FINAL REPORT

Grant Agreement number: 228536

Project acronym: NEPHH

Project title: NANOMATERIALS RELATED ENVIRONMENTAL POLLUTION AND HEALTH HAZARDS THROUGHOUT THEIR LIFE CYCLE

Funding Scheme: Small medium scale focused collaborative project

Period covered: **from 1st September 2009 to 31st August 2012**

Name, title and organisation of the scientific representative of the project's coordinator: JUAN ANTONIO GASCÓN, Mr. EKOTEK INGENIERÍA Y CONSULTORÍA MEDIOAMBIENTAL

Tel: 0034 902 998 368 / 0034 94 4041919

Fax: 0034 944 780 025

E-mail: jagascon@ekotek.es

Project website address: www.nephhf-fp7.eu

TABLE OF CONTENTS

List of figures

Final publishable summary report

1 Executive Summary

The **nanomaterials and nanotechnology** area is an important market for the chemical industry, and the EU as a whole. In fact, many sectors are involved ranging from mature high volume markets like automotive applications, high added value parts like space and aeronautic components or even emerging activities like new technologies for energy. Nevertheless, while nanotechnology offers a number of beneficial applications, the potential impact on the environment and human health of certain nanomaterials is not yet fully understood.

In the past two decades, polymer nanocomposites (reinforced polymers with low quantities of nanosized organic or inorganic ingredients dispersed into a thermoplastic or thermoset polymer) have emerged as a new class of materials. The use of nanomaterials –NMs- in composites production offers enormous advantages over traditional macro- or micro-particles and applications across a wide range of sectors are currently on the market.

To date, although NMs are well implemented in some markets, limited know-how exists on the **environmental and human health risks** these materials pose from a life cycle perspective. The main reason is that most of the research activities related with toxicological evaluation of nanotechnology have been focused on NMs synthesized at laboratory scale or directly supplied from industrial providers, which represent the most relevant form of exposure in production facilities.

With the demand growing fast, the **consequent exposition of human and environment** to these novel materials will undoubtedly increase, not only in the production phase but also throughout all the life cycle of nanocomposites, from NMs manufacturing, to their surface modification for a better dispersion in the polymer matrix, to the production of a variety of consumer products that undergo different end of life processes after usage stage. Therefore, the evaluation of the plausible **release of NMs embedded into solid matrixes** through the different life cycle stages and the associated **toxicological and ecotoxicogical impact** of these released materials is fundamental for risk assessment. In this sense, NEPHH Project has identified and rated important forms of nanotechnology-related environmental pollution and health hazards that could result from activities involved in nano-structures throughout their life cycle, and it has also suggested means that might reduce or eliminate these impacts.

NEPHH Project aimed to identify and rate important forms of nanotechnology-related environmental pollution and health hazards that could result from activities involved in silicon-based polymer nanocomposites throughout their life cycle, and also to suggest means that might reduce or eliminate these impacts. **Silicon based polymer nanocomposites** have been selected because they are a new field of research and development in which great hopes have been placed for significant social and environmental benefits linked to a greater economic development in plastics manufacturing industry. Generated knowledge contributes to better understanding the human health and environmental impacts of the selected NMs. NEPHH Project's results will set a basis for the establishment of required actions for the efficient management and minimisation of risks, at the time that it also contributes to the **acceptance of nanotechnology by the wide public**, thus assuring its **safe and sustainable introduction into market**.

2 Summary description of project context and objectives

While nanosciences and nanotechnologies-N&N- offer a number of beneficial applications, the potential impact on the environment and human health of certain nanomaterials-NMs- and nanoproducts is not yet fully well understood. Conversely, there exist apprehensive domains with a planetary impact like **environment** and new products, and functions for **health and safety of people**. Not only should nanotechnologies be **safely applied** and produce results in the shape of useful products and services, but there should be also **public consensus on their overall impact**. In fact, the **risk assessment of NMs** has become the focus of increasing attention. To date, the widely accepted view is that there are many unanswered questions on the potential environmental and health risks associated with the manufacture, use, distribution and disposal of NMs.

At present, the exposure of the general population to nanoparticles-NPs- originating from dedicated industrial processes is marginal in relation to those produced and released unintentionally e.g. via combustion processes and recycling centres. The exposure to manufactured NPs is mainly concentrated on **workers in nanotechnology research and nanotechnology companies**. Over the next few years, more and more consumers will be exposed to manufactured NPs, at then time that it is inevitable that in the future manufactured NPs will be released gradually and accidentally into the environment. The biggest concern is that free NPs could be inhaled, absorbed through the skin, dissolved in fluids or ingested.

Because of their small size and large surface area, engineered NPs may have chemical, physical, and biological properties distinctly different from larger particles of similar chemical composition.

The NEPHH Project has the following key motivations:

1. In early studies, engineered **NPs have shown potentially toxic properties.** They can enter the human body in various ways, reach vital organs via the blood stream, and possibly damage tissue. Due to their small size, the properties of NPs not only differ from bulk material of the same composition but also show different interaction patterns with the human body. The risk assessment for bulk materials is therefore not sufficient to characterise the same materials in nanoparticulate form. Information on the bioaccumulation and potential toxic effects of inhalation and/or ingestion of free engineered NPs and their long-term implications for public health is needed. The environmental consequences associated with the ultimate disposal of these materials also need to be evaluated carefully. There is a dearth of evidence about effects of pollution NPs on environment. Moreover, in common with other chemicals, NPs may reach humans and other organisms by a wide variety of environmental routes.
2. **Prioritizing and obtaining materials to evaluate are major challenges** when studying NMs. Specific NMs with the highest exposure potentials are not well known, making it difficult to identify the most important materials to study. Obtaining materials is also an impediment**.** In many cases, information about the nanoscale material is proprietary. Consequently, the EU may be unable to study those materials that pose the highest potential exposure to humans. In other cases, the material may be available, but not in sufficient quantities to allow an adequate hazard evaluation, particularly regarding long term, repeated exposure studies.
3. **Characterization of NMs has proven to be more difficult than anticipated for several reasons**. First, a standard nomenclature has not been developed. Second, biologists, physicists, and

materials scientists working in this area do not always communicate effectively. In addition, an analytical infrastructure to allow characterization is not consistently available or well-located. The high degree of variability in size and surface chemistry of nanoscale materials and in the coatings, crystal structure, shape, and composition used in preparing these materials increase both their complexity and the multiple permutations that must be considered in their evaluation.

