LIFE Project Number

# LIFE17 ENV/GR/000285

# Final Report

## Covering the project activities from 01/09/2018[1] to 30/09/2022

Reporting Date[2]

## 31/12/2022

LIFE PROJECT NAME or Acronym

# NanoEXPLORE

Data Project

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|---|---|
| **Project location:** | Greece |
| **Project start date:** | 01/09/2018 |
| **Project end date:** | 28/02/2022 **Extension date:** 30/09/2022 |
| **Total budget:** | 2,254,607 € |
| **EU contribution:** | 1,350,645 € |
| **(%) of eligible costs:** | 59.91% |

Data Beneficiary

| | |
|---|---|
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[1] Project start date
[2] Include the reporting date as foreseen in part C2 of Annex II of the Grant Agreement

# 1. Table of contents

# 2. List of key-words and abbreviations

**Key-words:**

Nanomaterials, Engineered Nanomaterials, Biomonitoring, Exposure, Biomarkers, Health Effects, Eligible Populations, Sensors, Environmental Release, Biomarkers Reference Values, Adverse Effects, Occupational Exposure, Portable Measurement Device, Field Studies, Nanotechnology, Nanoenabled Products

**Abbreviations:**

| | |
|---|---|
| ENMs | Engineered Nanomaterials |
| NPs | Nanoparticles |
| UFPs | Ultrafine Particles |
| BMs | Biomarkers |
| DNEL | Derived no-effect Level |
| CLP | Classification, Labelling and Packaging of substances and mixtures |
| SAC | Surface Area Concentration |
| PNC | Particle Number Concentration |
| PSD | Particle Size Distributions |
| PBZ | Personal Breathing Zone |
| REACH | Registration, Evaluation, Authorisation and Restriction of Chemicals. |
| ECHA | European Chemicals Agency |
| OELs | Occupational Exposure Limits |
| OEBs | Occupational Exposure Bands |
| RELs | Recommended Exposure Levels |
| EPHT | Environmental Public Health tracking |
| EPHESUS | EU/EEA Public Health Surveillance |
| OECD | Organisation for Economic Co-operation and Development |
| EBC | Exhaled Breath Condensate |
| OPEA | Oxidative Potential in the Exhaled Air |
| EUON | European Union Observatory for Nanomaterials |
| NIA | Nanotechnology Industry Association |
| RCR | Risk Characterisation Ratio |
| TEM | Transmission Electron Microscopy |

# 3. Executive Summary

NanoEXPLORE aims to improve the understanding of levels, nature and possible adverse effects after ENMs exposure in indoor workplaces and urban areas, with a holistic approach, considering the integration of human biomonitoring studies with measured data as an instrument for developing consisting risk management guidelines. Thus, it proposes the implementation of a network of measurement devices for ENM levels in air together with the use of biomarkers selected and validated to detect early effects on pulmonary and cardio-vascular system and to support a proper risk assessment in the monitoring sites.

The project **preparatory actions** include four steps as follows:

1. **Selection of a set of 11 ENMs** considering the current production volumes (use), likelihood of exposure, availability of biomarkers and information on the (eco)toxicological properties. A **priority list of processes** with high exposure potential to ENMs in industrial settings and urban areas was defined and the **identification of European companies willing to participate in biomonitoring studies** was achieved through an online survey. (Action A.1).
2. **Critical evaluation of existing methods and tools to measure ENMs**, their aggregates and agglomerates (NOAA) in workplaces and urban environments (Action A.2). A complete **analysis of existing exposure assessment strategies and measurement technologies** was documented and the type of information needed was identified. The most relevant metrics for NMs evaluation were defined and the specific requirements to incorporate the data measured into the existing databases and protocols were specified.
3. **Review of the state of the art on existing candidate biomarkers for assessing exposure and potential human health effects following exposure to ENMs** (Action A.3). This analysis was indispensable to select the most appropriate biomarkers and tools, in field studies. All candidate biomarkers were identified and grouped according to their physiological meaning and pathophysiology and only those which are sufficiently robust for consideration for integration into field studies and/or health registries for nanomaterial workers have been selected. The feasibility of biomarkers in field studies was investigated, by considering barriers for providing biological samples (including ethical considerations and invasiveness of sampling) and practical/logistical considerations (e.g. collection, storage conditions) and technical feasibility (e.g. availability and accessibility of analytical techniques).
4. **Identification and analysis of end-users and stakeholders needs, identification and definition of the minimum requirements of the NanoExplore wireless sensor network and web-based application and definition of the implementation plan of the project** (Action A.4). The analysis of the end-users and stakeholders needs, mainly professionals, in relation to the characterization of potential adverse effects and exposure monitoring, was carried out through an online survey. Based on the analysis results, the definition of the main functionalities of the NanoExplore wireless sensor network and web-based application was conducted and the implementation plan of the project considering the results of the preparatory actions, as well as the technical amendment, was prepared.

During the **implementation phase**, the main outcomes are the following:

1. **Design and development of a low-cost wireless sensor for measuring particle number concentration and mass concentration of relevant ENMs** (Action B1)**.** During the finalization of the device specifications, Partector 2 was chosen, instead of DiSCmini, to enhance the reliability of the device. Though, this solution has increased the budget of the sensor and, therefore, it became mandatory to reduce the number of units to 8, instead the

initially foreseen (up to 20). However, the lower number of units did not affect the project's scheduled actions. Moreover, an **LC Impact Assessment of the wireless sensors was conducted**.

2. **Assessment and optimization of the specific requirements for carrying out studies on nanomaterial workers** (Action B2)**.** Human studies and epidemiological investigations of exposed workers have been reviewed and their study design was assessed. The most efficient and feasible **strategy of recruitment of companies and individuals** was elaborated. Despite the difficult situation due to COVID19, a biomonitoring pilot study was carried out in a company, producing paints and using UF TiO2. The results obtained prove the reliability of the NanoExplore integrated approach. Based on the above, the fundamental deliverable **"Harmonized Study Protocol"** was produced and published. **This protocol paves the way for reliable and consistent biomonitoring studies with comparable results.** Finally, two studies were conducted to determine **reference values of the biomarkers, selected in the preparatory phase, in urine and exhaled breath condensate (**the first attempt to define reference values for these biomarkers using meta-analytical methods) and of **OPEA**, for the first time**.** The estimated reference intervals are very important for the interpretation of the NanoExplore results but also for the broad scientific, pharmaceutics and medical communities.
3. **Design and development of the NanoEXPLORE intelligent web-based platform** (Action B3). This web platform aimed at supporting the acquisition, management and processing of data on the concentration of ENMs monitored by the sensor prototype network in industrial settings and the environment. The platform developed is a user-friendly tool, dedicated for automatic operation in combination with the NanoExplore device, however it provides the possibility to receive data from other sensors too.
4. **Pilot biomonitoring studies to assess the feasibility of the harmonised protocol of the collaborative study and refine the NanoExplore integrated approach** in a well characterized limited number of unexposed and exposed workers to nanoparticles, including workers from production facilities, workers from office positions, working in civil infrastructures, and control participants (Action B.4). Field campaigns were conducted as soon as the restriction measures due to the pandemic were lifted, in summer and fall 2021. **The biomonitoring study sample included 141 adults of both sexes in 5 industrial facilities and 1 laboratory.** The studies showed a significant dose-response relationship between IL-10, IL-1β and TNF-α measured in EBC with both, particle number concentration and LDSA. An absence of oxidative stress is also observed in the exhaled air. Regarding urine, a significant negative association was observed between both ENM exposure metrics and Total Antioxidant Potential (TAP). The damage on lung function observed was due to the cumulative long-term exposure over the last 10 years rather an acute effect of short-term ENM exposure.
5. **Derivation of recommended exposure levels and risk rating to support EU policies** (Action B.5). **New values for Occupational Exposure Limits for a list of selected nanomaterials were defined.** The derivation of the OELs was based on the calculation of the Human Equivalent Concentration (HEC) and the use of the Multiple-path Particle Model. Moreover, a proposal of Occupational Exposure Bands (OEBs) for nanomaterials, based on data reported in literature, was included. **Definition of risk ratios for common industrial processes at all stages of the ENMs life cycle was accomplished for the installations of the field studies.** All information was structured and organised following the structure of the NanoExposure & Contextual Information Database (NECID) developed under the EU NANOSH project and used as the database under the EU project NanoREG. Based on the risk rating results, **even though the plants have high background concentration values, not all scenarios seem to represent an acute risk for workers,**

**but they might represent a long-term risk, as they are constantly exposed to high dusty environments with toxic components such as titanium and silicon.** Based on the above, specific measures were proposed towards ENMs levels abatement.

6. **Implementation and demonstration actions to evaluate and validate all the methodologies, strategies and tools developed under representative situations over the entire life cycle of targeted ENMs** (Action B.6). The demonstration studies were performed for **3 production facilities**, **1 laboratory, 4 urban sites and a quarry area.** Based on the indicators evaluation, **the NanoExplore device demonstrated its reliability to measure remotely in indoor and outdoor conditions**. Finally, **the biomonitoring protocol was validated as an appropriate protocol to support biomonitoring studies,** highlighting the use of simple methodologies for sampling and the use of a limited number of biomarkers which can be easily measured in laboratory.
7. **Replicability and Transferability of project actions** (Action B.7). In the concept and design of the NanoExplore device and web platform and of the biomonitoring studies, all the critical factors were considered to guarantee the replicability and transferability of the project approach and to ensure that the technology and methods applied and refined within the project are applicable in other places, locations etc. as well as for substances other than ENMs. To further enhance the replicability and transferability of the integrated approach, pilot studies were conducted, a good practice manual for exposure to ENMs and human health monitoring, considering the specificities of areas other than those represented in the project, was developed and dissemination actions to promote the integrated approach and the ideas and results of the project were implemented.
8. **Business plan and commercialization** (Action B.8). The commercialization strategy was selected and the business model was developed. Three alternative options are included in the business model: a) NanoExplorer purchase, b) NanoExplorer renting and c) NanoExplorer as a service. The main goal of the exploitation strategy is to sell Nanoexplore globally in the long term, after expanding to Europe and the local market. Considering the performed market analysis, the segment of occupational health and safety companies was chosen to establish our first entry into the market and demonstrate our capabilities, before making the leap to the other segments analysed. One of the cornerstones for the business exploitation will be an effective communication plan with the aim of achieving a reputation in the market and making Nanoexplore known to all key players. For this purpose, a detailed dissemination plan is foreseen.

# 4. Introduction

NanoExplore focuses on the likely effects derived from exposure to ENMs, a new type of chemicals of concern, whose properties differ significantly from those of bulk chemicals of the same composition due to their much larger specific surface area and surface activity (Hsieh et al. 2013) or much larger deposition rate in the respiratory systems (Silva et al. 2013), which may lead to unanticipated effects in human health, like pro-inflammatory effects or development of fibrosis and /or cancer (Shi et al. 2013),as well as to significantly alter ecosystems, causing adverse effects on the metabolism of a living being (Sauvé, 2014). The environmental problem targeted is due to the increasing worldwide use of ENMs in products, the lack of information on current exposure levels on both workers and population, and the scarcity of ENMs potential negative effects data on human health and environment. Nowadays, WHO and EU are monitoring PM2.5 and PM10 and reporting on their negative health effects and their ability to penetrate our lungs and cause respiratory and cardiovascular morbidity and disease. However, there is a lack of data on the effects of PM1 to deadly diseases like heart attacks, lung cancer, dementia, emphysema, edema and other serious disease, leading to premature death.

The project aimed to improve the understanding of levels, nature and possible adverse effects after ENMs exposure in indoor workplaces and urban areas, considering the integration of human biomonitoring studies with measured data on the Particle Number Concentration (PNC), mass and Particle Size Distributions (PSD) in the Personal Breathing Zone (PBZ) as an instrument for developing consisting risk management guidelines. So, it proposed the implementation of a network of measurement devices for ENM levels in air together with the use of biomarkers selected and validated to detect early effects on pulmonary and cardio-vascular systems.

Considering candidate biomarkers, experts from the consortium (Università degli Studi di Torino / Unisanté) suggested the pre-selection of biomarkers applied in air pollution studies related with combustion-derived UFPs, as well as of those of interest on the basis of relevant health endpoints that have been tentatively ascribed to ENMs including cardiovascular, pulmonary, and inflammatory effects. Under this context, the exposure assessment approach, validated under the project, was based on the generation of job-exposure matrices containing large series of data on the concentration of ENMs in the Personal Breathing Zone, area samples (far field measurements), respirable fractions, inhalable fractions and total suspended particles.

To date, the establishment of ENM exposure monitoring programs was hampered by the lack of devices able to discriminate background aerosols from manufactured NMs, and by the fact that biological and environmental effects were still limited due the lack of standardized testing methods. Background aerosol (BA) concentrations, originating from natural sources, contribute to the concentration levels measured by the current monitoring systems (e.g. CPCs, DISCmini, or nanotracer) used in exposure assessment campaigns and/or air quality monitoring systems, hampering a proper evaluation of the real levels of exposure to ENMs, and normally causing an overestimation of the exposure levels. The wireless sensor prototype developed by RAMEM and ITENE under NanoExplore generate background corrected measurements, discriminating background aerosols from ENMs and supporting a proper risk assessment in the monitoring sites. Therefore, NanoExplore has provided evidence of the potential detrimental effects of UFPs and ENMs on human health, as well as robust devices to measure the exposure, considering a high progress beyond current laboratory measurement devices.

To date, toxicity of ENMs to aquatic organisms at different trophic levels such as bacteria, algae, and fish has been widely investigated. Growth inhibition parameters and different levels of effect concentrations, uptake, transport and ENMs distribution in various organisms have been reported (Z.Wang et al, 2012; R.Kaveh et al 2013). There is also scientific agreement that ENMs production, use and disposal lead to environmental release of ENMs (Gottschalk and

Nowack, 2011), accumulating in soil, water or biota, endangering the health of living organisms and ecosystems (Köhler et al, 2008).
Regarding effects on human health, several adverse effects have been observed for a number of different organs and organ-specific cell lines, including human epidermal cells, human embryo kidney cells and human bronchial cells, causing cellular toxicity (Ricarte, 2016) and DNA damage (Lindberg, H.K., et al, 2017). Pulmonary disease and death were observed in mice, as well as dose-dependent inflammatory reactions (FP7 NanoMICEX-Final Report, 2015).
Routes of exposure are well characterized, being inhalation the most common one. Dermal uptake has also been investigated, but studies have shown little to no transdermal absorption (Karjalainen et al, 2012). Recent studies show that the most extensive exposure occurs in the workplace, particularly research laboratories, start-up companies, pilot production facilities, where ENMs are handled.
Therefore, there is an urgent need to provide stakeholders, including regulatory bodies and companies, with an integrated approach to generate robust data on the exposure levels and related health effects, supporting the risk assessment. An adequate evaluation of ENMs is a major concern being addressed by regulatory agencies from all EU Countries, and international organizations (e.g. OECD), in order to ensure that society can benefit from novel applications of nanotechnology, whilst a high level of protection of health, safety and the environment is maintained.
On the other hand, the theme addressed by the project is aligned with the priorities published by the EU NanoSafety Cluster on March, 2017, as well as with the information published in the EUON, launched by EU authorities, where it is stated that ENMs are covered by the same rigorous regulatory framework that ensures the safe use of all chemicals. Within REACH, the EU defines an exposure level below which no adverse effects are expected (DNEL). Under the EU 'Framework Directive' (89/391/EC) on occupational health and safety, occupational exposure levels (OELs) have to be applied. Moreover, when substances have hazardous properties, CLP requires them to be notified to ECHA and labelled and packaged to promote a safe use.
The project addressed relevant issues related to the European environmental policy and legislation including 1) the elucidation of possible ENMs adverse effects posed by means of validated biomarkers, 2) the definition of a quantitative exposure limit value for a set of ENMs for workers and the consumers protection, and 3) the generation of data on the levels of exposure to ENMs in workplaces and urban areas by setting up a robust wireless sensor network for the real-time remote monitoring of the concentration of ENMs.
Moreover, the activities within the project have supported the implementation of the 7th Environment Action Programme (Union Environment Action Programme to 2020) which is the first to set a long-term vision for policy-making in the field, until 2050 but its objectives are not yet fully met. NanoExplore could be placed in the third key action area of the programme, which covers challenges to human health and wellbeing, such as water pollution, and sets out a long-term vision of a non-toxic environment, proposing to address risks associated with the use of chemicals in products and chemical mixtures.
Finally, it should be noted that the outcomes of the project have been extended to new scenarios and conditions as part of the replicability and transferability activities. In this sense, the network sensors developed can be implemented in different indoor areas and urban environments, considering significant variations of temperatures, conductivity, pressure or humidity. The elements of the network and guidelines developed for human monitoring are specifically designed to ensure the transferability to other conditions. Moreover, the harmonized protocol for the implementation of biomonitoring studies and the guidelines provided in the course of the project guarantee the dissemination of the new knowledge gained and its use by policy makers and stakeholders.

