this may support industry activities in the range of sectors where nanotechnologies play an enabling role, i.e. food, cosmetics, consumer products, medicine and medical devices, biocides, etc.

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