4. **Adequate methods to detect NMs in cells and tissues also need further development**. Some of these impediments could be addressed by, for example, the development of a repository of well characterized model of NMs for use in both toxicological and biomedical research/ reference standards for nanoscale particles targeted for the biomedical and toxicological research.
5. Health, safety and environmental risks that may be associated with products and applications of N&N need to be addressed upfront and throughout their life cycle. Doing complete **Life Cycle Analysis- LCA-** on newly developed products, and considering all the ecological as well as the socio-economic components, **will help to ensure growth and employment in the European Economic Area**.
6. The implications of the **special properties of NPs with respect to health and safety** have not yet been taken into account by regulators. Size effects are not addressed in the framework of the new European Community Regulation on chemicals and their safe use (REACH). Although production volumes for the most commonly used NMs are already approaching the REACH threshold of 1 tonne per year per company. This is why NPs raise a number of safety and regulatory issues that governments are now starting to tackle.

Within this context, the NEPHH Project targets to identify and rate important forms of **nanotechnology-relate environmental pollution and health hazards** that could result from activities involved in nano-structures throughout their **life cycle**, and suggest means that might reduce or eliminate these impacts.

The **specific objectives** for of the project are listed next:

1 To develop a systematic and continuous practice for selecting and prioritizing NMs to **assess their safety, environmental and human health implications**.

2 To contribute to the **standardization and validation of test methods and schemes for NMs** as adaptation of the current physicochemical sampling protocols to present research is envisaged.

3 To collect nanocomposites samples, including laboratory and industrial Silicon based materials. These targeted materials represent an innovative selection supplementing ongoing investigations and setting a basis for future ones.

4 To achieve a better understanding of the health impacts of the selected NMs. *In vitro* methodologies were established for the regulatory demands for the safety assessment of nanotechnology products.

5 To **assess the human and environmental exposure throughout the life cycle** (synthesis and manufacturing stages) of targeted NMs according to the ISO 14.040:2006 and ISO 14.044:2006 standardized methodologies.

6 To assess the potential of NMs to damage the environment (or human health through the environment).

7 To select and disseminate the **Best Practices** (in the fields of manufacture and disposal mainly), and actuation guidelines for exposed workers, to minimise the exposure of workers, in a safe and economic manner. This included the generation and transfer of knowledge, regarding engineered NM safety issues, supporting research and regulation.

8 To contribute to the **'Code of Conduct for Responsible Nanosciences and Nanotechnologies Research'** action to ensure that nanotechnologies are developed in a safe manner. This objective aligned with the EC aims at reinforcing nanotechnology and, at the same time, boosting support for collaborative R&D into the potential impact of nanotechnology on human health and the environment via toxicological and ecotoxicological studies.

9 To contribute to the **regulatory frameworks applicable to NMs** (chemicals, worker protection, environmental legislation, product specific legislation, etc). Important elements as the test methods and the risk assessment (hazards and exposure) methods serve as a basis for implementing legislation, administrative decisions, manufacturer's obligations or employer's obligations.

The NMs selected are **silicon based laboratory materials** which have been supplemented with **NMs from industry**. On the one hand, silicon based NPs including nanosilica (SiO2), layered silicates or montmorillonite (MMT), glass(nano)fibres and foam-glass-crystal materials were selected. On the other hand, a total number of three engineering polymeric matrixes were selected, including **polyamides and polypropylenes** as bilsk materials and **polyurethanes as foamed polymeric materials**. According to this selection, **12 polymer composites** were produced on the combination of all NMs and polymeric matrixes.

The NEPHH Project Consortium was made up of ten entities from seven countries, amongst which there are important benchmark references in nanotechnology research such as the University of Cranfield, the University of Technology of Cracow, the Institute of Biochemistry of the Ukrainian National Academy of Sciences, the Polytechnic University of Tomsk, the Tecnalia Research & Innovation Technology Centre and the CNRS. Furthermore, there is an important presence of industrial partners, represented by 3 SMEs, Ekotek, Grado Zero Espace and Association for the Prevention of Accident and one large company Laviosa Chimica.

Regarding the project's workplan, NEPHH Project is organized in 8 Work Packages (WP) which have covered the following activities:

- The development of a Technological Surveillance System-TSS- during the **WP1**, resulting in a systematic, continuous practice for the selection of NMs in order to assess their safety, environmental, and human health implications. Within this WP a survey was carried out to assess the occupational health and safety procedures in place.
- During the **WP2**, the selected NMs were synthesised and macro-scale structural specimens were manufactured. This enabled the consortium to analyse the implications of such NMs from synthesis to disposal.
- The **WP3** involved the generation of **nanoscale dust particles** from the macro-scale fibre reinforced nanostructures fabricated in WP2, to consider the exposure throughout the whole life cycle of NMs in as near 'real life' exposure as possible.
- The health implications (**WP4**) and environmental implications (**WP5**) of the selected NMs were assesed in parallel. The health effects of NPs (1) on lungs; (2) the structural study of cells and

protein expression, and (3) the genotoxicological effects were studied. During WP5 the potential of NMs to damage the environment (or human health through the environment) was assessed, based on persistence, bioaccumulation and eco-toxicity studies. Moreover, the environmental performance of nanocompounds from cradle to grave was also evaluated.