# 5. Administrative part

The overall objective of the consortium management tasks has been to guarantee the efficient and productive functioning of the project to ensure that the objectives of the different actions are reached, tasks and deliverables completed and milestones achieved in accordance with time schedule. The main aim of the management is to ensure that the consortium is working as a team and that the entire related tasks are performed successfully. The project is coordinated by ALCON Consultant Engineers Ltd. and Project Coordinator (PC) is Dr. Athena Progiou assisted by the Project Coordination Committee (PCC). The Coordinator is the single point of contact between the European Commission (EC) and the Monitoring Team as well as the Consortium members, reports to the EU and maintains a Project office to provide the necessary support for the day-by-day project management and activities to the EC. The responsibility for each action is attributed to one of the partners, being in charge of the coordination of the action, the Action Leader (AL) who is responsible for the work done by other participants bringing contribution to the specific Action. AL establishes, in coordination with the participating partners and other ALs, the detailed schedule of his Action and presents the Action progress when required by the PCC and the external reviews. Moreover, the Coordinator is responsible for heading the PCC, executing decisions made by the PCC, preparing and submitting -with the collaboration of the ALs- the activity and financial reports to be sent to the EC and assumes the overall responsibility of analyzing and approving the results of each of the actions. The mechanism to control and monitor the progress of work and objective achievements is carried out on a three-monthly basis and is based on:

- Internal technical reporting: Technical Progress Reports describing the work done per action were prepared by all beneficiaries. After completion of each action, more detailed information was provided by the AL to the PC on the progress of the action and main results achieved.
- Internal financial reporting: Every three months costs incurred for the established period were be reported to the PCC. This includes all the financial reports for the various actions and cost categories along with all the relevant documents required (e.g. salary slips, timesheets, contracts, invoices etc.).

The project consortium is composed of eight (8) members with proven track records and EU projects. The involvement of six (6) European countries is an added value for the project because it allows us to compare the interactions between REACH and other environmental regulations existing in this country. It is to be noted that the project includes a Swiss partner Unisanté (former IST) due to their high experience in human biomonitoring. Moreover, the transnational approach gives us the possibility to involve several industrial sectors and to cover all the expertise areas required in the project. The collaboration will create new opportunities for further future collaborations that may enhance science and technology and eventually economy and living standards in European area. Finally, the actions, methods, approaches and techniques developed within NanoExplore will be totally applicable in every country of the European Union. A partnership amendment was accomplished due to IST Incorporation into Unisanté, as from 01.01.2019, and the split of Yordas (UK) to Yordas UK and Yordas DE, as from 01.07.2019. Moreover, a technical amendment concerning Task B.2.3 was undertaken. More specifically, Task B.2.3 “In vitro Validation of causal link between nanomaterial and selected biomarkers in human workers” (as originally submitted) was replaced by the “Determination of reference values for biomarkers selected”. This amendment did not modify the objectives of the project and fully falls under the scope and objectives of the project as described in the proposal. A second amendment to the Grant Agreement was requested, on November 22, 2021, concerning: a) the “National Centre of Environment and Sustainable Development (NCESD)” name change to “Natural Environment and Climate Change Agency (NECCA)” and b) the extension of the project duration for seven (7) months, up to September

30, 2022, due to the unforeseeable conditions implicated by the pandemic. The most important challenge NanoExplore faced was the pandemic restrictions which hampered the production of the prototypes and the implementation of the field studies and augmented the project cost. However, during this period, frequent teleconferences among partners were taking place to ensure the continuation of the project, guaranty the quality of its results and further motivate all beneficiaries who put all their efforts to overcome the significant problems occurred. This approach enabled the accomplishment of the field studies and finally the production of all the important project results.

# 6. Technical part

## 6.1 Technical progress, per Action

In this chapter, the activities undertaken and outputs achieved are described. A more detailed description of the results is provided in the deliverables as foreseen in the Grant Agreement.

### 6.1.1 Action A.1 Identification and definition of target nanomaterials, processes and exposed workers

**Action status:** Completed
**Foreseen start date:** 01/09/2018 **Actual start date:** 01/09/2018
**Foreseen end date:** 18/01/2019 **Actual end date:** 18/01/2019

**Objective**: The aim of this action is to: a) **identify, select and describe a priority list of engineered nanoparticles to be considered under the scope of the project**, b) define **a priority list of processes for human biomonitoring** and c) identify **potentially eligible populations** and characterise their propensity and barriers to participate into a longitudinal study.

**Activities conducted and progress:** A set of three tasks were completed within action A1, including the selection of ENMs under task A1.1, the selection of a priority list of processes for human biomonitoring on the basis of potential exposure and available toxicological information under task A1.2, and the identification of companies with interest in participating in biomonitoring studies under task A1.3. ITENE was in charge of ST A1.1 and A1.2 whereas Unisanté was responsible of ST A1.3.

Under **Task A1.1**, a set of 11 ENMs was selected considering the current production volumes (use), likelihood of exposure, availability of biomarkers and information on the (eco)toxicological properties (Table 1). A thorough review of information published in relevant publications, in peer reviewed journals, reports from FP7 and H2020 projects, abstracts and communications for relevant conferences, as well as data from technical documentation on ENMs and nano-enabled products was carried out complemented with direct consultation with companies using tailored designed questionnaires distributed among relevant stakeholders, companies, as well as public and private research organizations. A detailed description of the methodology applied to select target ENMs is provided under deliverable DA1.

Under **Task A1.2**, a priority list of processes with a high exposure potential to relevant ENMs in industrial settings and urban areas was defined. To this end, a scoring system to rank the potential exposure to ENMs, their agglomerates and/or aggregates was applied to support prioritization after mapping the uses of the core set of ENM selected in the project. Table 2 shows a list of relevant activities considering likelihood of exposure across the life cycle of selected ENMs. This consisted in mapping the known downstream and consumer uses, employing the standard terminology of the descriptor system used under REACH regulation.

To complement the data compiled from literature, the data retrieved from the questionnaires distributed among the target audience were compiled and analyzed in detail. Although the action was completed on time, we considered useful to extend the duration of the survey till February 2020, in order to increase the participation and collect more data on the finalisation of the definition of the processes linked to ENMs release. This delay was not considered significant as the targeted populations for the pilot studies were foreseen to be finalised from February to May 2020.

Finally, under **Task A1.3**, Unisanté worked on the identification of European companies willing to participate in biomonitoring studies, as well as on the analysis of the issues limiting the participation of companies on biomonitoring studies. In this framework, a questionnaire had been sent to workers who are potentially exposed to ENMs to 200 companies of the registry of companies related to ENMs (Annexed Table to Action A1 Deliverables submitted in MTR). The survey was held online and, in Table 2, an example of the populations selected after the analysis of the information retrieved from questionnaires is shown. Twenty-seven companies out of the 200, provided at least one answer to our invitation to participate; amongst them, 21 confirmed that they produced or used ENMs; 14 of the respondents were senior managers, 7 H&S managers.

**Results and deliverables:** All foreseen outcomes, namely the list of engineered NMs, the priority list of processes with a high exposure potential and the eligible populations were defined. The foreseen **Deliverable A1** has been submitted and revised according to the PA's comments in July 2019 and in 2020, as well as the update of the information on the uses and properties of targeted ENMs. Milestone **MS1** List of engineered nanomaterials and eligible populations defined was achieved. A publication entitled "Producers of Engineered Nanomaterials—What Motivates Company and Worker Participation in Biomonitoring Programs?", was published on April 2021, in International Journal of Environmental Research and Public Health 18(8):3851, DOI: 10.3390/ijerph18083851 (attached).

*Table 1 Target Engineered Nanomaterials*

| | EXPOSURE (50%) | | HAZARD (50%) | | | ENM SCORE |
|---|---|---|---|---|---|---|
| | Production / Use | OELs | Biomarkers availability | Toxicological effect | Freshwater Acute ecotoxicity (Daphnia) | |
| scoring | 40.0% | 10.0% | 12.5% | 25.0% | 12.5% | |
| SWCNTs | 4 | 1 | 1,25 | 0,78125 | 1,25 | 8,28 |
| MWCNTs | 4 | 1 | 1,25 | 0,9375 | 0 | 7,19 |
| Ag | 4 | 1 | 1,25 | 0,9375 | 1,25 | 8,44 |
| $TiO_2$ | 4 | 1 | 1,25 | 1,09375 | 0 | 7,34 |
| ZnO | 4 | 1 | 1,25 | 0,78125 | 1,25 | 8,28 |
| $CeO_2$ | 4 | 1 | 1,25 | 0,15625 | 1,25 | 7,66 |
| Metal oxides | 0 | 0 | 0,3125 | 0 | 0 | 0,31 |
| Si | 0 | 0 | 0 | 0 | 0 | 0,00 |
| $SiO_2$ | 4 | 1 | 1,25 | 0,9375 | 0,625 | 7,81 |
| Graphene | 4 | 0 | 1,25 | 0 | 1,25 | 6,50 |
| Graphite | 0 | 0 | 0 | 0 | 1,25 | 1,25 |
| Fullerene | 0 | 1 | 0 | 0 | 1,25 | 2,25 |
| $Fe_3O_4$ | 0 | 0 | 0,3125 | 0 | 1,25 | 1,56 |
| $Fe_2O_3$ | 0 | 0 | 0,3125 | 0,46875 | 1,25 | 2,03 |
| $CaCO_3$ | 0 | 0 | 0,3125 | 0,15625 | 0,625 | 1,09 |
| BaTiO3 | 4 | 0 | 0,3125 | 0 | 1,25 | 5,56 |
| CuO | 0 | 0 | 0,3125 | 0,46875 | 1,25 | 2,03 |
| SrO | 0 | 0 | 0,3125 | 0 | 1,25 | 1,56 |
| $SnO_2$ | 0 | 0 | 0,3125 | 0 | 1,25 | 1,56 |
| MgO | 0 | 0 | 0,3125 | 0 | 1,25 | 1,56 |
| $ZrO_2$ | 0 | 0 | 0,3125 | 0 | 1,25 | 1,56 |
| Cu | 0 | 0 | 0 | 0,46875 | 1,25 | 1,72 |
| Au | 0 | 0 | 0 | 0 | 0 | 0,00 |
| Ni | 0 | 0 | 0 | 0 | 1,25 | 1,25 |
| Co | 0 | 0 | 0 | 0 | 1,25 | 1,25 |
| Cd-Se QDs | 0 | 0 | 0 | 0 | 1,25 | 1,25 |
| Indefinite NMs | 0 | 0 | 0 | 0 | 0 | 0,00 |
| $Al_2O_3$ | 4 | 0 | 0,3125 | 0,78125 | 0 | 5,09 |
| Nanoclays / Nanocellulose | 4 | 0 | 0 | 0 | 1,25 | 5,25 |

*Table 2 Targeted population*

| POPULATION INVOLVED | ENMs / Nano-enabled products | SHORT TITLE | PROCESS OR ACTIVIT |
|---|---|---|---|
| WORKERS FROM SMEs | Graphene | Weighing operations of graphene platelets | Bag Filling<br>Weighing operation |
| | Graphene | Weighing Graphene | Bag Filling<br>Weighing operation |
| | Nano-TiO2 | $TiO_2$ synthesis in liquid state | Mixing / stirring operation<br>Harvesting |
| | Nano-SiO2 | $SiO_2$ synthesis in solid state | Mixing / stirring operation /Harves |
| | | $SiO_2$ synthesis in solid state + vacuum cleaning + LEV | Mixing / stirring operation<br>Harvesting / Cleaning |
| WORKERS FROM A SMEs | Nano-TiO2<br>Silver | Weighing TiO2 / Silver | Weigh, charge, mix |
| | | Mixing operations | Weigh, charge, mix<br>Mixing / stirring operation |
| WORKERS FROM RESEARCH ORGANIZATIONS | Metal Oxides | Synthesis | Unpacking<br>Weigh, charge, mix, homogenisatio<br>Cleaning |
| | Nano-ZnO | Application | Weigh, charge, mix<br>Homogenisation<br>Spraying / Dip Coating<br>Cleaning |
| SUBWAY STATION WORKER | Metal Oxide<br>Metal based NMs | Maintenance operations | Maintenance operations<br>Cleaning |
| | Ultrafine carbon based NMs | Ticket office | Ticket dispensing |
| TRANSPORTATION NODES | Ultrafine carbon based particles | Airport fueler | Fueling process<br>Flight Supervision |
| VEHICLE INSPECTIONS AND CONTROL OPERATORS | Ultrafine carbon based particles | Weight station control | Weigh control |
| | | Vehicle Inspections | Regular inspections |

### 6.1.2 Action A.2 Evaluation of exposure measurement strategies and data quality requirements to perform human biomonitoring studies

**Status:** Completed
**Foreseen start date:** 01/11/2018 **Actual start date:** 01/11/2018
**Foreseen end date:** 28/02/2019 **Actual end date:** 28/02/2019

**Objectives** The aim of Action A.2 is to perform **a critical evaluation of existing methods and tools to measure ENMs, their aggregates and agglomerates (NOAA) in workplaces and urban environments**. Within Action A2, a complete analysis of existing exposure assessment strategies and measurement technologies was conducted and documented.

**Activities conducted and progress:** Task A.2 includes three Tasks: A.2.1. Definition of exposure metrics and contextual information "meta-data" needed for human biomonitoring and regulatory risk assessment, A.2.2. Limitations of current exposure assessment strategies to conduct epidemiological studies and A.2.3 Characterization of information requirements and quality criteria for use measured data under REACH and human biomonitoring studies. Within **Task A.2.1**, the type of information needed to link exposure to nanoparticle hazard in different settings, such as occupational, consumer and environmental settings, for the purpose of a consistent approach in human biomonitoring studies was identified by UNITO. By using WEB sources, Projects Reports or Regulatory documents, we have assessed exposure metrics relevant for conducting epidemiological studies, with special emphasis on the metrics used for continuous monitoring such as size distribution, particle number concentration, mass concentration and surface area as key descriptors associated with possible health outcomes. This preliminary identification of the above key parameters along with other physicochemical features such as charged surface, surface reactivity, agglomerate particle size etc. have been used to support the identification of a detailed list of the exposure metrics and minimum meta-data needed to use measured data during epidemiological studies. From the study conducted, it was concluded that the two alternative metrics for NMs evaluation in worksites are surface area concentration (SAC) and number concentration (PNC). However, surface area concentration is likely to be the best metric for predicting health effects of ENMs in humans, since experimental toxicological studies suggest that their biological effects are mainly related to this parameter.

Within **Task A.2.2**, RAMEM has carried out research on current state of the art regarding exposure strategies and measurement technologies. Dr Silvia López Vidal has implemented this research and has also prepared the corresponding deliverable. The conclusion drawn was that there was no commercially available technology able to fulfil the requirements for the exposure strategies needed, therefore the development of prototypes is mandatory. RAMEM´s proposal includes the range of ultrafine particles, but also a range of larger particles: PM1, which are particles that are not considered in the current air quality regulations, but also PM2.5 and PM10, which are considered particles in the present regulations. The equipment developed broadens the measurement range while linking them with the existing measurements given by the actual monitoring stations present in the air quality networks.

In **Task A.2.3** the definition of the specific considerations to be taken into account to incorporate the data measured into the existing Environmental Public Health tracking (EPHT) protocols, EU/EEA Public Health Surveillance Systems (EPHESUS), in the case of epidemiological studies, as well as the risk assessment process under REACH according to ECHA guidance on information requirements and chemical safety assessment, the OECD report on the use of Monitoring Data in the Exposure Assessment of Industrial Chemicals, OHS Directives, as well as any other recommendations included in current or forthcoming guidelines on the use on measured data, was conducted by ITENE. These considerations include among others physicochemical information of ENMs, workplace observations, health status etc. Finally, a complete description of the criteria was defined for the classification of measured data on the concentration of ENMs as valid without restriction, valid with restrictions or non-valid. These criteria have been identified for urban and occupational exposure as well as for epidemiological exposure and health surveillance and include measurements information, location specific characteristics, sampling system, reference and limit values, type of biological sample, health endpoints etc. Metadata include the target chemical, analytical method and

performance information for the analysis; sampling protocol; sampling location and time; information on the nature of the sample; and other relevant information. Finally, the identification of the target chemical limit of detection (LOD) and limit of quantification (LOQ), defined by analytical method; sampling location and sampling time, are key elements for using the data for exposure assessment confidently.

As described by the OECD, completeness of the meta-data, associated information to the monitoring data, are critical for data quality. In the framework of NanoExplore, generated data were collected according to requisites of information and data quality required by REACH Regulation (for measured exposure concentrations use) and main monitoring programs (for the incorporation of generated data in the databases).

**Results and deliverables:** The main results of the action include: a) a detailed list of the exposure metrics and minimum meta-data needed under epidemiological studies, b) shortcomings of current monitoring approaches to quantify the nature and extent of exposure in epidemiological studies and c) a stepwise procedure to evaluate the adequacy of existing and new monitoring data for risk assessment purposes on a regulatory basis. The foreseen **Deliverables DA2a and DA2b** have been submitted on time and were accordingly revised and resubmitted with regard to the PA's comments.

### 6.1.3 Action A.3 Critical evaluation of current data on biological effects and existing biomarkers of nanomaterial exposure

**Status:** Completed
**Foreseen start date:** 01/09/2018 **Actual start date:** 01/09/2018
**Foreseen end date:** 28/02/2019 **Actual end date:** 28/02/2019

**Objectives** The aim of Action A.3 is to **review the state of the art on existing candidate biomarkers for assessing exposure and potential human health effects following exposure to ENMs (T A.3.1).** This analysis is needed to identify and select appropriate biological indicators and tools, to assess the feasibility of using them in field studies of nanotechnology workers and finally **identify the candidate biomarkers (T A.3.2)** and **propose methods for monitoring biomarkers of exposure and effect (T A.3.3).** Thus, **Action A3 is a foundation action for the implementation of the project, after having defined the ENMs of interest and the processes and eligible populations (Action A1) as well as exposure strategies and measurement techniques.**

**Activities conducted and progress:** Within Action A3, all tasks foreseen were conducted and successfully accomplished.

The main findings of this review are summarized below:

i. **Twenty-five human studies** were identified to be reliable in terms of methodology and number of participants,
ii. **The primary targets of ENM exposure were the respiratory and cardiovascular systems**,
iii. **Changes in biomarkers levels compared with controls were also observed**; however, limited exposure data and the relatively short period since the first exposure may have influenced the incidence of adverse effects found in epi studies,
iv. Although it is possible to identify **candidate biomarkers**, their **suitability in terms of pathophysiological meaning, specificity and sensitivity towards health outcomes, should be assessed in well designed field studies**.

Moreover, **this review provided all the information required for:**

v. **The identification of candidate biomarkers associated with exposure to UFP/ENM (T A.3.2)** and
vi. **The development of methods for biomarkers monitoring and their feasibility in field**
vii. **studies (T A.3.3).**

It is to be noted that the review presented in Deliverable A.3 was based on the deep experience and expertise of Prof. Bergamaschi and his group and their former works. In the costs incurred, the above fact has been taken into account.

In **Task A.3.1**, carried out by UNITO, **a systematic literature review of mechanistic (in vitro and in vivo) studies, human studies and epidemiological investigations of exposed workers dealing with possible adverse effects for the most widely used ENMs has been performed** through searches of major scientific databases in order to refine a biomarker database and develop the candidate biomarkers table. Biomarkers have been identified from the peer reviewed literature, taking into account the particle forms (nanoscale and non-nanoscale), target organs and relevant/attributable health effects, and including occupational, environmental and field studies. These biomarkers have been grouped according to their physiological meaning and pathophysiology and the biomarker database was structured (Table 5 of Deliverable A3).

Biomarkers of exposure include blood and urine concentrations of the substance itself, in our case particle concentration at the main target organ (lung). Local and systemic effect markers have been subdivided according to their position in the exposure-effect pathway, to address early and reversible effect markers, clinically relevant markers and markers with a prognostic meaning.

In **Task A.3.2**, conducted by Unisanté, the **critical evaluation of the putative biomarkers, identified in T.A.3.1, was performed based on their pathophysiological meaning, potential health effects and the assessment of their suitability for incorporation into worker (health) monitoring schemes has been accomplished**. Only those biomarkers which are sufficiently robust for consideration for integration into field studies and/or health registries for nanomaterial workers have been selected from the database of Task A.3.1. In order to assess the intrinsic quality of the biomarkers, the following features have been considered: specificity and sensitivity towards the exposure/effect of interest (e.g. reflecting exposure to nano-objects only vs. combined exposure, organ and disease specificity). Unisanté focused on identifying and validating oxidative stress biomarkers (e.g. Heme Oxygenase, SOD, GPX) using non-invasive sampling (EBC, exhaled air, urine). Finally, a further, systematic review on the variability of these biomarkers was launched to complement the work conducted by UNITO in Task A.3.1 and the validated biomarkers are identified. In the following table (Table 3) a snapshot of the list of biomarkers investigated and validated is presented (Table 5 of Deliverable A3).

*Table 3 Appraisal of biomarkers of exposure or effects investigated and validated (**in bold**) in in-vivo or human studies on people exposed to different ultrafine or fine particulates or known fractions, and biomarkers specifically investigated in relation to ENMs (snapshot)*

| Quality of Biomarker | Biomarker | Physiological / Pathological meaning |
|---|---|---|
| EXPOSURE | Exhaled particles and/or elements in EBC | estimate of the "deposited dose" or "target tissue dose" |
| | Metallic Elements analysis in biological fluids | excretion, body burden |
| | Lipid peroxidation products in EBC or blood (**MDA**, **T-BARS**, LTB4, F2- and **8-isoprostane**; saturated aldehydes: 4-hydroxy-trans-nonenale (**HNE**) and 4-hydroxy-trans-hexenale (HHE) | Oxidative stress and airways inflammation (EBC) vs. Systemic (blood) |
| | **IL-6**, **IL-8**, sTNF-RII, **TNF**-α; High-sensitivity C-reactive protein (**hsCRP**) in plasma | Systemic inflammation |
| | DNA excision base products in plasma/urine: 8-hydroxy-2-deoxyguanosine (**8-OHdG**), 8-hydroxy-guanosine (8-OHG) | Systemic oxidative stress; oxidation of nucleic acids |

In **Task A.3.3**, conducted by UNITO, the feasibility of biomarkers in field studies has been investigated, taking into consideration potential barriers for providing biological samples (including ethical considerations and invasiveness of sampling) and practical/logistical considerations, such as pre-analytical factors affecting biomarker stability (e.g. collection, storage conditions) and technical feasibility (e.g. availability and accessibility of analytical techniques). Practical issues, such as the adequate sampling strategy, the non-invasiveness of sampling for biological media were also privileged, as to obtain specimens in sufficient amounts under routine conditions and without unacceptable discomfort and health risk for the individual or worker ("ethical issue").

It should be mentioned that, at this point, only candidate biomarkers could be identified. Validity and reproducibility of biomarkers were not assessed in the studies reviewed, due to the

generally small study sample used and other methodological limitations such as the limited time since first exposure (<15 years). However, the field studies conducted during the Project implementation allowed to validate specific biomarkers measured in biological fluids collected in non-invasive way (exhaled breath condensate, urine), therefore, whereas the objective of feasibility is achieved, their validity has been further investigated and confirmed in field studies.

Finally, from the critical review implemented in the framework of Action A3, the problem of unknown baseline values for most candidate biomarkers was identified. This finding led to the amendment of Task B.2.3 Determination of Reference Values for Biomarkers selected, which added value to the project outcomes.

**Results and deliverables:** The foreseen **Deliverable A3** was submitted on time. Deliverable A.3 summarizes in a quasi-comprehensive way the state of biomonitoring studies and biomarkers in order to lead to reliable conclusions and hypotheses. The conclusions drawn are based on this (pre-existing) work which finally led to the preparation of the list of candidate biomarkers. Therefore, through the input data (previous work) evaluation and critical analysis, the main deliverable was accomplished, summarising all the foreseen tasks in the framework of Action A3. In addition, obviously, this information makes part of the expertise and background of the authors and reflects their high-level scientific knowledge. If this was not the case, this work would be very difficult to implement under the specific time and cost frame. It is also to be noticed that the cost incurred to this Action is based solely on the work done beyond the current knowledge and this is why it was kept low. Milestone **MS2** "MS2. List of candidate biomarkers hierarchically ordered available" has been achieved.

### 6.1.4 Action A.4 Conceptual design of the NanoExplore approach and implementation plan

**Status:** Completed
**Foreseen start date:** 01/12/2018 **Actual start date:** 01/12/2018
**Foreseen end date:** 31/03/2019 **Actual end date:** 30/04/2019

**Objectives** The main goal of the action is to define the **functionalities and specifications of the wireless sensor network, the structure and functionalities of the web-based data management tool and the implementation plan of the NanoExplore approach**, including the definition of pre-selected locations to conduct demonstration studies.

**Activities conducted and progress:** Action A4 includes **the identification and analysis of end-users and stakeholders needs (Task A4.1), the identification and definition of the minimum requirements of the NanoExplore wireless sensor network and web-based application (Task A4.2) and the definition of the implementation plan of the project (Task A4.3)**. It is an important Action as it concludes all previous preparatory actions and prepares the implementation phase. All tasks were accordingly implemented.

In **Task A4.1** the analysis of the end-users and stakeholders needs, mainly professionals, in relation to the characterization of potential adverse effects and exposure monitoring when dealing with ENMs, was carried out by Yordas in collaboration with ALCON and all the other partners. To identify and analyse the needs of stakeholders, a survey was created to support the conceptual design of the NanoExplore approach. The survey addressed ENM types and forms used/ manufactured, questions on health and safety aspects of handling of ENMS such as exposure, health and safety plans, engineered controls as well as the use of personal protection equipment. Additionally, the survey included general regulatory and environmental aspects, i.e.

environmental protection measures and exposure monitoring programmes. The survey has been sent to 200 companies and it was available online and made public via social media. Despite the promotion done, only 27 answers were received and the results of the survey were included in the MTR. The propensity of companies to participate in biomonitoring studies has already been identified in previous works and is associated with the lack of a regulatory framework to support the organization of such studies and the lack of awareness of the target populations (see publication annexed). Additionally, all partners conducted informal interviews with relevant stakeholders to gain their feedback on the needs for the NanoExplore approach, i.e. interviews were held with the NanoIndustry Association, a major chemical manufacturer, Hellenic Association of Chemical Industry, Italian Association of Chemical Industries, Cosmetica Italia, ANSES France etc. All feedback from the survey as well as from informal interviews were fed back to the project team to be considered for the development of the NanoExplore integrated approach for exposure and health effects monitoring of ENMs in workplaces and urban areas. Although this survey elicited a limited participation, the results are consistent and useful for designing appropriate biological surveillance protocols for implementation in occupational settings.

The second task, **Task A.4.2**, undertaken by ITENE and RAMEM along with ALCON, consisted in the definition of the main functionalities to be implemented in the NanoExplore wireless sensor network and web-based application. The derivation of the requirements was conducted considering a 3 stages approach: Stage 1. Expert panel and interviews, Stage 2. Technical feasibility analysis and Stage 3: Technical specifications document. The first stage focused on the selection of an expert panel, including expert staff from the project, as well as end users from targeted end-users, including occupational, health and safety (OHS) advisors and air quality experts from the Valencian Community air quality network. The expert panel finally selected included the following persons: The functionalities considered were based on the information retrieved from task A.4.1 and current experience and expertise of the members of the consortium. In addition, a consultation process with the main end-users (industry, regulatory bodies, environmental agencies, policy makers) has taken place to ensure that the preferences, interests and perspectives are considered. The second stage focused on the analysis of the technical feasibility of the requirements identified considering the budget available and the current state of the art of the technologies. This analysis was conducted by expertise staff from RAMEM and ITENE. The last stage focused on the preparation of a technical specification sheet that was discussed with action leaders to get the approval to start with the development process.

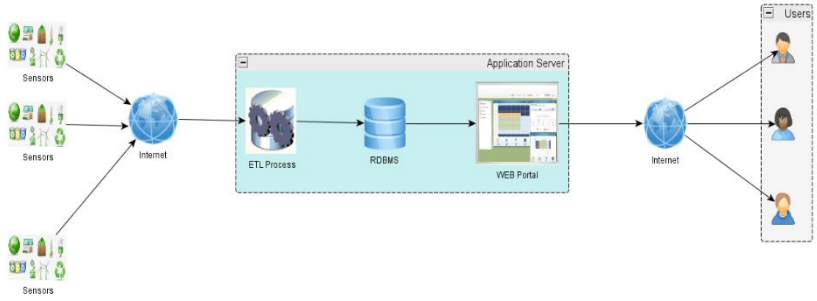
Based on the above, the main characteristics of the monitoring station design and functionality defined under action A4 are depicted below: a) compact size station b) able to operate 24 hours/ 7days, c) battery powered, d) wireless remote-control systems to provide real-time information on the concentration of ENMs in outdoor and indoor areas, providing data on the number concentration (number/cm3), mass concentration (µg/cm3), lung deposited surface area (µg/cm2) and average particle diameter (nm), e) particle collection unit, f) tailored designed software to control the instrument settings, g) remote and local access, h) identification of relevant events during operation, i) integrated cooling unit to climatize the particle sensor monitoring unit, i) reduction of the background noise, j) being low cost. The above characteristics of the monitoring station design and functionality are depicted explicitly in the corresponding Deliverable DA4a, “Report on the functionalities and system requirements of the NanoExplore integrated system Part 1” as foreseen in Task A4.2.

The on-line software application (webserver) will be based on a flexible and modular architecture to support the processing of data on ENMs concentration and the collection,

analysis and interpretation of health-related data needed for the planning, implementation and evaluation of public health practice. The identification of the web based tool functionalities is based also on the information gathered during task A4.1, as well as the information retrieved from task A2.3, including specifications of the type of data used by public health surveillance systems, recommendations published in the ECHA guidance on information requirements and chemical safety assessment, as well as any other recommendations included in current or forthcoming guidelines on the use on measured data. The main web tool ensures accessibility over the Web, availability of processed and/or raw data from various data sources, user friendliness, estimation/elaboration of values such as concentrations, (from given emission estimates), forecasts or historical data, different groups of users, map layout, search tool, statistical tools.

The programming requirements include compatibility of versions for personal computers, tablets and mobile devices, operation on several browsers, data extraction in excel or csv files.

A high-level diagram of the solution in relation with the external entities' interoperability is shown below:




The main characteristics of the web portal are depicted explicitly in the corresponding Deliverable DA4a, "Report on the functionalities and system requirements of the NanoExplore integrated system Part 1 and Part 2" as foreseen in Task A4.2.

*Figure 1. Scheme of the web-based application proposed*

Finally, **Task A.4.3**, undertaken by ALCON, has provided the implementation plan of the project considering the results of the preparatory actions, as well as the technical amendment. This detailed plan is presented in Deliverable A4b to ensure the implementation of the outcomes of the project by the industry and stakeholders. The implementation plan includes the definition of the scope of the activities to be conducted to validate the specifications of each element in the NanoExplore integrated approach, as well as the list of actions to support the demonstration of the applicability of the outcomes of the project for human biomonitoring, including the selection of the specific locations and study cohorts, the definition of monitoring indicators to measure the progress of the project, as well as the definition of the steps needed to ensure the implementation.

**Results and deliverables: Deliverables A4a and A4b** have been submitted with a slight delay and have been accordingly updated after the technical amendment and with regard to PA's comments. Finally, **MS3** on the software and monitoring network requirements has been achieved.

### 6.1.5 Action B.1 Design and development of the wireless sensor network to monitor ENMs

**Status**: Completed
**Foreseen start date:** 01/04/2019 **Actual start date:** 01/04/2019
**Foreseen end date:** 30/06/2020 **Actual end date:** 30/06/2021

**Objectives** The main goal of the action is **to design and develop a low-cost wireless sensor for measuring particle number concentration and mass concentration of relevant ENMs**

by combining low pressure impactors, diffusion charging, miniaturized particle measurement units and data transmission elements.