- **WP6** aimed to make available the understanding of the safety, environmental and health implications of NMs in order to define the appropriate measures and minimise the exposure of workers. Guidelines for responsible management of waste NMs were produced.
- Finally, **WP7** dealt with the dissemination of the research results and the Project Management was performed by the **WP8**.

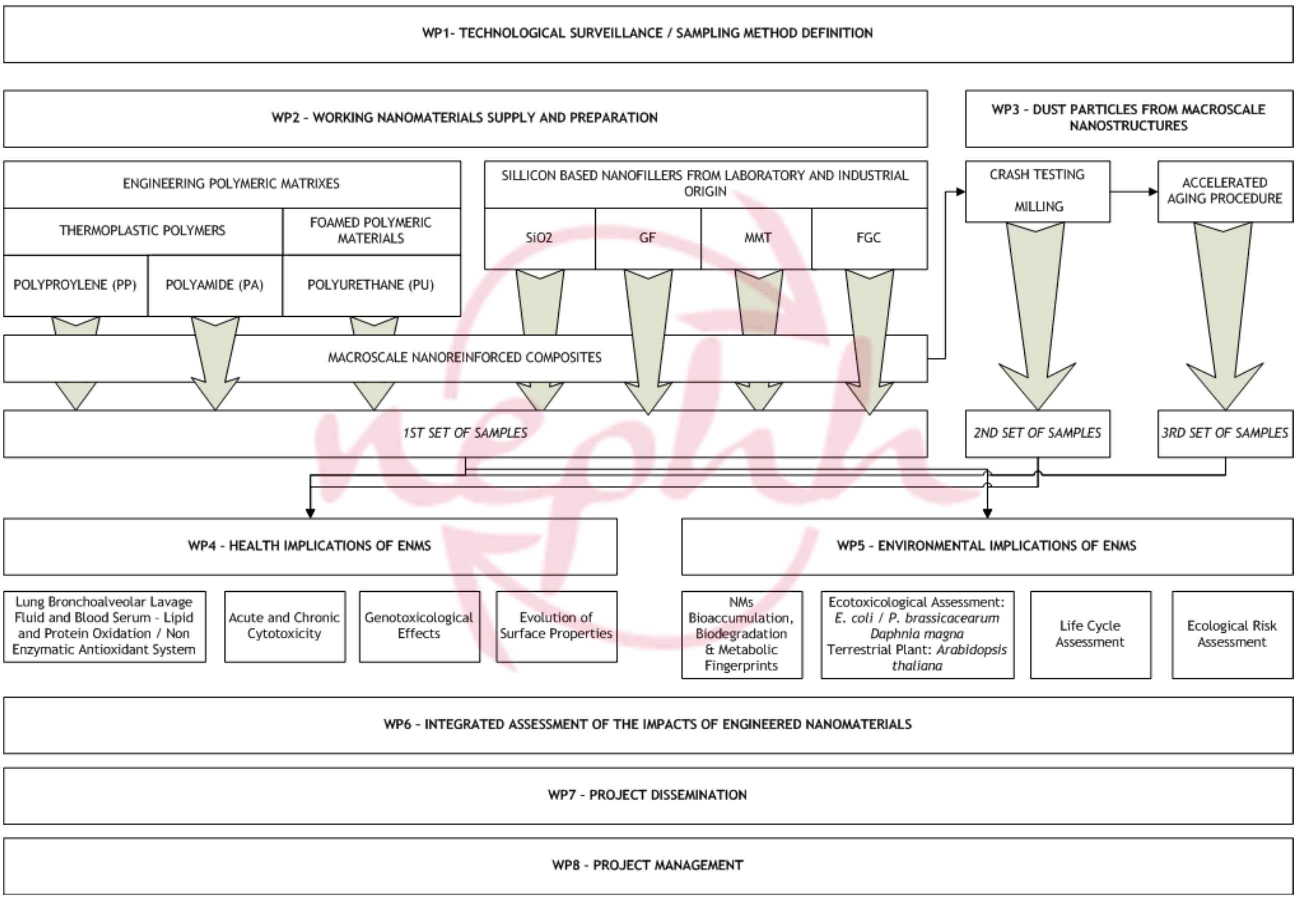


Figure 1- NEPHH Project Workplan

3 Description of the main S&T results /foreground

The aim of NEPHH is to identify and rate important forms of **nanotechnology-related environmental pollution and health hazards** that could result from activities involved in nano-structures **throughout their life cycle**, and to suggest means that might **reduce or eliminate these impacts**. During its execution, the project has considered the safety, environmental and human health implications of nanotechnology-based materials and products.

The NMs selected are **Silicon based NMs** which will be supplemented with **NMs from industry**. On the one hand, Silicon based NPs including **(nano)silica (SiO2)**, **layered silicates (MMT)**, **glass (nano)fibres** and **foam-glass-crystal materials** have been selected. On the other hand, a total number of three engineering polymeric matrixes have been selected, including **polyamides** and **polypropylenes** as bulk materials and **polyurethanes** as foamed polymeric materials, which will be used to produce nano-induce polyurethane foams. According to this selection, several polymer composites have been produced on the combination of all NMs and polymeric matrixes.