**Activities conducted and progress:** Action B1 includes the following tasks:

***B1.1.*** *Definition of the specifications of the wireless sensor prototype.* In this task RAMEM has worked together with ITENE in the definition of the specifications, selection of commercials and consumables, electronic and software (SW) design for the prototype development. Dr Silvia López Vidal and Dr Pavla Dohányosová have worked in this task and have written the deliverable DB1a in collaboration with ITENE. Drs López and Dohányosová have also worked with a Systems´ Engineering University professor to ensure the due level of quality for the deliverable and for the development. The task has gone as planned in terms of results and deliverables although with a delay due to the decision made to include Partector 2 instead of DiSCmini (nanoparticle detectors), in order to ensure the use of more robust electronics. Although the selection of Partector 2 is more reliable for the operation of the device, as it includes an industrial communication protocol, it does not need much power, it possesses a better operating system and a most adequate programming language, this solution has increased the budget of the NP sensor and, therefore, it became mandatory to reduce the number of units to 8, instead the initially foreseen (up to 20). However, the lower number of units did not affect the project's scheduled actions. Finally, after the completion of field studies, RAMEM has taken into consideration the feedback obtained from the users and implemented the changes/improvements required.
Progress in T B1.1 compared to Action A4 has been made in the selection of the specific system parameters. The selected solution and its justification are provided in detail in section 8 of DBa.

***B1.2.*** *Design and development of the portable measurement devices and communication modules.* RAMEM has started with this task in January 2020. An updated version of the deliverable (D.B.1.a) has been submitted with the agreement of the consortium. The new development includes new hardware (electronics) and new software in Python, Linux based Operating system and utilities to make the data analysis easier. Additionally, a new NPs sensor was selected, a TEM grids holder was included and battery and solar cell were added for better autonomy.

The layout and the electronic scheme are shown in the pictures below (*Figures 2 and 3*). During the design phase, an **LC Impact Assessment of the wireless sensors was conducted**. The LCA was developed in accordance with internationally accepted ISO standards 14040 and 14044 and it is included in Deliverable DB1b. The development of the prototype was delayed due to the pandemic and "**Task B1.3**. Internal testing and validation" was delayed too, as it started after the first prototype is produced. This task was implemented by ITENE, under controlled conditions, and it was finally completed after the accomplishment of the field studies to include all failures and/or weaknesses occurred. All problems arisen were resolved and have been included in the corresponding report of Action B6. Furthermore, **Task B1.4** Scaling-up was accomplished by the end of June 2021.

*Figure 2 Layout of the instrument*

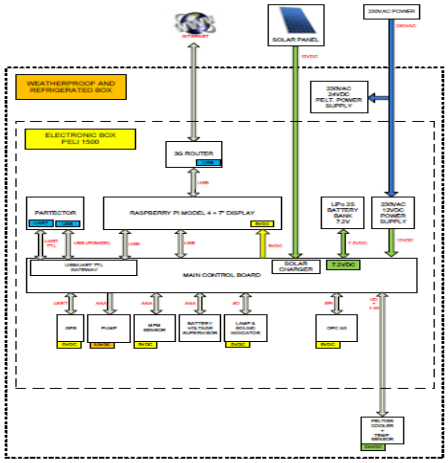



*Figure 3 Electronic scheme of the instrument*

**Results and deliverables:**

We have produced 8 units of indoor portable NanoExplore Device (1 prototype unit and 7 series units), 4 units of outdoor enclosure. The original proposal doesn't correspond very much to the real production cost. The OEM DiSCmini cost (in 2018) was about 6500 €, whereas its commercial version 12000 €. We purchased Partector 2 from Naneos for 6350€ with a big discount whereasits normal price is currently 13600 €. So, we have changed the sensor from DiSCmin to Partector 2 for reasons explained in DB1a (the OEM version is not anymore disponible, the full version costs 13600€, maintenance and compatibility). As a result, we were obliged to adjust the number of units to our budget which was limited and did not permit fabrication of more devices as it can be seen from the financial justification.

However, the production of fewer devices did not cause any deviations on the implementation plan of the project field studies. The pilot and case studies were organized considering the limitations on the number of devices available. To this end, the consortium decided to concentrate the pilot studies conducted under B4 in two months, making possible to use additional devices during the case studies scheduled under action B6. Moreover, UNISANTE and ITENE supported the monitoring campaigns using available devices, including particle counters and sampling pumps, to generate the necessary amount of data to evaluate the potential exposure to nanoparticles, their agglomerates and aggregates.

The pilot studies were conducted using two instruments, identified as NE1 and NE6. The device NE1 was used in the pilot study conducted in four selected facilities of Kerakoll, all located in Italy, as well as the campaign conducted in the facilities of the research organization ICN2, located in Barcelona, Spain. The device NE6 was installed in the company Applynano, located in Alicante (Spain).

The case studies conducted under B6 were also organized considering the limited number of devices. The monitoring studies related with urban areas were conducted using devices NE3 and NE4, as both devices include an environmental enclosure to protect the devices, as well as a solar panel, making possible the operation in the event of limited access to electricity. These devices were installed for 3 weeks, sufficient time to analyze and test the robustness of the device. NE1 and NE5 were also dedicated to measure the concentration of nanoparticles, PM2.5 and PM10 in two areas located in Athens and Thessaloniki respectively. In this case, both stations were installed and running correctly. NE2 and NE6 were used to perform monitoring campaigns in Laurentia, Grupo Antolin and AD Biocomposites, where the operation of the tool was analyzed.

**Deliverables DB1a and DB1b** were submitted on time and a revised version was submitted recently. DB1c has been submitted and updated after the finalization of the devices production. DB1d foreseen is also submitted with a delay. Similarly, milestones **MS4 and MS5** "MS4. Beta version of the wireless sensor available and validated" (03/2020) and "MS5. Wireless sensor network and controlling unit available" (05/2020) were achieved with delay.

### 6.1.6 Action B.2 Validation of biomarkers for human biomonitoring studies

**Status:** Completed
**Foreseen start date:** 01/01/2019 **Actual start date:** 01/01/2019
**Foreseen end date:** 30/06/2021 **Actual end date:** 31/03/2022

**Objectives** The main goal of the action is **to assess and optimize the specific requirements for carrying out studies on nanomaterial workers**.

**Activities conducted and progress:** Action B2 includes Tasks B.2.1 Assessment of existing studies and the most critical issues in designing epidemiological studies in nanomaterial workers, B.2.2 Integrate and develop a harmonized protocol of the collaborative study of human biomarkers with respect to nanoparticle exposure and B.2.3 Determination of reference values for biomarkers selected.

Action B2 is the most important prerequisite for the implementation of biomonitoring studies, with the development of the study protocol and the reference values for the biomarkers selected.

In **Task B.2.1**, human studies and epidemiological investigations of exposed workers dealing with possible adverse effects for the most widely used ENMs have been reviewed by UNITO, with focus on studies identified in Action A3, and their study design was assessed with regard to the risk related to ENMs exposure. The main conclusions drawn from the results of Task B.2.1 are presented below:

- To date, the most critical issues in epidemiological studies on ENMs are: particle heterogeneity, temporal factors, data collection and analysis, a correct exposure characterization, definition of disease endpoints, and identification of study population.
- The heterogeneity of ENMs and the ability to recruit pooled cohorts of adequate size with similar and long-term exposures, remain among the greatest methodological and practical challenges. Furthermore, there is lack of a consistent industry-wide exposure assessment program and no harmonized registration systems for workers employed in these industries are available. As a result, current studies suffer of questionable statistical power related to small workforce sizes and short latency for disease occurrence.
- A clear knowledge on ENMs specific models of action or health effects and early markers of effect is lacking and companies' participation to studies is still low.
- Scientific and political coordination at national and international level would be essential to harmonise the research and collection protocols upstream of data collection.
- Large-scale longitudinal prospective epidemiologic designs would be the best choice for studies investigating workers exposed to ENMs, forming cohorts with potential exposure and following them forward, available also in the future.
- Panel studies and cross-sectional studies in populations of exposed workers can also be applied in this early stage.

**Task B.2.2**, led by Unisanté, aimed at elaborating the most efficient and feasible strategy of recruitment of companies and individuals with accounting for 1-number of potentially exposed individuals in different settings/companies, 2-propension to participate, 3-barriers to participate, and 4-support of stakeholders, and with respect to the national/regional specificities.

All partners have participated in the preparation, review and translation of three questionnaires to be used to assess the degree of awareness of stakeholders and their interest to participate in field survey and/ to training activities. Despite the difficult situation due to COVID, UNITO carried out a biomonitoring pilot study in a small company near Torino, producing paints and using UF TiO2. Results from biomarkers, available at the beginning of 2021, support the idea that an integrated approach relying on both personal exposure and biomarkers assessment can improve the risk characterization in occupational settings in which TiO2 is handled. Task B.2.2 which has been delayed for a number of reasons such as delays in previous tasks, e.g. T B.2.3, and the pandemic restrictions, has successfully been accomplished and the fundamental deliverable "Harmonized Study Protocol" was produced and published with special emphasis on procedure characterisation, sample representativeness and recruitment feasibility. This harmonized protocol paves the way for reliable and consistent biomonitoring studies with comparable results.

In **Task B.2.3**, Unisanté and UNITO worked on **Study 1 (Determination of reference/baseline values for biomarkers measured in urine and exhaled breath condensate).** In particular, for the candidate biomarkers selected from Action A3, six systematic reviews and metanalyses were conducted to determine their reference values in EBC and urine. This was the first attempt to define reference values for these biomarkers using meta-analytical methods. In the framework of **Study 2 (OPEA study)** implemented by Unisanté, a first study in general population where OPEA has been measured, a large and likely representative sample of individuals participated. The estimated reference interval was provided for the first time, enabling comparison of OPEA values obtained in other studies and different settings with this interval. The estimated reference intervals are very important for the interpretation of the NanoExplore results but also for the broad scientific, pharmaceutics and medical communities.

**Results and deliverables:** The main results of this action dealt with the design and planning of the field studies by identifying and taking into account the most critical issues, developping the harmonized protocol for conducting human biomonitoring studies and determining the reference values of biomarkers selected. Deliverable "DB2a Harmonized protocol for the human biomonitoring studies" has been submitted (publication is annexed) as well as Deliverable DB2b Determination of reference values for biomarkers selected submitted in 2 parts (urine and EBC and OPEA). Seven articles resulting from this work were published in the international peer-reviewed journals (publications are annexed).

### 6.1.7 Action B.3 Development of the NanoExplore web based platform

**Status:** Completed
**Foreseen start date:** 01/07/2019 **Actual start date:** 01/07/2019
**Foreseen end date:** 30/06/2020 **Actual end date:** 14/05/2021

**Objectives** The main goal of the action is to **design and develop the NanoEXPLORE intelligent web-based platform aimed at supporting the acquisition, management and processing of data on the concentration of ENMs monitored by the sensor prototype network in industrial settings and the environment**.
**Activities conducted and progress:** Action B.3 aims at the actual platform development. In this context the following activities were undertaken per task by ALCON in collaboration with GETMAP, in accordance with the action description.
**Task B3.1. Preparation and definition Tasks**

- Definition of the data sources and the communication protocol through information exchange between RAMEM and ALCON. Data are uploaded via FTP and JSON/CSV file format is used. Up to approximately 50 parameters are expected.
- User/Role/Rights definition. The following roles were identified: a) Administrator, b) Public (unregistered users), c) Companies, d) Stakeholders. Each role has different privileges in accessing the data.
- User Requirement analysis. Functional and not functional requirements analysis was performed. Main modules include: Real Time Dashboard, Map, Detailed Data presentation, Data filtering and export, Statistics, User registration/Management, Sensor monitoring, Alerting.

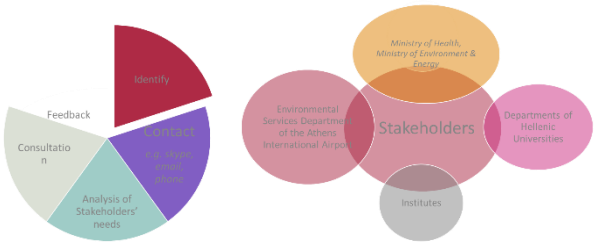



*Figure 4 Stakeholder engagement and user requirement*

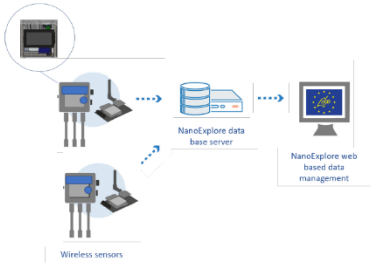



*Figure 5 System Architecture*

**Task B3.2. Software development and implementation**

- Investigation for IoT best practices and standards for conceptual and logical modelling. ISO/OGC Observation and Measurement (O&M) model [OGC and ISO 19156:2011] was selected as the basis for the development.
- Design of the architecture of the platform. An open architecture design was adopted to support maintenance and scaling. Free and Open Source (FOSS) technology used.
- Database implementation (on PostgreSQL). The multitenant schema structure allows for addition of things and sensors with various characteristics and measured properties, on custom types of places (e.g. village, industrial facilities), thus ensuring the reusability of the platorm. Special timeseries of the observed values is indexed by TimeScaleDB to maintain high performance.

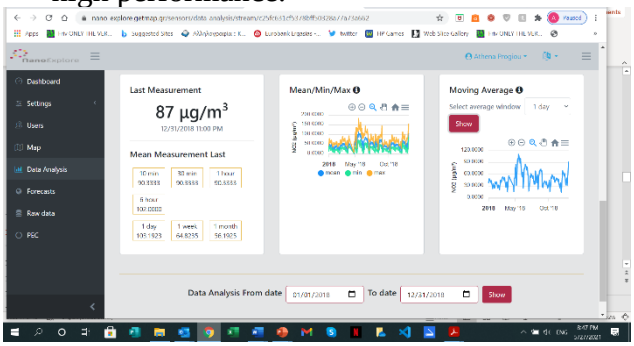



*Figure 6 Web Platform snapshot*

Implemented Modules: Registration and User management Module, Map module, Real Time Dashboard, Data filtering and export, Sensor.

**Task B3.3. Testing and Put in operation**

The activity refers to the continuous integration and testing of the modules, the unified platform until is set to full production operation.

**Results and deliverables:** The platform developed is dedicated for operation in combination with the NanoExplore device, it receives automatically all the monitoring data, it stores them and it processes them by conducting statistical analyses on demand by the user. It is a user-friendly tool that provides the possibility to see, assess and elaborate all data generated within the project. Of course, the platform can also receive data from other sensors, but in this case, if the file produced is not compatible, the upload should be manual, this was the case of the data received from the monitoring station of the Piraeus Port. Deliverables "B3a Users manual and beta version release" and "B3b Performance Evaluation Report" are submitted and "MS8

Operational version of the NanoExplore platform" was achieved. The delays encountered are connected to the pandemic and the delays in the implementation of Action B.1. However, this delay did not affect the implementation of the field studies.

### 6.1.8 Action B.4 Screening biomonitoring studies in industrial facilities and urban areas

**Status:** Completed
**Foreseen start date:** 01/01/2020 **Actual start date:** 01/01/2020
**Foreseen end date:** 31/03/2021 **Actual end date:** 30/06/2022

**Objectives** The main goal of the action was to conduct **pilot biomonitoring studies to assess the feasibility of the harmonised protocol of the collaborative study and refine the NanoExplore integrated approach in a well characterized limited number of unexposed and exposed workers to nanoparticles, including workers from production facilities, workers from office positions, working in civil infrastructures, and control participants.**

**Activities conducted and progress:** Action B.4 comprised several subsequent tasks, namely:
**Task B4.1**. Recruitment of the study participants (coordinated by Unisanté) aimed to apply the strategy of recruitment of study participants elaborated in Task B2.2 to identify eligible workers in different settings;
**Task B4.2**. Exposure assessment (coordinated by ITENE), dedicated to the exposure characterization at the workstations where exposed workers performed the task of concern;
**Task B4.3**. Measurement of Biomarkers and of their relevance with respect to nanoparticles (coordinated by UNITO) focused on the sampling and analysis of the effect biomarkers in biological fluids, including exhaled air, exhaled breath condensate (EBC), and urine in order to detect potential early effects on the pulmonary and cardiovascular systems;
**Task B4.4.** Exposure Registry (coordinated by ITENE), dedicated to the compilation of the data on the levels of exposure and biomarkers in a registration form to support further studies.

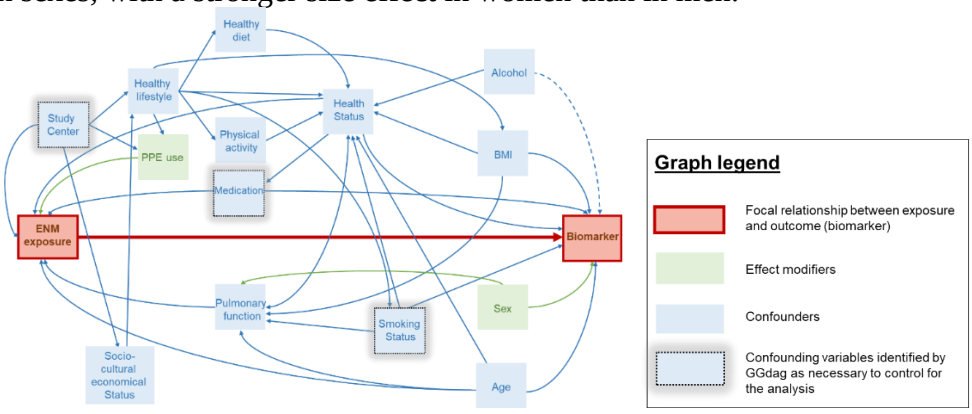
Among these tasks, the Task B4.3 was subdivided in two sub-tasks:
**Sub-task B4.3.1** Sampling and measurement of selected biomarkers in laboratory and, in parallel, the characterization of the health status of workers, creation and completing of a log-book of exposure activities and
**Sub-Task B4.3.2** Statistical analysis of the relationship between nanoparticles exposure and selected biomarkers based on the individual data collected within the tasks B4.1, B4.2, and B4.3.1 and merged in a database for descriptive and etiological analyses. This sub-task is particularly crucial for the Action B4, as its results should allow concluding on the relevance of each biomarker for biomonitoring of ENMs exposed workers.

Field campaigns were conducted as soon as the restriction measures due to the pandemic were lifted, in summer and fall 2021 (Tasks B4.2 and B4.3). In Task B4.2, a comparison was made between the estimation of the exposure of theoretical scenarios using the NanoSafer tool and the estimation of risk from real scenarios obtained from the two measurement campaigns carried out in Italy and Spain using the three-tiered approach proposed by the OECD and the formula from the Tier 2 approach by nanoGEM. The results obtained from the monitoring campaigns had as main objective to characterise nanoparticle emissions in the most representative production areas, the determination of the potential exposure to nanoparticles during the different processes, including quantitative data related to the levels of exposure of a particulate material in the range of 10nm to 10µm, thus, in the inhalable and respirable range. Another objective was to determine the spatial variability of nanoparticles in the air and the dustiness of the material, as well as the state of aggregation/agglomeration in the air.

Additionally, chemical and morphological characterization of the materials was implemented through samples taken in filters for each scenario. From the scenarios studied, the most significant in terms of exposure level, are the mixer area and the cellulose transfer at the Zimella plant (production of paints and coatings), as well as the mixers and the powder transfer at the plant in Brugine (production of adhesives and paints). In ICN2 (Research Institute in Nanotechnology), the only scenario with no significant exposure was that of the Novel-Energy Oriented Materials Group. In Deliverable B4a, detailed information is given on the exposure studies.

The biomonitoring study sample included 141 adults of both sexes (Task B4.3). Regarding the comparison of the exposure-related variables between the three ENM exposure groups (no ENM exposure, low and moderate to high exposure), the group with moderate to high ENM exposure had the highest exposure to nanoparticles, regardless the metric used (i.e., particle number concentrations or LDSA). Moreover, the systemic use of personal protection was reported by 80% of participants in this group. Regarding the type of ENMs handled by the participants, CaCO3 and SiO2 were the most common chemicals reported by companies, followed by TiO2 and iron oxides. SiO2 and TiO2 were also the most often reported ENM exposures by the study participants. The use of the Directed Acyclic Graph (DAG), shown in Figure 7 below, was proposed in order to visually explore the whole causal network that links ENM exposure and its outcome. The DAG was used to determine variables that can be identified as potential confounders or effect modifiers, to overcome the revealed between-group differences in the exposure-effect and dose-response analyses.

In conclusion, this study showed a significant dose-response relationship between IL-10, IL-1β and TNF-α measured in EBC with both particle number concentration and LDSA. This pattern of dose-response in EBC was differing between men and women, with the association being stronger in the latter. Another biomarker in EBC was also marked by a gender effect, hs-CRP was significantly and positively associated with both particle number concentration and LDSA only in men. These results suggest an innate immune response rather than oxidative stress in the EBC. An absence of oxidative stress is also observed in the exhaled air. Regarding urine, a significant negative association was observed between both ENM exposure metrics and Total Antioxidant Potential (TAP). This dose-response relationship was also statically significant in both sexes, with a stronger size effect in women than in men.




*Alcohol was linked to Biomarker with a dotted arrow because the scientific evidence is uncertain.*

*Figure 7 Directed acyclic graph showing the causal relationship between ENM exposure and a biomarker and the variables identified as confounding variables Figure 4.*

The damage on lung function (decrease of FEV1 and FEF25-75% compared to LLN) observed was due to the cumulative long-term exposure over the last 10 years rather an acute effect of short-term ENM exposure, these data confirm what reported by others who, in a similar exposure scenario, did not find significant changes in lung function tests in a 4 years follow-

up. More detailed information on the numerous data produced and their analysis is included in Deliverable B4b.

We recommend continuing the follow-up of this study, as a prospective cohort, both improving the exposure characterization also at individual level as well as recruiting additional companies and participants, to increase the statistical power. The harmonised protocol elaborated within the NanoExplore project should facilitate this development. Further analysis of collected samples and data could also usefully complement and confirm the study findings. Moreover, this could allow to build a more consistent dose-response relationship analysis.

**Results and deliverables:** Deliverables "B4a. Report on Exposure Assessment", "B4b Report on Human Biomonitoring Studies" and "B4c. Registry of Workers" are submitted and "MS9 Screening Biomonitoring Studies" was achieved. The delays encountered are connected to the pandemic and the delays in the implementation of previous Actions.
Follow-up of biomonitoring studies was not possible to be accomplished in the time plan of the project due to the significant delays and difficulties because of the pandemic and the corresponding cost increase as well as the propensity of facilities to participate in the studies. However, these field studies are included in the After Life Plan and are going to be implemented, on partners' own costs in the beginning of 2023.

### 6.1.9 Action B.5 Derivation of recommended exposure levels and risk rating to support EU policies

**Status:** Completed
**Foreseen start date:** 01/04/2020 **Actual start date:** 01/04/2020
**Foreseen end date:** 30/09/2021 **Actual end date:** 30/06/2022

**Objectives** The main goal of the action is to **conduct a systematic review** of the data on the toxicological profile, exposure levels and effects observed using biomarkers **in order to propose recommended exposure levels and risk ratios to support decision making by competent authorities.**

**Activities conducted and progress:** The main tasks undertaken for the derivation of Recommended Exposure Levels (RELs) are: i. Review of proposed Occupational Exposure Levels and identification of data needed to derive RELs (T B.5.1), ii. Analysis of project data and results from literature review and evaluation of RELs and Occupational Exposure Bands (OEB) for the types of ENMs studied (T B.5.2), iii. Grouping studied ENMs into similar toxicity groups (T B.5.2), iv. Risk Characterisation Ratios (RCRs) and database construction (T B.5.3) and v. Transfer of project results to policy stakeholders (as EEA, ECHA, JRC and OECD) through e-mails, reports, phone-conferences or face-to-face meetings (six-monthly) – Non confidential reports to EEA, ECHA and Regional governments (T. B.5.4).

**Task B5.1** includes the review of available frameworks for the development of occupational exposure limits and bands for ENMs and their aggregates and agglomerates (NOAA), as well as current nano-OELs proposed by national and international bodies, including an in-depth review of the information used to derive recommended values for specific groups of ENMs. Based on the review results in Task B5.1, the methodology for the calculation of the OELs for workers was determined and the uncertainty factors considered were selected according to the approach proposed by Weldon et al. (2016) and NIOSH 2013 (pls. see DB5a).

In **Task B5.2**, new values for Occupational Exposure Limits for a list of selected nanomaterials were defined. The derivation of the OELs was based on the calculation of the Human Equivalent Concentration (HEC) and the use of the Multiple-path Particle Model (*Table 4*). Calculations

were based on data from a subchronic inhalation toxicity study using a lung dosimetry model. Moreover, a proposal of Occupational Exposure Bands (OEBs) for nanomaterials, based on data reported in literature, has been included. In Table 4, the OELs derived for a list a selected ENMs is presented. More details for both Tasks B5.1 and B.5.2 are included in Deliverable B5a.

In **Task B5.3**, risk rating of selected ENMs was performed. Definition of risk ratios for common industrial processes at all stages of the ENMs life cycle was accomplished for the installations of the field studies (*Table 5*). Information on available levels of exposure, physicochemical and toxicological properties, risk management measures implemented and risk characterisation ratios elucidated, was structured and organised following the structure of the NanoExposure & Contextual Information Database (NECID) developed under the EU funded NANOSH project, and used as the database under the EU project NanoREG. The primary target audience of this database is occupational safety and health professionals in governments, industry and academia. The data included in the database will facilitate the uptake of the outcomes of the project in the occupational risk management decision-making for ENMs, their aggregates and agglomerates (NOAAs). Based on the risk rating results, it can be concluded that even though the plants have high background concentration values, not all scenarios seem to represent an acute risk for workers, but they might represent a long-term risk, as they are constantly exposed to high dusty environments with toxic components such as titanium and silicon. On the other hand, the ICN2 laboratories seem to have a big problem with the cross contamination being evidenced in the microscopy results, where particles from other laboratories were detected. According to these results, first we have identified the need to implement a more efficient general ventilation in all plants, to reduce the background concentration values, therefore the number of air changes per hour should be increased. A localised exhaust ventilation (LEV) for the scenarios with the higher RCRs in Kerakoll Brugine and ICN2 would also be an alternative. Regarding ICN2 laboratories, the rotavapor from ES2 must have an adequate ventilation in order to eliminate the presence of FeO nanoparticles in the air. In the meanwhile, until measures are taken to improve ventilation in the designated areas, individual protection equipment must be used, such as respirable masks suitable for the sizes of the released particles. More detailed information on the above can be found in Deliverable DB5b.

*Table 4 Summary of the OELs values derived from ITENE for a list of selected NMs*

| Nanomaterial | Size (nm) | NOAEL(mg/kg) | NOAEL(mg/m$^3$) | HEC (mg/m$^3$) | OEL (mg/m$^3$) |
|---|---|---|---|---|---|
| **Ag** | 56 | 30 | 26,45 | **6,742** | 0,2247 |
| **Ag** | 55 | 0.1 | 0,1 | **0,025** | 0,0008 |
| **Ag** | 60 | 30 | 30 | **7,955** | 0,2652 |
| **Ag** | 10 | 250 | 220,39 | **37,89** | 1,2631 |
| **$TiO_2$** | 145 | 1000 | 881,58 | **227,658** | 7,5886 |
| **$CeO_2$** | 100 | 1000 | 881,58 | **235,177** | 7,8392 |
| **$CeO_2$-NM212** | 28.4 | 1 | 1 | **0,226** | 0,0075 |
| **ZnO** | 40 | 268.4 | 236,62 | **57,422** | 1,9141 |
| **ZnO** | 20 | 1000 | 881,58 | **182,109** | 6,0703 |
| **ZnO** | 100 | 31.25 | 27,55 | **7,178** | 0,2393 |
| **$SiO_2$** | 15-20 | 1000 | 881,58 | **181,365** | 6,0455 |
| **$SiO_2$** | 20 | 2000 | 1763,16 | **364,218** | 12,1406 |
| **$SiO_2$** | 100 | 2000 | 1763,16 | **459,396** | 15,3132 |
| **Graphene Oxide** | 136,4 | 3.02 | 3,02 | **0,777** | 0,0259 |
| **Graphene** | 123 | 1.88 | 1,88 | **0,503** | 0,0168 |
| **SWCNT** | 100 | 1000 | 881,58 | **185.331** | 6,1777 |
| **MWCNT** | 300 | - | 0,1 | **0,016** | 0,0005 |
| **MWCNT** | 200 | - | 0,1 | **0,019** | 0,0006 |
| **MWCNT** | 240 | - | 0,98 | **0,200** | 0,0067 |

*Table 5 Risk Rated Exposure Scenarios*

| Company | Scenario | Material | Ratio Cact/Cbg | Ratio Cactmax/Cbg | Exposure |
|---|---|---|---|---|---|
| Kerakoll (Zimella) | ES1: Mixer (2nd floor, Plant A) | TiO2 | 3,04 | 3,84 | Significa |
| | ES2: Big Silos (passing a door to the left, Plant A) | | 0,66 | 0,82 | Not Significa |
| | ES3: Cellulose power transfer (1st floor, Plant A) | Cellulose | 4,20 | 8,42 | Significa |
| | ES4: Calcitec refilling (Plant B) | Calcium Carbonate | 1,41 | 2,53 | Not Significa |
| Kerakoll (Brugine) | ES1: Powder transfer – Site B | | **7,82** | **58,87** | **Significa** |
| | ES2: Mixer°1 (FeO, amorfous silica, amina, etc) - Site B | | **7,97** | **51,73** | **Significa** |
| | ES3: Mixer°2 (TiO2) - Site A | | 1,37 | 3,87 | Not Significa |
| | ES4: Big Mixer - Site D | | **12,41** | **74,21** | **Significa** |
| | ES5: Mixer°3 (TiO2) - Site C | | **7,67** | **46,09** | **Significa** |
| Kerakoll (Sassuolo) | ES1 Mixing | CaCO3 | 1,88 | 3,35 | Not Significa |
| | ES2 Filling | CaCO3 | 1,05 | 1,72 | Not Significa |
| Kerakoll (Reggia Emilia) | ES1 Filling | silica-carbonate | 1,17 | 3,01 | Not Significa |
| ICN2 | ES1: Novel-Energy Oriented Materials Group (Lab. 1) | AgNPs, AuNPs | 1,17 | 2,59 | Not Significa |
| | ES2: Magnetic Nanostructures Group (Rotavapor) | FeO | **19,09** | **143,04** | **Significa** |
| | ES3: Supramolecular Nanochemistry Group (Lab. CM3/009) | Biodegradable polymers | 1,61 | 2,02 | Significa |
| | ES4: Nanostructured Functional Materials Group (Lab. CM3/007) | Methacrylate and acrylate polymers | 1,45 | 1,73 | Significa |
| | ES5: GO synthesis - Nanomedicine group | GO | 0,95 | 1,12 | Not Significa |

Finally, **Task B5.4** included the transmission of the outcomes of the project towards EU policy agencies, including the European Chemicals Agency (ECHA) and the EU Environmental Agency (EMEA) as well as international organisations (USEPA and NIOSH) and the

compilation of information received by the stakeholders (DB5c). The main outcomes communicated and inputs received focus on: i) the use of the suite of candidate biomarkers and the biomonitoring protocol, validated under Action B2, to investigate the potential health effects due to exposure to ENMs, ii) the new functional wireless sensor (Action B1) to monitor the concentration of ENMs and other PM fractions, iii) the on-line platform to support the processing and analysis of monitoring data (Action B3), iv) the new refined Recommended Exposure Levels (RELs) defined under B5.

**Results and deliverables:** The main results include the derivation of OELs and OEBs, the Risk Rating Characterisation of selected ENMs, the assessment of the scenarios studied and the interaction with stakeholders. Deliverables "B5a Report on recommended nano OEL and nano OEB", "B5b Library of risk rated exposure scenarios" and "B5c Inputs from the relevant agencies" are submitted and "MS10 Validated nanoOELs/OEBs defined" was achieved. The slight delays encountered for DB5a and DB5b were associated with the pandemic and delays in the implementation of other actions.