The NEPHH Project's approach is represented graphically by the next figure:

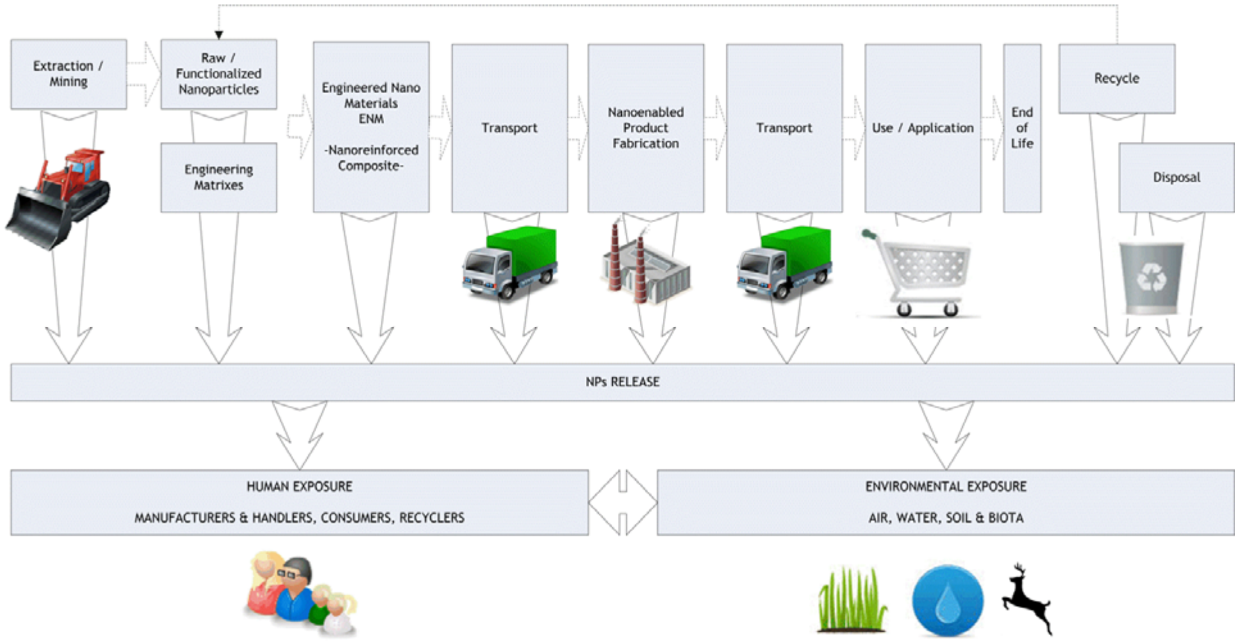


Figure 2- **NEPHH Project's approach**

In the following section a description of the main activities and S&T results obtained is presented.

3.1 WP1 – Technological Surveillance System

The first WP of the project dealt with three main activity groups: **(1)** the design and management of a **Technology Surveillance System-TSS**-, **(2)** the assessment of the occupational health and safety procedures for Silicon based NMs at industrial and research organizations within EU, and **(3)** the evaluation and development of sampling protocols for silicon based NMs. This WP set up the boundaries and scope of the project in terms of current situation of silicon based NMs.

1- Within the TSS development, a complete automated system was developed to capture, evaluate and disseminate information released about a number of topics related to nanotechnologies and, more specifically, materials of interest within NEPHH including: Health and environmental implications, Sampling and sample preparation methods, Physicochemical and Toxicological Characterization, Protective systems and working practices when manipulating NMs, Regulation and standardisation in nanotechnologies, New uses and applications of nanomateriales and Events.

The developed vigilance system is a tailor-made one, designed and implemented in order to identify all the innovations regarding health and environmental implications of nanotechnologies, but, specifically focusing on **Silicon based NMs**. Around 100 information sources have been tracked continuously, producing a database of 857 documents classified in aforementioned topics. With regards to the information collected, six monthly bulletins were prepared to be disseminated through the consortium and Nanosafety Cluster, focused on information relating to Silicon based NMs although additional general new information of special interest was also included. Moreover, to provide the consortium with the newest information, relevant information was sent to the partners at the moment it was captured.

The TSS has contributed to the research developed in other WPs, as well as in the **public dissemination of the project**, i.e. gathering information for the newsletters of the project and the “Guide of actuation for people working on nanomaterials” and ”Guidelines for responsible management of waste nanomaterials” produced in WP6. .

2- Within the WP1, a second group of activities targeted to asess the **occupational an environmental health and safety procedures in force** in the global Nanotechnology Sector, by means of a survey sent to more than 800 organizations. A structured questionnaire to collect information on Environmental Health and Safety -EHS- Programs, Engineering Controls, Personal Protective Equipment -PPE-, waste management, workplace monitoring, risk characterization and product stewardship, was specifically developed and distributed together with the NEPHH Project information.

Most organizations reported having a general EHS Program, but only half of the organizations of these reported having a **nano-specific EHS Program**. Overall, most organizations communicated having **PPE recommendations for their employees** while working with NMs, although conventional lab wear was most often reported as the recommended means of protection. Regarding the **waste management practices**, the majority of respondents dispose of waste containing NMs in the same manner as any other waste (either as hazardous waste or as non-hazardous waste). Finally, the majority of respondents did not perform monitoring of the workplace for NPs, due to the fact that little amounts of NMs were used and only NMs in aqueous solutions were handled.

In general, the collected information shows that there is an **important lack of information and knowledge about the risks associated to the NMs handling**, and that high quality research and legislation development is needed. However, most of organizations surveye are willing to improve their nano-specific practices and are currently planning or implementing new measures or considerations to improve their EHS Programs. The results obtained represented the current situation related to health and safety procedures, providing a baseline for the development of the Guidelines of actuation for people and entities working with NMs in WP6.

3- The last activitiy within the WP1 drew and evaluated **sampling protocols replicating the different life cycle stages of nanocomposites**. NEPHH Consortium has defined the envisaged procedure for samples production, collection, storage, labelling and transference amongst partners. Given the importance of the samples and unknown risks associated with these, special procedures were developed covering the samples identification, maintenance and storage, as well as samples transfer to Project partners’ laboratories for further toxicitity and eco-toxicity investigations. This procedure is a relevant highlight of the project, as it supposes the basis for the **standarisation of the testing approaches** within the project that can be later replicated at a major scale. It is expexted that further activities in the assessment of the toxicological and ecotoxicological potential of nanomaterials from a life cycle perspective will contribute to an international consensus in the area of nanomaterials testing which turns this protocol into a living document that would ideally be updated and completed as new knowledge becomes available beyond NEPHH.

For this purposes, series of experiments were conducted to evaluate and optimize the samples collection and handling procedures. Mechanical performance and morphology investigations were conducted to allow successful particles generation and collection to happen (PU Foams). A **crash chamber** was specifically designed and constructed for the purpose of generating and sampling of NPs through drop weight impact test.

For the rest of matrixes -PP and PA- a specific chamber for particles generation and handling while composites and nanocomposites drilling was designed. The generated particles from mechanical loadings were supplied to Project partners in suspension and in dry form.

Additionally protocols for macrosamples abrassion followed by accelerated ageing have been designed, representing different approaches for the simulation of real life cycle stages possibly conveying the release of embeded nanomaterials in solid matrixes.

3.2 WP2 – Working Nanomaterials: Supply and Preparation

NEPHH's WP2 involved the synthesis and/or acquisition of selected NMs and the manufacturing of the **macro-scaled structural specimens** for the subsequent experiments in WP3 (dust particles generation), WP4 (heath implications assessment) and WP5 (environmental implications assessment). With this approach, different nanocomposites were produced, composed of a NM (nanofiller) and a polymer or composite (matrix). This technical approach of the project can be seen in the following scheme:

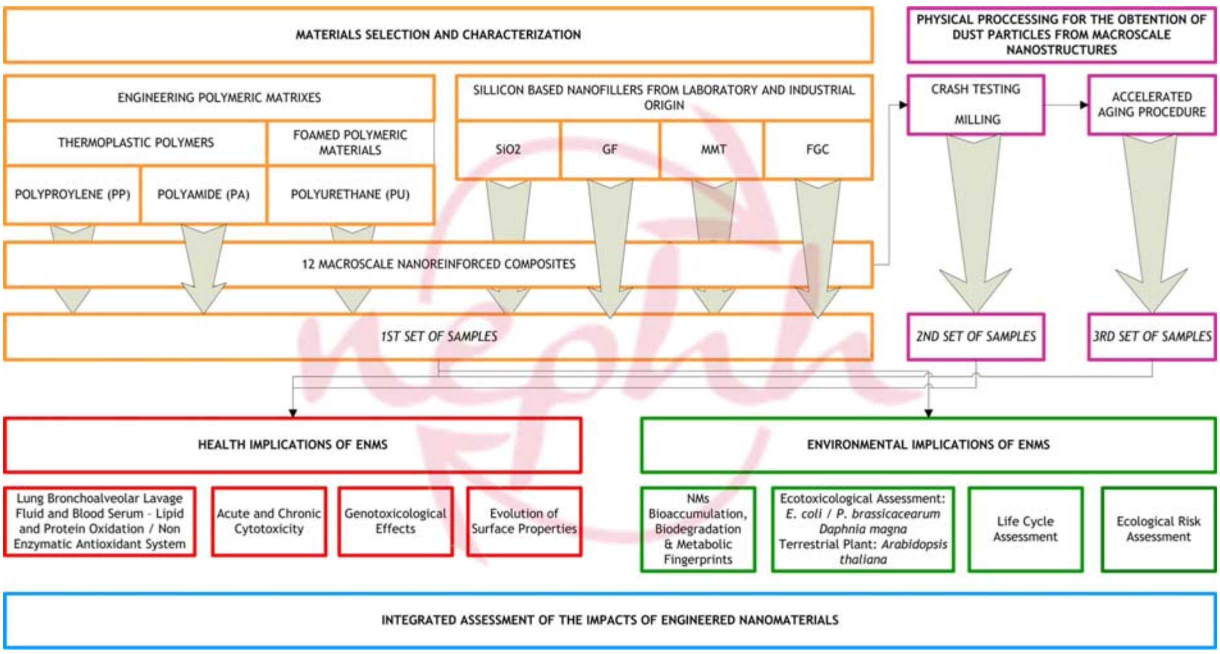


Figure 3- **Technical Approach of NEPHH Project**

The production of **macro-scale structural specimens** involved a number of steps as hereby listed:

(1) Selection and characterization of **Silicon based NPs**: (Nano)Silica (SiO2), Layered Silicates (MMT), Glass (nano)Fibres (GF) and Foam-Glass-Crystal (FGC) materials to be used as **nano-reinforcing agents.**

(2) Selection and characterization of engineering polymeric matrixes – **Polyamides (PA) and Polypropylenes (PP) as bulk materials and Polyurethane (PU)** as a foamed matrix was carried out.

(3) Polymer nanocomposites preparation by using polymers and NPs described in points 1 and 2.

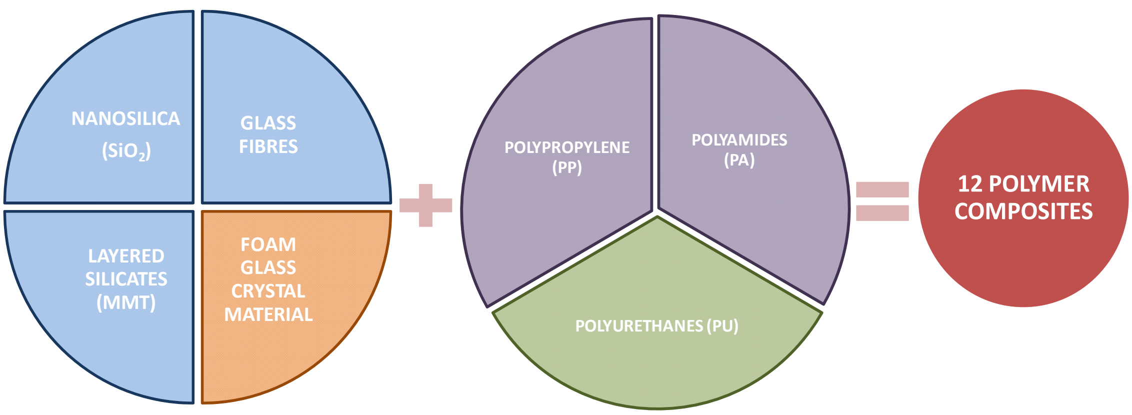


Figure 4- **Polymer nanocomposites selected**

Afterwards, developed polymer nanocomposites were used to fabricate **macro-scale structural specimens** to be physically processed (WP3).