### 6.1.10 Action B.6 Implementation and demonstration actions in 4 EU countries: Greece, Italy, Spain, and Switzerland

**Status:** Completed
**Foreseen start date: 01/07/2020** **Actual start date: 01/07/2020**
**Foreseen end date: 30/09/2021** **Actual end date: 31/07/2022**

**Objectives** The main goal of the action was to **evaluate and validate all the methodologies, strategies and tools developed under representative situations over the entire life cycle of targeted ENMs,** including: i) the definition of a representative set of foreseeable scenarios (production, use, accidental spills, consumer use and end-of-life treatments), ii) implementation of local demonstration studies, involving ENMs manufacturers and users, public research laboratories, and civil infrastructures and iii) performing remote validation studies based on logic analysis, reliability and sensitivity assessment.

**Activities conducted and progress:** The main goal of this Action was to describe the results of the validation and demonstration studies conducted under Action B6. Action B.6 comprised several subsequent tasks, namely:
**Task B6.1**. included the definition of: i) the performance indicators to support validation studies and ii) the scope and goal of the case studies to fulfill the main scope of the Action which is the evaluation and validation of the NanoExplore approach. The indicators selected relate to the hardware and software specifications. For the hardware, among the indicators selected are: i) dimensions of the device (if they fit easily to the studied environment), ii) isolation and protection level of the device components, iii) communication, iv) general specifications (e.g. temperature range, minimum flow, calibration requirements), v) data storage capacity. For the device software, the main indicators are: i) accessibility and user friendliness, ii) autostoring function, iii) connectivity with the database, iv) alarms and errors communicated by the device, v) connection to the web-portal (ITENE, ALCON, NECCA).
**Task B6.2.** comprises the implementation of demonstration studies with the selection of representative cases to be studied and the recruitment of facilities, companies, institutes and organisations (*Table 6*). This task was implemented by all partners. In total demonstration actions were performed for 5 case studies, namely 3 production facilities (case studies 1-3), 1 laboratory (case study 5), 4 urban sites (case study 4) and a quarry area (case study 4), (ITENE, ALCON, RAMEM).

**Task B6.3** includes the monitoring, analysis and evaluation of performance indicators and thus, the evaluation of capacity of the methodologies, strategies and tools developed to safeguard the production and use of ENMs along their whole life cycle, (ITENE, ALCON, NECCA).
**Task B6.4** includes the final refinement and improvements of the monitoring procedure based on the evaluation of the performance indicators (all partners).

The results obtained from the implementation of Tasks B6.1-B6.4, demonstrated the effectiveness of the developed system, which adds useful new information to the scientific community, air quality agencies and to the companies' personnel. The last version of the NanoExplore device has demonstrated its ability to measure remotely in indoor and outdoor conditions during (relatively) long periods (2 weeks), with the operational coverage being more than 80%. In all cases examined, the evaluation process detected several failures or misfunctions; the problems identified and the remediation actions taken are presented in the below *Table 7*. However, based on the indicators evaluation and the percentages of successful operation of the device, the accuracy and reproducibility of the data generated confirm the reliability of the NanoExplore devices to detect and monitor ENMs in various environments.

The data generated provided valuable information on mean ENMs values and their temporal evolution throughout the days (e.g. weekend effect). The maximum values of hourly mean concentrations were recorded in the urban-traffic environment (20.000-40.000 particles/$cm^3$), followed by the urban-industrial environment (10.000-25.000 particles/$cm^3$) and by the suburban-traffic environment (5.000-12.000 particles/$cm^3$). On the contrary, in rural-background areas, the maximum hourly mean values ranged between 100 and 1.700 particles/$cm^3$, In workplaces, the respective maximum hourly mean values ranged from 25 particles/$cm^3$, in a downstream user of carbon nanofibers, to 35.000 in an industrial facility dealing with Cu compounds and metallic NPs. It is noteworthy that ENM levels in urban areas are significant, demonstrating that these substances should be taken into account in the air quality legislation.

Finally, the biomonitoring protocol developed was validated as an appropriate protocol to support biomonitoring studies when dealing with ENMs, highlighting the use of simple methodologies for sampling and the use of a limited number of biomarkers which can be easily measured in laboratory. More explicitly, the team of the Foundation for the Promotion of Health and Biomedical Research of Valencia Region, FISABIO, a non-profit scientific and health care entity, was approached to study the technical viability of the use of the information generated and protocols implemented in NanoExplore to support the epidemiological studies. In addition, a research team from the University of Valencia was also appointed to evaluate the use of the set of biomarkers and the biomonitoring protocol developed in the project. To perform the evaluation of the proposed biomonitoring approach the following activities were implemented:

- Analysis of the potential use of the biomarkers proposed in epidemiological studies.
- Applicability of the data generated through the NanoExplore biomonitoring protocol.
- Use of candidate biomarkers in in vitro tests.

*Table 6 Case studies scenarios*

*Table 7 Main problems encountered*

| Scenarios | Cases | Outcomes |
|---|---|---|
| C1. Production facility of metal oxide ENMs | Laurentia Technologies | Proper detection of airborne particles |
| C2. Applicability of wireless sensor to measure the levels of ENMs in a downstream user of CNT | Grupo Antolin | Good operation for Particle Breathing Zone studies |
| C3. Applicability of a network of 12 wireless sensors for monitoring in real time the exposure to ENMs in laboratories working with ENMs | Xenobiotics | Good operation of the filtering module<br>Good quality background measurements |
| C4. Applicability of a network of sensors to monitor in real time the exposure to ENMs in parking infrastructures and urban areas with important road traffic loads | Urban area in Valencia (ES) and Greece | Long term operation validated |
| | Parking facilities in Alicante (ES) | Ability to measure carbon based particles validated |
| | Airborne dust exposure in mines and quarries | Ability to monitor particles from far sources |
| C5. Interoperability of the NanoExplore approach with current public databases to manage human biomonitoring data | University of Valencia (ES) | Available metadata with direct application in biomonitoring studies |

| **Problems** | **Remediation actions** |
|---|---|
| Connectivity problems to the server | A new user was created and the problem stopped. It seems that there was a problem in the user description. |
| If the equipment is stopped for a long time, it has a hard time starting, showing "server error" for a long time before allowing to connect again. It is necessary to restart it several times. | This problem has disappeared with the new user. |
| The Touch Screen has poor sensitivity | If there is a lot of dust in the environment or dusty hands, it does not work well. The problem is resolved after cleaning the screen. |
| Particles pump encounterd problems | The device was repaired and a new pump type was used. |
| Partector 2 needs regular maintenance | The device users have received detailed guidance on the maintenance required. |

**Results and deliverables:** Deliverable "B6. Report on the evaluation by case studies" is submitted with detailed information on the data produced and "MS11.Validation activities in the target audience completed" is achieved despite the delays encountered as a result of previous actions delays due to the pandemic. The main outcome of this action was that **the device operated successfully in different settings with different conditions and users and for the few problems identified, the remediation actions have substantially improved the reliability of the device**. The NanoExplore team members had to travel often to fix the problems occurred but, finally, this was a very useful exercise in real conditions and the device operation standards were considerably improved.

### 6.1.11 Action B.7 Replicability and Transferability of project actions

**Status:** Completed
**Foreseen start date:** 01/01/2021 **Actual start date:** 01/06/2021
**Foreseen end date:** 31/12/2021 **Actual end date:** 31/07/2022

**Objectives** The main goal of the action is to **guarantee the applicability of the NanoExplore approach in areas other than those studied in Action B4 (replicability), as well as on particles of a larger diameter (transferability).** To this end, a tailored designed approach, based on the determination and analysis of critical factors, to promote replicability, capacity building and training and performance of case studies, was applied.

**Activities conducted and progress:** The workflow of the tasks included in Action B.7 is the following: **Task B7.1 Replicability in terms of applying the NanoEXPLORE approach in other places, locations, countries, etc.** In the concept and design of the NanoExplore device and web platform and of the biomonitoring studies, all the critical factors were considered to guarantee the replicability of the project approach (ST B7.1.1). Such factors are the technical factors, addressed since the beginning of the project to guarantee that the technology applied and refined within the project is applicable in other places, locations etc. Moreover, to enhance replicability, during the design and implementation of the NanoExplore approach, the economic factors on the cost of the wireless sensors and personal resources expected to analyse biomarkers as well as factors related to regulation and acceptance of stakeholders in other industrial sectors, cities and countries, were considered. In the framework of this task the following activities were foreseen/undertaken:

i. Pilot studies: Transfer of the human monitoring protocols and measurement units to relevant institutions in EU countries other than those represented by the project, including Germany, France, Portugal and Belgium. Due to long delays in previous actions, as a result of the pandemic, replicability studies were carried out in Spain, in industrial facilities, urban areas and a research organisation as well as in Greece where other institutions, such as the Municipality of Thessaloniki and the Hellenic Ministry of

Environment and Energy have participated in the monitoring campaigns (see also Action B6), (ITENE, ALCON, RAMEM).

ii. Development of a good practice manual for exposure to ENMs and human health monitoring considering the specificities of areas other than those represented in the project (UniTo, Unisanté, ITENE)

iii. Identification of supporting institutions to promote the dissemination of the approach (Yordas and all partners).

iv. Dissemination of the ideas and the results of the project through the partners' scientific publications, participation in conferences, networking, publication of informative leaflets/booklets and through the NanoExplore website (all partners). Additionally, ST B7.1.2 Capacity building and training (Yordas and all partners) included the organisation of three (3) workshops: Workshop 1 and Workshop 2 held online and Workshop 3 on 9th July in Thessaloniki, and an E-learning environment set up on website.

Moreover, in the first semester of 2023, a monitoring campaign is going to be organized with the Laboratory of Analytic Chemistry of the National Kapodistrian University of Athens (NKUA), under Prof. Evangelos Bakeas, to assess NMs' levels due to biogenic emissions in the forest area of the campus of NKUA. This field study is going to provide new data on NMs of physical origin and will further enhance the replicability of the NanoExplore approach.

**Task B.7.2 Transferability of the project approach for other substances** (ITENE, RAMEM, ALCON)**.** In the concept and design of the NanoExplore device and web platform and of the biomonitoring studies, all the critical factors were considered to guarantee the transferability of the project approach. Such factors are the technical factors, addressed since the beginning of the project to guarantee that the technology applied and refined within the project is applicable for substances other than ENMs as well as the selection of biomarkers and the sampling methods.

In this context, in ST B7.1.3 pilot studies were conducted to assess the applicability of the project approach to other substances. The field activities conducted under Action B6 (and T B7.1) focused on the analysis of the applicability of the NanoExplore approach to other scenarios, locations etc. including industrial settings, lab scale facilities and urban areas. In T B7.2, the field activities focused on the validation of the specifications and performance of the NanoExplore approach for PM1 PM2.5 and PM10. In this context, two pilot studies were conducted, one in a company from the Natural Stone Sector, where a number of relevant processes such as washing and grinding were selected due to the potential release of PM10, PM2.5 and PM1, and the other in a research organization related to building industry. From the results, the reliability of the NanoExplore device for monitoring PM, was validated, and, therefore, the device can be used for long term unsupervised PM measurements. Finally, the biomonitoring strategy is adequate to monitor the health impacts due to PM exposure, since the biomarkers selected are also dedicated for particulate matter biomonitoring.

The above tasks (B.7.1 and B.7.2) were accomplished and their results were appropriately described in Deliverables B7a E-Learning Platform and B7b Report on the transferability studies, as well as in the relevant deliverables of Action A4 and B1 and the dissemination deliverables.

**Results and deliverables:** Deliverables "B7a. E-Learning platform for the promotion of the use of the outcomes" and "B7b Report on the transferability studies" are submitted and "MS12 Transferability studies completed" was achieved. Similarly, in this action, the delays encountered are associated with delays in all previous implementation actions due to COVID19 pandemic.

### 6.1.12 Action B.8 Business plan and commercialisation

**Status: Completed**
**Foreseen start date: 01/07/2020 Actual start date: 01/07/2020**
**Foreseen end date: 28/02/2022 Actual end date: 30/09/2022**

**Objectives** The main goal of the action was to **develop the commercialization strategy and business model of the nanoparticle wireless sensor and the associated web platform**.

**Activities conducted and progress:** Action B.8 comprised the following tasks: i) **Task B8.1**. Definition of the Commercialisation Strategy, ii) **Task B8.2.** Property rights and protection (IPR) comprises the development of the IP agreement among the contribution partners (RAMEM, ITENE and ALCON) and iii) **Task B8.3** Business model includes the development of the business model which is based on the direct sales system of RAMEM as well as on the official distributors network in Germany, Korea, Japa, China and the USA. In the business plan it is foreseen that RAMEM will be responsible for the production of the devices.

In the framework of this Action, the strategy finally selected was that RAMEM commercializes the product and shares the benefits with ITENE and ALCON via royalties. This scheme presents the advantage that it is simple and it will use the already recognised brand IONER.

The business model includes three alternative options:
a) NanoExplorer purchase
In this case the customer will pay for the Nanoexplore monitor for its own property and will make use of it to his own criteria. Maintenance (to be provided by ARQUIMEA) will be contracted independently.
Normally this option is expected to be selected by R&D centers and institutions permanently oriented to environmental research, based on nanoparticles control and monitoring, whose staff is familiarised with the technology. Also, companies that produce or handle nanomaterials may be interested in this option, depending on their own capability to make use of the Nanoexplore monitor. In these cases, sales will be performed by directly by ARQUIMEA or its local agents (as applicable). Orders will be managed through a dedicated web page.
b) NanoExplorer renting
Mainly for customers who need to monitor nanoparticles for a limited period of time, for instance, in the framework of R&D projects or for occupational safety and health monitoring. In this model, maintenance will be included in the baseline renting price. Sales to R&D groups will normally be performed by ARQUIMEA through a dedicated web page, while sales to OHS will be performed by ITENE, since OHS are its common customers.
c) NanoExplorer as a service
The third possibility is to commercialise the Nanoexplore monitor as a service. This means that the customer will just look for the monitoring data in the given area(s) and for a given period. In this case, ARQUIMEA or ITENE (whoever is the seller) will implement the measurements scenario and will handle all data to report the results to the customer.

Additionally, the main goal of the exploitation strategy is to sell Nanoexplore globally in the long term, after expanding to Europe and the local market. In the short term, our commercialisation strategy will start in the European market, given that it is the closest and the most familiar. We are aware of the advantages of our technologically advanced product, compared to the existing ones in the market; however, the NanoExplore device must compete with mature technologies, already on the market, which have built up a reputation of trust and efficiency after years of development. Considering the performed market analysis, the segment

of occupational health and safety companies was chosen to establish our first entry into the market and demonstrate our capabilities, before making the leap to the other segments analysed.

One of the cornerstones for the business exploitation will be an effective communication plan with the aim of achieving a reputation in the market and making Nanoexplore known to all key players. For this purpose, a detailed dissemination plan is foreseen (Table 4, Deliverable B8a).

**Results and deliverables:** Deliverables "B8a. Business model, market analysis and detailed commercialisation strategy" and "B8b. Marketing materials" are submitted and "MS13. Business model and commercialisation strategy defined" is achieved.

### 6.1.13 Action C.1 Monitoring LIFE Project Performance Indicators

**Status:** Completed
**Foreseen start date:** 01/09/2018 **Actual start date:** 01/09/2018
**Foreseen end date:** 28/02/2022 **Actual end date:** 30/09/2022

**Objectives** The main goal of the action is to **regularly monitor the LIFE Project performance indicators** which contribute to evaluating the impact of the project, both during its implementation and beyond project's end.

**Activities Conducted and progress:** Action C1 includes the identification and analysis of relevant sources for the analysis of the project performance indicators (**Task C.1.1**), the monitoring of the project performance indicators (**Task C.1.2**) and contingency planning (**Task C.1.3**) as well as **Task C.1.4**, evaluation of the project performance indicators. Finally, in **Task C.1.5** the update of the LIFE KPI Webtool takes place in the MTR and in the FR. Additional Life Project Indicators are presented below and in Deliverable C.1.1, C.1.2 and C.1.3.

Project monitoring is conducted continuously and periodically. Several qualitative and quantitative indicators have been selected. Their data have been provided within the LIFE project specific indicators call 2017 Microsoft excel table and the KPI Webtool. The table was developed considering existing information on production volumes of ENMs and nano-enabled products, current guidelines on the RA of ENMs, as well as current regulatory requirements established under REACH, air quality and environmental regulations.

To update the performance indicators defined, first the proper sources of data (peer reviewed publications, OECD guidelines and reports, ECHA guidelines and databases, reports from public authorities, etc.) have been specified. An indicative list of these sources is presented below:

- Ex-post evaluation of the European Union Observatory for Nanomaterials (EUON), July 2019,
- Critical review of the relevance and reliability of data sources, methods, parameters and determining factors to produce market studies on manufactured nanomaterials on the EU market, July 2018,
- Considerations about the relationship of nanomaterial's physical-chemical properties and aquatic toxicity for the purpose of grouping (Hund-Rinke, et al., 2017),
- Éléments issus des déclarations des substances à l'état nanoparticulaire RAPPORT D'ETUDE 2017 (MTES, 2017),
- World market for nanomaterials: structure and trends Elena Inshakova and Oleg Inshakov,

MATEC Web of Conferences 129, 02013 (2017) DOI: 10.1051/matecconf/201712902013 ICMTMTE 2017,

- Review article Quantitative material releases from products and articles containing manufactured nanomaterials: Towards a release library Antti Joonas Koivisto, Alexander Christian Østerskov Jensen, Kirsten Inga Kling, Asger Nørgaard a , Anna Brinch, Frans Christensen, Keld Alstrup Jensen, NanoImpact 5 (2017), p. 119-132.

The project specific indicators are appropriately updated and reported in deliverable C.1.1. where information on any modification on the starting assumptions is included. The monitoring of the project indicators was conducted and periodically reported with the planned reports (DC1.2 and DC1.3).

In the contingency plan applied in the project, a number of solutions and remediation actions, in case of deviations on the performance indicators, have been included. The assessment team responsible for the application of contingency actions, consists of the project coordinator and the action leaders. The contingency plan is included in Deliverable C1.1 and updated, due to the pandemic, in Deliverable C1.2.

The update of the LIFE KPI Webtool (Task C.1.5) is presented in the corresponding chapter 7. In Action C1, ALCON is the Action leader along with Yordas DE and Yordas UK. However, all partners contribute to data collection and the assessment and evaluation of the project indicators.

From the assessment and evaluation of the indicators, it is concluded that NanoExplore has either overpassed or achieved the estimated end values, showing that, despite the difficulties and the COVID19 “force majeure”, the project delivered the expected outcomes and succeeded with regard to the goals set.

**Results and deliverables:** The expected results from this action are mainly a compendium of reliable values for each one of the indicators considered regularly updated according to the project progress. The deliverable foreseen up to the end of 2019 (30 September 2019), DC.1.1 Updated table with life project specific indicators, was submitted as well as the upcoming deliverables DC.1.2 and DC.1.3 which were submitted on time, according to the project progress.

### 6.1.14 Action C.2 Monitoring the impact of the implementation actions on improving the use of chemical monitoring data in the protection of human health and the environment

**Status:** Completed
**Foreseen start date:** 01/09/2019 **Actual start date:** 01/09/2019
**Foreseen end date:** 31/08/2021 **Actual end date:** 31/08/2022

**Objectives** The main goal of the action is to **monitor the impact of the project actions in the promotion of the use of exposure/environmental monitoring data for risk assessment purposes**. Assessment will be conducted by means of a compendium of indicators defined specifically to quantify the use of the data generated by the project.

**Activities Conducted and progress so far: Task C2.1.** Definition of the monitoring indicators, includes the definition of adequate indicators to evaluate the use of the data available into the

NanoExplore web based application and related database A pre-list of indicators was examined and the final list of indicators was delivered in the first months of 2020. In the list of indicators the indicators included are:

- Number of stakeholders who agreed to use the web-based platform
- Number of registered users/organizations using the web-based platform outside the consortium
- Number of visitors of the web-based platform
- Communication with the stakeholders (emails)
- Feedback from stakeholders (emails)
- Candidate Biomarkers (BM) applicable to stablish cause-effect relationships with diseases
- OELs and bands defined: up to 4 bands of exposure levels (bands for carbon based ENMs, insoluble and persistent NMs, for partially soluble NMs, and for high aspect ratio (3:1) NMs
- Web references of the NanoExplore project
- Scientific publications related to NanoExplore project (reviews, journal articles, book chapter, Conference, Report)

In **Task C.2.2**, survey campaigns were carried out for monitoring the use of the data generated. In **Task C.2.3,** the analysis and reporting of the data collected were implemented for the calculation of the indicators. Finallys in **Task C.2.4,** the reduction of adverse effects of ENMs on human health and the environment was examined. From analysing the project results, it has been estimated that if the installations, where the exposure and biomonitoring studies were carried out, would adopt the protective measures proposed by the NanoExplore team, this would lead to at least 10% reduction of exposure in workplaces. However, the Project Coordination Committee (PCC) after having examined the potential impact due to exposure to ENMs on human health, they have concluded that the evaluation of the ENMs impact before the project implementation as well as the evaluation of the impact of the project on reduction of potential adverse effects ENMs might have on human health and the environment, after the end of the project, is not scientifically pertinent, with regard to data available before and the data generated in the framework of the project. A direct impact of the project on the potential environmental effect of ENMs is expected, to the extent that environmental policies in national and European scale will support the implementation of mitigation measures in areas with higher ENMs concentrations, as well as the definition of safety procedures to be implemented in scenarios with risk ratios generation concerns. NanoExplore has generated important information on the risk rating of ENMs and the corresponding scenarios with the more significant emission rates and has also communicated this information to relevant organisations and agencies and to the industrial installations of interest. From this point of view, the project results can propose the selection of ENMs forms with lower emission potential, which is directly related to the composition, shape and conditions of use of each ENM. Of course, other factors affect ENMs concentrations: safe-by-design may be a way to reduce ENMs hazardousness, by modification of their physical properties, hence choose to manufacture less hazardous nanoforms of the substance. Another way is assuming that introduction of mitigating measures or safety procedures will primarily reduce exposure to the ENM. These measures would be appropriate for the environment in which the ENM is being used or measured. For example, in a production environment, where the virgin ENM is being manufactured and exposure to workers is of concern, such measures might be introduction of improved ventilation, better enclosure of the substance or introduction of low energy maintenance/house-keeping protocols.Where the environment of concern is around the use of nano-enabled mixtures or articles, the measures might be improved PPE for workers during activities that release dusts or introduction of natural water settling tanks to prevent release to the environment (many nanomaterials will agglomerate and settle in the presence of humic acids found in natural waters).

Unfortunately, reduction of the levels of release could not be defined, because the follow-up studies are not yet implemented, an effect of the COVID19 pandemic which excluded the timely implementation of these studies, and thus there is no possibility to estimate the impact of the mitigation measures on exposure and human health (through biomonitoring). Still, in the deliverables of Actions B4, B5 and B6, important conclusions have been drawn regarding the exposure levels, risk rating and the impact of exposure on human health on the basis of the outcomes from the case studies conducted in the screening and demonstration actions. Moreover, in Deliverable C3, the estimation of change in occupational injury related to direct exposure to ENMs is -5% at the end of the project and -20% in the period after 5 years.

Difficulties in Action C2 (Deliverable C2a updated) were encountered concerning the redefinition of the indicators proposed to measure how and how often the data produced by the project are utilised in activities that aim to protect human health and the environment, as implied by CINEA. However, in some cases such indicators cannot reveal the project impact in the course of the project but only in the medium and long term.

**Results and deliverables:** Main results come from the analysis of the data retrieved from the indicators and survey:

- Quarterly reports on the promotion of use of measured data and BM, including a detailed analysis of the intended use of the data and user profile. However, it should be noted that these reports could be completed due to lack of data as pilot studies and campaigns were rescheduled due to the pandemic restrictions for 2021. For this reason, in the MTR we had proposed that these reports should be prepared yearly in order to reflect a substantial change of data.
- A complete data record of the nº of references used by target audience
- A detailed report on how the project contributes to the reduction of the potential effects on ENMs in pulmonary and cardiovascular diseases

The analysis of available information concerning exposure and (eco)toxicological data, biomarker and OEL/REL was conducted, concluding that most of the references are information about workplaces, with an evident lack of knowledge about the concentration of ENMs in urban areas. Most of the data published in both cases are in the quarters Q2, Q3 and Q5. We can also conclude that there are very limited references regarding the relationship between the cause and effect of the disease. The same situation can be observed concerning the OELs for risk assessment purposes. A report on the above analysis is included in deliverable DC2a.

### 6.1.15 Action C.3 Monitoring the socio-economic impact of the project actions

**Status:** Completed

**Foreseen start date:** 01/07/2020 **Actual start date:** 01/07/2020
**Foreseen end date:** 28/02/2022 **Actual end date:** 31/08/2022

**Objectives** The main goal of the action is **the assessment of the socio-economic impact of the project actions**. In this sense, a set of socioeconomics indicators have been developed in order to measure the impact of the project to the local economy and population. The conclusion extracted from these indicators was collected and explained in the socio-economic impact report delivered.

**Activities Conducted and progress:** The action includes two tasks: **Task C3.1**: Identification of indicators of socio-economic benefits, where the indicators that will be monitored were defined with regard to their relevance to the project goals and the availability of reliable data, necessary to conduct their evaluation (ALCON). Three different groups of indicators were selected:

- **Direct cost for REACH implementation**
  - Change in the level of employment at ENM producers and downstream users
  - Direct jobs generated in the context of the project
  - Change in business opportunities and competitiveness (Market share)
  - Change in insurance cost related associated with exposure to ENMs
- **Social Indicators**
  - Change in occupational injury related to direct exposure to ENMs
  - Change in consumer acceptance of REACH and nanotechnology based products
  - Promotion of a safe environment and change in exposure
- **Environmental Indicators**
  - Changes in the amount of environmentally hazardous nano-materials, $PM_{2.5}$ and $PM_{10}$ releases in industrial and research workplaces
  - Enhancement of the performance of risk management measures

In **Task C3.2**: Monitoring of the Socio-economic benefits of the NanoExplore project, each of these indicators were used to assess the socio-economic impact of the project actions over the project lifetime. To this end, for each one of the indicators defined, information was collected and compiled (Yordas)

The indicators were specified with regard to the availability of reliable data, necessary to conduct their evaluation.

In the long-term, the main goal of the project is to reduce significantly the potential adverse effects derived from the exposure to ENMs by setting up a harmonized health surveillance system and promote new EU policies for the safe use of ENMs. The impacts of the project on society and economy are directly linked το the benefits of the project in the safety of workers and consumers dealing with ENMs, as well as το the increase in the business opportunities due to the increase of the consumer acceptance of nanotechnology and the reduction on the lack of data to fulfil relevant policies at EU scale such as REACH regulation, CLP, food safety and safety at work. The effectiveness of NanoExplore in reducing occupational injury was estimated at 20% in the long term, resulting in a considerable reduction of the number of workers being affected by a disease caused by direct exposure to ENMs. This will result in a significant positive impact on the society, as well as a direct reduction in the insurance cost associated with the exposure to ENMs. A major economic impact for companies dealing with ENMs is the reduction on the direct costs associated with the implementation of REACH, which will likely result in a direct increase of the R&D investments, and therefore, an increase in the number of added value products available on the market.

Finally, the reduction on the release of ENMs to the environment, estimated at 15% average reduction in the medium term, should be considered as a key long-term positive impact for the project. The expected increase production levels of ENMs and nano enabled-products will likely result in new sources of emission, but the use of recommended risk controls will play a key role to guarantee a high level of protection of the human health and the environment.

**Results and deliverables:** Main results come from the analysis of the data retrieved from the indicators. Deliverable C3.1 Regularly updated data related to the socio-economic impact of the project was submitted on time.

### 6.1.16 Action D1. Dissemination and awareness raising activities to the general public and stakeholders

**Status:** Completed
**Foreseen start date:** 01/09/2018 **Actual start date:** 01/09/2018
**Foreseen end date:** 28/02/2022 **Actual end date:** 30/09/2022

**Objectives:** The main aim of this action was to **manage the communication and dissemination activities related to the NanoExplore project**, and thus to ensure the quality of the activities to develop.

**Activities conducted and progress:**

**Task D1.1 Planning and execution of Communication and Dissemination activities:** This task focused on the design and development of the dissemination and communication strategy, including the development of standard dissemination channels and target group identification. The second reporting period saw the continued implementation of the dissemination and communication as set out in the plan developed at project start.

**Task D1.2 Preparing and keeping the project website**: Yordas, with support of all partners had set up the project website by month 3 and since then has been continuously updated it with news, events, results and other relevant information. The website is the project's main presence. It helps to spread information about the project, to inform target audiences about the project and its results.

**Task D1.3 LIFE Dissemination Materials:** This task's aim was to elaborate the materials that was used to disseminate the project. We have developed posters, pull up banners, a flyer, a fact sheet, several press releases, newsletters, videos and email campaigns.
Additionally, we run on and offline communication campaigns supported by social media to disseminate the materials and project results. All material can be found on the project website.

**D1.4 Evaluation and monitoring process**: The aim of this task was to ensure the compliance related to project dissemination and prove the success of the dissemination and communication actions. Therefore, two indicators have been chosen; Feedback from stakeholders and alignment with the dissemination plan. The table below shows the progress so far:

*Table 7 Indicators and progress made*

| Indicator | Measure of success | Results | Status |
|---|---|---|---|
| Feedback form stakeholders | Positive Feedback of stakeholders:<br><br>a) Present at events<br>b) Collect feedback via one to one conversations | We presented the project at several events e.g. NSC meetings and collected feedback of stakeholders during the events from one to one conversations. and through a survey. | Completed |

| Indicator | Measure of success | Results | Status |
|---|---|---|---|
| | c) Stakeholder survey | | |
| Alignment with the disseminatio n plan | Quarterly reports to ensure compliance | The updates to the dissemination plan (if needed) have been included in the quarterly progress reports. Figures about website visitors, press releases, events and other indicators were collected and updated and trigger action if necessary | Completed |

**Results and deliverables:** All deliverables of Task D1 were successfully delivered by project end. Deliverable DD1.2 Project website was successfully implemented in time by Dec 2018 as foreseen. The website is continuously updated with latest events, results, news or other relevant content. The tracking code was only implemented in April 2019, the website had 5900 visitors since then. Project Brochure (DD1.4a), Project Factsheet (DD1.4c), Newsletter (DD1.4b) have been completed on time in the first reporting period. Layman's report and summary report via email newsletter (D1.4f and DD1.1b), as well as Videos (DD1.4e) and notice boards (DD1.4e) were produced as foreseen in the second period. Action D1 was successfully completed as foreseen by project end. Nevertheless, we will continue to update the website and social media channels according to the After Life Plan.