Firstly, a systematic and continuos practice for **selecting and priorizniting NMs** was set up, and the selection of the industrial NMs, required for the manufacturing of nanocomposites, was carried out, according next criteria:

- The presence of nanostructures available to from uniform dispersion in polymer matrix.
- Anticipated compability with polypropylene, polyamide and polyurethane matrices.
- The possibility of using the nanofiller in extrusion technology – material can be quantitatively dosed to the polymer melt or a premix masterbatch of polymer and nanofiller can be prepared.
- The detailed knowledge abouth the technology of production of NMs.

The selection of Silicon based NPs acquired from several producers are listed below:

- Purified sodium montmorillonite and organically modified montmorillonite from Laviosa, Souther Clay and ZGM Zebiec.
- Pyrogenic silica with hydrophilic and hydrophobic surface from Degussa
- Glass fibres from Taiwan Glass Ind. Corp.
- Foamglass from Dennert Poraver GmbH.

NMs acquired were subjected to initial testing towards their further application in polymeric nanocomposites. The **physicochemical characterization** of the industrial NMs to be applied for preparation of polymer nanocomposites by Wide-Angle X-ray Diffraction (WAXD), Scanning Electron Microscopy (SEM) and Thermogravimetry (TG) methods was carried out. The main goal of the analysis was to ascertain the nanostructure of the supplied nanofillers and investigate their **thermal stability**, especially in the range of typical processing temperatures of thermoplastic polymer matrices used in this project (PA, PP and PU). Furthermore, this activity was focused on finding possible thermal processes that could influence the structure formation and final properties of polymeric nanocomposites which would be prepared and tested in the forthcoming stages of this WP.

The following conclusions were drawn from the **structure, morphology and thermal behaviour analysis** of these industrial nanofillers:

- Structure analysis carried out by WAXD and SEM methods confirmed the presence of NPs (organobentonites, fumed silica) or nanopores (foamglass) in the tested fillers,
- Nanofillers and NMs differ in shape and dimensions of elementary particles which, in original state of filler, form bigger porous or branched aggregates and agglomerates of complex morphology, that can be further dispersed in polymer.
- As resulted from the TG and Differential Scanning Calorimetry (DSC) analysis, all of the selected nanofillers do not undergo any significant thermal processes, particularly thermal degradation, in the temperature range up to 200°C, that could influence the miscibility of nanocomposite components and render difficult the formation of nanostructure.

According to the physicochemical characterization a set of representative and well-characterized fillers from industrial origin were selected, due to their structure, morphology and thermal behavior, for their plausible application in polymer engineering nanocomposites as listed below:

- Dellite 72T and Dellite 43B from Laviosa (**montmorillonite**)
- Aerosil 974 and Aerosil 200 from Degusa (**nanosilica**)
- TGFS 202P and TGFS 473H from Taiwan Glass Ind. Corp. (**glass fibres**)
- Poraver2-4 from Dennert Poraver GmbH (**foam glass**)

The following activity within WP2 dealt with laboratory NPs supply and physicochemical modification. Chemical modification of NPs using designed modification procedures can lead to desirable changes in their structure/morphology towards better compatibility with polymer matrix. In a second group of activitities within WP2, **physicochemical modification of laboratory NPs**, specifically montmorillonite (MMT) and foamglass, was carried out.

In the case of **montmorillonite** (Sodium MMT - from Southern Clay deposits) was modified by exchange of inorganic metal cations with ammonium cation as an organic modifier (trimethyloctadecylammonium chloride) in water medium. The parameters of preparation of organically modified montmorillonite were experimentally tested. Lyophilization was found to be most convenient method of drying producing fine powder and restraining formation of compact agglomerates. The obtained laboratory NPs were characterized by WAXD, SEM and TG techniques. The physicochemical modification procedure was completed by the evaluation of different ammonium modifiers and general optimization of the procedure inding the drying process.

In the **foamglass** area, well-founded selection of optimum raw materials was done and tested during the controlled experiment. Facile preparation routes were developed implementing chemical and X-

Ray–phase analysis techniques. A two-stage method for producing foamglass via the intermediate product (quenched cullet) synthesized by thermal treatment of the mixture of the certain composition was developed. Theoretical design and experimental testing of raw mixtures (including selection of chemical components) for producing foamglass and foam-glass-crystal materials were performed. Initial tests towards development of a new method of low temperature foamglass synthesis were done and be continued in following activities. This approach intended to prepare raw materials for energy saving in the technology of foam-glass production by using natural and anthropogenic waste material. At processing temperatures not exceeding 950ºC, a residual crystal phase is preserved resulting in mechanical enhancement of foam glass crystal material along with a possible reduced impact on human health and environment.