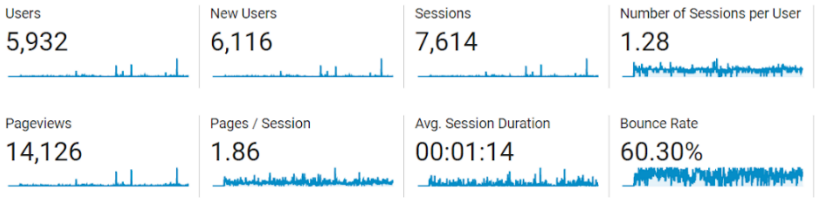




*Table 8 Summary of Dissemination actions achieved*

| Dissemination actions achieved | | |
|---|---|---|
| **Name** | **Information** | **Online link** |
| ***Website of the project*** | The project's main point of contact is its website. The website is used to spread information about the project and inform target audiences, as well as the general public, of the progress made by the project. As it is presented below, scientific publications, the poster of the project and factsheet, newsletters, new alerts, videos etc, are available via project website. | https://www.lifenanoexplore.eu/ |
| ***Partners' websites: 1. Unisante website*** | Partners' websites are linked to the project website for presenting and further disseminating the NanoExplore results. All the websites are linked to the project website. | Integrated approach for exposure and health effects monitoring of engineered materials in workplaces and urban areas (NanoExplore) \| Unisanté (unisante.ch) |

| Dissemination actions achieved | | |
|---|---|---|
| **Name** | **Information** | **Online link** |
| ***2. ITENE website*** | | https://www.itene.com/casos-de-exito/lifenanoexplore-exposicion-nanomateriales/ |
| | | https://www.itene.com/en/solutions/outdoor-industrial-urban-indoor-air-quality/ |
| ***3. Comunitat Valenciana website*** | | https://www.itene.com/actualidad/institutos-tecnologicos-coordinan-proyectos-life/ |
| ***4. N.E.C.C.A. Website*** | | https://necca.gov.gr/en/erga/sustainable-development-and-climate-change/life-nanoexplore-integrated-approach-for-exposure-and-health-effects-monitoring-of-engineered-nanomaterials-in-workplaces-and-urban-areas-life17-env-gr-000285/ |
| ***5. Ο.ΦΥ.ΠΕ.Κ.Α. Website*** | | https://old.necca.gov.gr/en/air-quality/life-nanoexplore-integrated-approach-for-exposure-and-health-effects-monitoring-of-engineered-nanomaterials-in-workplaces-and-urban-areas-life17-env-gr-eng/ |
| ***6. Yordas website*** | | https://www.yordasgroup.com/webinars/nanoexplore |
| ***7. ALCON website*** | | https://www.alcon-engineers.gr/ |
| ***Social Media Profiles of the project*** | Two social media profiles were created for the project. One on Tweeter and one on LinkedIn. Both channels were used to post project updates, event notifications, publications and other information relevant to the project regularly. Should be noted that the aforementioned social media profiles are linked to the project website. Partners also used their own channels to distribute the messages. | https://twitter.com/LFnanoexplore |
| | | https://www.linkedin.com/company/life-nanoexplore/ |
| ***Publications of scientific papers*** | All publications submitted to scientific, peer-reviewed journals are included in the project website, as it is shown in the next column. | https://www.lifenanoexplore.eu/library/publications |

| Dissemination actions achieved | | |
|---|---|---|
| **Name** | **Information** | **Online link** |
| ***Videos*** | Several videos were produced during the NanoExplore project. All the videos are available on the video section of the NanoExplore website and on the YouTube playlist of NanoExplore, as shown in the next column. | **Youtube channel:** https://www.youtube.com/playlist?list=PLqkPl6PutClTut-7-0AevS2OJQjoA4itz |
| | | **Website of the project:** https://www.lifenanoexplore.eu/library/videos |
| ***Layman's report*** | The final project results were released in an easy-to-read format as the "Layman's report". It was made publicly available by the project website, as shown in the next column | https://www.lifenanoexplore.eu/app/nano/files-module/local/library/NanoExplore_Layman's_Report_final.pdf |
| ***Press releases/new alerts*** | News alerts were created at regular intervals and presented with three different ways. More specifically they could be found as news alerts or in the project newsletters on the website of the project, and in the newsletter of the Nanosafety Cluster (NFC). In these sections, information about new webinars, conferences, workshops etc. were presented. | https://www.lifenanoexplore.eu/news |
| | | https://www.lifenanoexplore.eu/library/newsletters |
| | | https://www.nanosafetycluster.eu/outputs/nsc-newsletter/nsc-newsletter-archive/ |
| ***Newsletters*** | During the project period, nine newsletter were created and disseminated via email campaigns. All newsletters are also available on the progect website, as shown on the link presented on the next column. | https://www.lifenanoexplore.eu/library/newsletters |
| ***Posters*** | The roll-up banner of the project was developed and disseminated to all partners. | https://www.lifenanoexplore.eu/app/nano/files-module/local/library/Nanoexplore%20a3%20portrait%20poster.pdf |
| | The project poster was developed as well. | https://www.lifenanoexplore.eu/app/nano/files-module/local/library/Nanoexplore%20a3%20portrait%20poster.pdf |
| ***Project factsheet*** | The NanoExplore project factsheet was created in order to provide an overview of the project. | https://www.lifenanoexplore.eu/app/nano/files-module/local/library/Factsheet%20Web.pdf |

### 6.1.17 Action D2. Public awareness and dissemination of results

**Status:** Completed
**Foreseen start date:** 01/09/2018 **Actual start date:** 01/09/2018

**Foreseen end date:** 28/02/2022 **Actual end date:** 28/02/2022

**Objectives:** The main aim of this action was to **develop high quality communication and dissemination activities of the project, but mainly to ensure adequate transfer of the lessons learnt to alternative sectors.** Action D2 is split into 2 subtasks.

**Activities conducted and progress:**
**Task D.2.1 Dissemination actions addressing stakeholders other than those directly affected by REACH and relevant EHS regulations:** The first half of the second reporting period was heavily impacted by the Covid pandemic, which resulted in the cancellation of all face to face events. Therefore, members of the consortium focused on intensifying existing contacts with relevant stakeholders and competent authorities and in the participation of online events. In the second half of the second reporting period, face to face engagement with stakeholders only slowly picked up.

*Table 9 Summary of the networking related action conducted.*

| Date | Action | Description | Location | Purpose |
|---|---|---|---|---|
| 22-27 March 2020 | Nanosafety Training school 2020 | Knowledge exchange with other EU funded projects | Virtual | Knowledge Exchange with projects in the NanoSafety Cluster |
| 2 July 2020 | Bavarian NanoNetwork Cluster | Presentation of the Project | Virtual | Knowledge Exchange with regional network and presentation to industry representatives |
| 20 - 22 April 2021 | NanoTox | Presentation of the project | Virtual | Networking and information exchange with other EU funded projects and with researchers and industry representatives. |
| 5-6 May 2021 | EuroNanoForum | Knowledge Exchange | Virtual | Information and Knowledge Exchange |
| 23-25 Juni 2021 | NanoTech France | Presentation of the project and its goals | Virtual | Networking and information exchange with industry |
| 15-20 May 2022 | NanoSafety Training School 2022 | Knowledge Exchange with other EU funded Initiatives | Venice, Italy | Knowledge Exchange with projects in the NanoSafety Cluster |
| 20-24 June 2022 | "Nano-week" & NanoCommons Final Conference | Knowledge Exchange with other EU funded Initiatives | Limassol, Cyprus | Knowledge Exchange with projects in the NanoSafety Cluster |

*Direct communication*

Concerning the direct communication with key stakeholders, phone meetings and email exchange took place with following institutions in this period:

- Hellenic Association of Chemical Industry
- Italian Association of Chemical Industries - Federchimica - Milan, Italy
- Cosmetica Italia – Italian Association of Cosmetic Industries), Milan, Italy
- Nanotechnology Industry Association
- Scientific committee "nanotechnolgy workers" of the International Commission for Occupational Health (ICOH)
- French Agency for Food, Environmental and Occupastional Health & Safety (ANSES)
- Suisse Ministry of Economy
- Cluster Nanotechnology - NanoInitiative Bayern - Bavaria, Germany

- CUSP – the European research cluster to understand the health impacts of micro- and nanoplastics (MNPs).

Additionally, 3 workshops (2 online and 1 in person) and 3 webinars were held in the second phase of the project as described in the project plan. These workshops and webinars attracted more than 250 attendees from all stakeholder groups.
A detailed description of the 3 workshops can be found in Deliverable D2.1 "Report on the project workshops with special focus on the transference to alternative sectors of the chemical industry".
Further information on the webinars is presented in Deliverable DDD1.1b "Summary report of dissemination activities conducted". Furthermore, a recording of the three webinars and the two online workshops can be found here: https://www.lifenanoexplore.eu/library/e-learning

**Task D.2.2 Networking with other projects (LIFE and/or H2020):** The aim of this task was to establish direct contacts with other European projects and European research groups working in the field of REACH, nanotoxicology, air quality assessment and other initiates related to NanoExplore.
Members of the consortium are very well linked in the Nano community, e.g. the NanoSafetyCluster and thus with projects related to NanoExplore. Direct communication took place with coordinators from other LIFE projects such as Fit for REACH, CHEREE, AskREACH, LIFE ASTI and H2020 projects such as NanoFASE, Calibrate, Gracious, Biorima, Patrols, SbD4Nano and Sunshine. Furthermore, members of the consortium attended several events mentioned in Table 5 to share knowledge and to network with other EU funded projects, e.g. the annual NanoSafetyTraining School or the Nano-week.

**Results and deliverables:** Action D2 has been successfully completed. We have successfully initiated collaborations in European initiatives and projects as well as exchanged expertise in the relevant fields. Members of the consortium presented the project as described above. D2.1 has been delivered and completed as planned. D2.2b, the second report on the networking actions has been submitted according to plan in Q2 2022.

## 6.2. Main deviations, problems and corrective actions implemented

In the former phase of the project, up to the end of 2019 (submission of the MTR), with the exception of the technical amendment in Task B.2.3, no major deviations have been identified. A problem encountered was to accomplish the survey aiming at the harmonised protocol and the specification of field studies but it was resolved by extending the duration of survey and by trying to increase the participation in the survey through following-up and by disseminating the survey to more companies. A further issue was due to unknown baseline values for the most of biomarkers proposed as candidate, to enable interpretation of biological and clinical significance of a biomarker. This need was satisfied in Action B.2, Task B.2.3 as it was proposed in the 1$^{st}$ amendment approved.

Nonetheless, this situation has dramatically changed, due to the force majeure imposed by the COVID19 pandemic. In 2020 we had planned to finalize the production of the wireless sensors and the web portal (Actions B1 and B3) and to implement the exposure and biomonitoring studies (Action B4), which are critical for the validation of the NanoExplore approach and the generation of a significant amount of data required to characterise and assess the exposure, the risk associated with specific ENMS and the health effects due to exposure to ENMs. Action B4

required first the approval of the companies to participate in the pilot studies, given that the companies were in operation and that the strict epidemiologic protocol could allow such studies, and second the possibility of the project team to travel and to participate in these campaigns. *Despite this hard, unknown situation, with Italy and Spain who had the lead in the implementation of these studies being severely affected during the first pandemic wave, the NanoExplore team succeeded to plan and design the pilot studies, to receive all the ethical committees' approvals and finalize the companies recruitment as soon as the restriction measures were lifted. This preparation enabled the implementation of the pilot studies during the summer 2021, whereas the study for the universal control group was conducted earlier, in fall 2020. Still, this delay caused further delays in the next actions and additionally, this dysfunction has considerably increased the already limited project budget.*

More explicitly, the device development (**Action B1**), undertaken by the Spanish company RAMEM, faced difficulties due to the pandemic, with respect to the supply of components and suspension of the non-essential activities in Spain during the 2nd trimester of 2020, so the assembly of the instrument was stopped, since workers could not go to the workshop. Because of this delay, Action B1 was completed by the end of June 2021. Similarly, the web platform development (**Action B3**) was delayed due to the containment measures in Greece and the delay in Action B1. Overall, both actions exhibited a **12 month delay**. **Action B2** was **delayed by 9 months** due to i) limited access to the experimental laboratory (Action B.2.3 Study 2 OPEA), ii) no possibility to analyse the samples obtained from previous studies, necessary for building the harmonized protocol and iii) shutdown periods in 2020 and 2021 as well as travel restrictions have hampered scientific exchanges with partners and experts. **Action B4** (biomonitoring studies) required a lot of preparatory tasks in order to accomplish its final target regarding the ethical committees' approval in the involved countries, the companies recruitment etc. The most important constraint was the low response rate among companies that were invited to participate in pilot studies (Action B.4.1 Recruitment of study participants) as well as the limited or no access to the experimental laboratory and of course travel restrictions which led to a 12-month delay. Actions B.4.2, B.4.3 were similarly delayed. Due to the significant delays in Action B4 and the ongoing pandemic restrictions, delays in Actions B5, B6 and B7 have occurred: Action B5 finished with a 9-month delay, whereas Action B6 had a 10-month delay, and was accomplished by the end of July 2022, instead of the end of September 2021, with the demonstration actions taking place simultaneously to the pilot studies. Action B7, was accomplished by the end of July 2022, instead of the end of December 2021, with a delay of 7 months and, finally, due to the above delays, Action B8 was also delayed up to the end of September 2022.

### 6.2.1 Main deviations and corrective actions implemented

- Eight (8) units of indoor portable NanoExplore Device (1 prototype unit and 7 series units), 4 units of outdoor enclosure have been produced due to the selection of more robust and more expensive components which has limited the possibility to produce more devices. However, the limited number of devices did not affect the implementation of the field campaigns (more details are included in Annex I).
- The development of the monitoring stations has faced significant difficulties due to COVID19 restriction measures, leading thus to delays in the web portal development too. However, both actions have been accomplished and the delays did not affect the project progress as a whole, given that field campaigns were also delayed.

- The design and scheduling of the biomonitoring studies has encountered severe problems related to the pandemic. The core of the project are the field studies, which require joint efforts and interaction among partners. Travel restrictions have hampered the adoption of contingency actions.
- Extensive efforts were put to organise the field campaigns and recruit companies. This work was severely hindered due to the pandemic. However, the contingency plan adopted and the intense coordinated actions led to the accomplishment of this significant prerequisite and the campaigns were implemented, even so with an important cost increase.
- Follow-up of biomonitoring studies was not possible be accomplished in the time plan of the project, taking into account the significant delays and difficulties due to the pandemic and the corresponding cost increase as well as the propensity of facilities to participate in the studies (Please see justification below).
- Seven (7) demonstration actions (four case studies) were implemented in Spain, Greece and Italy (production facilities, laboratories and urban sites). Instead of the Athens airport maintenance area and parking infrastructures, we have chosen two urban traffic hotspots (Thessaloniki and Athens Greece) as we estimated that these two sites had much higher nanoparticles levels. No demonstration action was conducted in Switzerland, still this had no impact on the project results, as more demonstration studies were carried out in other locations.
- During the demonstration studies, technical and logistics problems occurred (e.g. problems with the pumps and need to regularly maintain Partector 2). We have resolved all of them but this has resulted in a delay of the demonstration studies completion and the data collection and elaboration with a cost increase too. Still, beside the difficulties, all the foreseen actions were accomplished.
- The indicators proposed in Action C2, were redefined to measure how and how often the data produced by the project are utilised in activities that aim to protect human health and the environment. However, in some cases such indicators cannot reveal the project impact in the course of the project but only in medium and long term after the end of it.
- In Action C3, the indicators were specified with regard to the availability of reliable data, necessary to conduct their evaluation.

### 6.2.2 Follow-up studies

According to the Harmonized Protocol (Deliverable B2a), the pilot study to demonstrate the feasibility of a harmonized approach and validate a choice of biomarkers for exposure and health effects monitoring of nanomaterials in workplaces and urban areas, includes also a 6/9-month follow-up after recommendations to reduce the nanoparticle exposure by at least 10%:

- The initial campaign will provide a baseline evaluation of relationships between exposure and biological parameters.
- The follow-up campaign will assess changes in exposure-biomarkers associations based on exposure controls implemented by companies.

Although foreseen and interesting in scientific terms, to confirm the previous findings, the follow-up studies are clearly bound due to the delays encountered as a result of the pandemic restrictions and the availability of the Companies. In the case of KeraKoll, they gave their potential availability only from the beginning of 2023 onwards; after the lockdown, they increased the production and included also a third shift which has made it difficult to agree for a second study.

Under these circumstances, all beneficiaries discussed together and concluded that, given the pandemic-related challenges, the better adjustment would be to focus on the recruitment campaign, to include as much as possible centers in our multi-center study. Thus, we had not enough time and no budget at all for preparing and conducting the second set of field campaigns. Moreover, Unisanté and Unito had to compensate the delay and the reduction of the number of prototype instruments for exposure assessment by providing 5 DiSCMini, for measuring the particle number concentration and lung deposited surface area and approximate on this base the individual exposure of the recruited participants. Unito had to struggle with analytical kit shortage and run several, instead of one, biochemical analyses, and have spent their own money for the extra analytical cost.

Despite the above constraints, the data analysis led to very interesting and original results which confirm that our strategy and study protocol were right and strong. These results will have an important impact on the "nano" community, and we hope that this will give us an opportunity to apply for additional funding and keep the "NanoExpore cohort" active by conducting regular follow-ups. It will be very important and extremely useful for research and nanotechnology development. However, this would need a sufficient resource supply to keep a high methodological standard that we tried to establish and maintain, despite all challenges we had to face.

All beneficiaries acknowledge the importance of the follow-up studies and have decided to conduct the campaigns in the framework of the After-LIFE Plan, on their own ressources. Prof Bergamaschi has already been in contact with the companies already participated in the first pilot campaigns and they have agreed to participate again in the second part.

Further on, in the first semester of 2023, a monitoring campaign is going to be organized with the Laboratory of Analytic Chemistry of the National Kapodistrian University of Athens (NKUA), under Prof. Evangelos Bakeas, to assess NMs' levels due to biogenic emissions in the forest area of the campus of NKUA. This field study is going to provide new data on NMs of physical origin and will further enhance the replicability and transferability of the NanoExplore approach.

In the After Life Plan, a more detailed description of the follow-up studies is provided.