After that, the next activity was focused on the **selection of the polymeric matrices for nanocomposite materials** (polypropylene (PP), polyamide (PA) and polyurethane (PU)) for the manufacturing of macro-scale structural specimens. This selection was based on the **melt flow index value and mechanical properties of the polymers** (given by the providers), and it also took into account suitable processing methods and possible application areas of different polymers' grades. The next characteristics were considered:

- Current potential application of nanocomposites (especially engineering applications) that make engineering polymers more competitive,
- Ability to disperse the filler in nanometric scale, associated with polymer molecular weight and melt flow index as a relative measure of molecular weight,
- Polymer grade suitable for extrusion and compounding,

Based on those criteria, the polymeric matrixes selected were the following ones:

1- **Polypropylene (PP) Moplen HP500J** from Basell Polyolefins (melt volume flow rate 4.3 g/10 min (230°C/2.16 kg), tensile modulus 1500 MPa, tensile stress at yield 34 MPa and Charpy notched impact strength 4 kJ/m2 (23°C))

2- Polyamide 6 (PA-6) Tarnamid T-30 from Zakłady Azotowe w Tarnowie-Mościcach (melt volume flow rate 25.0 g/10 min (275°C/5.0 kg), tensile modulus 1100 MPa, tensile stress at yield 28 MPa Charpy notched impact strength 5 kJ/m2 (23°C))

3- Rigid Polyurethane foams (PU) were considered for their study since all the prepared materials are designed for crash test and the ability of shock absorption is not desirable. Polyurethane foam (PU) was synthesized (after a set of optimization experiments) in a three-step process – first, to polyol (polyether RF-551) catalyst (N,N-dimethyl cyclohexylamine), water and surfactant (SR-321, Union Carbide, Marietta, GA) were added in order to prepare the polyol premix (component A). In the next step, n-pentane as a physical blowing agent was added to component A. In the third step component B (polymeric 4,4'-diphenylmethane diisocyanate (PM 200)) was added to component A and the mixture was stirred for 10 seconds with an overhead stirrer. Finally, the prepared mixtures were dropped into a mould. All the experiments were performed at ambient temperature of ca. 20°C.

The degree of dispersion of lamellar nanofillers in PA-6 melt was reported to be higher for polymers having higher molecular mass.

The selected grades of thermoplastic polymers should be characterized by the highest value of mass flow rate (MFR) at which an injection moulding of thick-walled details for e.g. cars is possible.

Moplen HP500J is a PP homopolymer with good stiffness in addition to good processability, suitable for compounding, whereby Tarnamid T-30 is PA-6 high quality engineering thermoplastic polymer fabricated in the process of ε-aminocaprolactam polycondensation. It shows high mechanical strength and high chemical/thermal resistance.

The **PP, PA-6 and PU polymer matrices** were characterized by WAXD, SEM, TG, and DSC techniques to confirm their structure and morphology.

The next activity dealt with the supplying of mineral charges and their chemical modification of the following NPs - Montmorillonite, Nanosilica and Glass Fibres - for the production of laboratory NPs to characterized latter, as well as Foamglass (laboratory material). Following, the modifications of these 4 Si-based industrial NPs are detailed:

a- Water dispersion of purified **montmorillonite** (MMT - from Southern Clay deposits) was modified by cation exchange of hydrated metal cations located in gallery space with the alkyl- and arylammonium salts with various chemical constitution (dimethyldioctadecyl ammonium chloride, N-p-hydroxybenzyl-N-octadecyl-N,N-hydroxyethylammonium chloride, N,N,N-trimethyl-N-alkylammonium chloride, where alkyl group incorporated amide group).

b- Nanosilica and **glass fibres** were modified by condensation of dichlorodimethyl silane with silanol groups present on the surface of silicon dioxide-based fillers.

The obtained laboratory NPs were characterized for what refers to structure, morphology and thermal analysis by WAXD, SEM, Fourier Transform InfraRed (FTIR), DSC and TG techniques.

c- A two-stage method for producing **foamglass** via the intermediate product (glass granulate) synthesized by thermal treatment of the mixture of a certain composition was developed at Tomsk Polytechnic University. This product acts as the raw material for the further sponging and obtaining foam-glass-crystal products with the pre-set characteristics. It has been found that the temperature of charges treatment calculated according to the base glass composition increases with the rise of SiO2 quantity and with the decrease of impurities content in silica component: from 800ºC for charges on the basis of zeolite (SiO2-63%) to 885oC for charges with marshallite (SiO2-95,7%).

Within the next activity, the preparation of **polymer nanocomposites** was accomplished. The **nanocomposites of PP and PA-6 with four types of fillers** (*organically modified montmorillonite, nanosilica, foam-glass crystal materials and glass fibres*) were obtained by direct melt mixing in twin-screw extruders. In order to maintain high dispersibility of nanofillers in both apolar (polypropylene) and polar (polyamide) matrix selected nanofillers were used with proper surface functionalization and macromolecular compatibilizer addition providing good compatibility with both types of polymers - nonpolar polypropylene and polar PA-6.

Injection moulding bars of nanocomposites prepared within this task have been examined in terms of structure, morphology and thermal properties by WAXD, Small-angle X-ray diffraction (SAXD), TG, DSC, SEM, Polarized Light optic Microscope (POLM) and FTIR methods.

On the basis of literature review and laboratory results **eight different compositions** (PP/MMT, PP/nanosilica, PP/FGCM, PP/GFs, PA6/MMT, PA6/nanosilica, PA6/FGCN and PA6/GFs) containing 5wt% of nanofiller were selected as a **suitable material for preparation of macrosamples** that would be further examined in the **scheduled physical processes replicating different stages of the life cicle of nanocomposites** in WP3. Selected filler concentration provided the highest content of additive in the form of well distributed and not agglomerated NPs. No other additives, eg thermal stabilizer, processing aids… were used in order to avoid their influence on material's toxicity. Macrosamples for physical processing (crash tests) were prepared by **compression moulding technique**.

The next task within WP2 included the preparation, through injection moulding process, of **glass fibre reinforced composite panels**. Two types of material were utilised to prepare the glass fibre reinforced panels: PP and PA-6 with/out 5% glass fibres. The glass reinforced PP and PA-6 composites were based on PP homopolymer (Moplen HP500J, Basell Orlen -Basell Polyolefins Group) and PA-6 (Tarnamid T30, Zakłady Azotowe w Tarnowie-Mościcach, Poland).

Additionally, commercial PP and PA-6 with glass fibres (MM-PP BI 24 and MM-PA I 1F30, 30% GF, MACOMASS Verkaufs AG, Germany) were also used to manufacture injection moulded thermoplastic panels. From the manufactured glass fibre reinforced panels sandwich structure were manufactured using PU/MMT foams.

Finally, polyurethane foam (PUR) was synthesized (after a set of optimization experiments) in a three-step process comprising (i) preparation of polyol premix with auxiliary components and nanoaditives, (ii) introduction of blowing agent into polyol premix and (iii) addition of isocyanate component. High speed mechanical mixer was applied in order to enhance dispersion of nanoadditives and ensure proper mixing of reagents in the course of synthesis of PUR.

Four types of nanoinduced polyurethane foams were prepared: polyurethane foam/montmorillonite (PUR/MMT), polyurethane foam/nanosilica (PUR/nanosilica), polyurethane foam/foam-glass crystal material (PUR/FGCM) and polyurethane foam/glass fibres (PUR/GF) with 5wt% of nanofiller. The foams were analyzed in terms of structure, morphology and thermal properties by WAXD, SAXD, SEM-Energy Dispersive X-Ray Analysis (EDX), POLM, TG, DSC and FTIR methods.

Figure 5- **Bars of PA6 for crash tests**

Macroscale samples for physical processing were cut from the large foam bar and their dimensions were adjusted to 10x10x1 cm, concluding that in the frame of this WP, a set of perfectly characterized **materials to be processed for nanosize dust generation** (WP3) were obtained.

3.3 WP3 – Dust Particles from Macro-Scale Nanostructures

The WP3 focused on **generating nanoscale dust particles** from the macro-scale nanoreinforced nanostructures fabricated previously, in order to analyse the **exposure throughout the whole life cycle of NMs** in near 'real life' exposure as possible. Mainly due to the fact that the potential exposure in high performance structures (aerospace, automotive, etc) is deemed to increase when material fracture occurs, WP3 focused on potential exposures in the **transport vehicles accidents**, **recycling centres** (especially composites ones), **milling, sawing, machining, manufacture and testing operations** of nanoreinforced composites. **Ageing protocols** were also performed to evaluate the effects of Silicon based NMs on recycling and reclamation at the end of the final product life-cycle.

A preliminary study comprised the **microscopic evaluation** of generated samples via mechanical processing as established in the Sampling Protocol defined in WP1, using electron microscopy along with EDX, X-ray photoelectron spectroscopy (XPS), laser-based particle size analysis and surface zeta potential. This study shown that the incorporation of different nanofillers into a PU foam matrix enhances the mechanical properties as compared with the neat polymer.

Furthermore, fracture studies of macrostructural systems using the standard system of foam glass crystal blocks fracture were accomplished. These mechanical tests were conducted to define mechanical properties and specifically to determine the tensile ultimate strength of foamglass. Foamglass samples underwent the test for compression down to their complete fracture with registering strain diagram in the automatic mode. The universal machine 'Instron 1185' with the load range between 0-100 N and 0-100 kN was used. For a comparative analysis three kinds of samples were chosen, namely: **(i)** foamglass-crystal materials (FGC), **(ii)** industrial foamglass and **(iii)** laboratory foamglass obtained from cullet. According to the research findings in relation to the fracture process of foamglass crystral samples obtained in vitro it can be concluded that:

- Foam glass sample have higher strength as compared to the foamglass obtained from cullet (i.e. traditional foamglass);
- Fracture mechanisms for foamglass crystal samples is well described by the synergetic models of deformation of a quasi-viscous (amorphous) solid;
- Relation between the interpore partitions of foamglass crystal material and the ultimate strength is similar to Hall-Ptech relation;
- Fracture stress is in direct proportion to the pore size whose diminishing raises the strength of cellular materials.

In addition, a literature review on impact behavior and end of life processes for relevant nanocomposites was conducted. The results showed that there is a strong dependency between **energy absorption capacity of nanocomposites and the physicochemical properties of the integrated nanostructures**. Numerous studies demonstrated that nanofillers have potential in improving both stiffness and energy absorption of polymer and/or conventional fibre-reinforced polymer composites. Regarding the end of life processes (recycling, combustion and disposal to landfill), several studies were reviewed but few data is available on the degradation of polymer nanocomposites, the state of the embedded NPs during environmental exposures, or how they will be released during their life cycles.

From the high performance nano and fibre polymer panels manufactured, NPs were generated by impacting polyurethane nanocomposites with different nanofillers (Montmorillonite, Nanosilica, Glass fibres, Foam Glass Crystal-FGC-) via **low velocity impact testing**. Also, NPs from polypropylenes and polyamide nano and fibre reinforced panels were generated by **mechanical drilling**, as impacting of these materials did not generate dust.

The **released particles** were sampled and extracted by suspending them in solution. The solution has been filtrated in several steps and the physical and chemical properties were characterized by means of SEM, transmission electron microscopy (TEM) measurements, Dynamic Light Scattering (DLS) and nanosight (SN). Additionally, the materials were investigated by WAXD, TG in inert and oxidative atmosphere DSC.

Additionally, dry NPs extraction was performed, as estimated necessary for the avoidance of the occurrence of changes in solutions due to storage time required during the accomplishment of the (eco)toxicological assessment: these changes include the occurrence of biological contamination and variations in the concentrations originally reported.

The results showed that **NPs can be generated by different mechanical processes**. Moreover the characterization revealed that the physicochemical properties of the generated particles significantly vary depending on filler and matrix material. It is worth to highlight that by integration of MMT into the PA-6 and PP matrices **particle quantity and particle size can be reduced**. In the particular case of PP panels the drilling of FGC, glass fibres and MMT reinforced panels generates a similar amount of NPs, being maximum for SIO2. However, PP unreinforced generates nearly no particles.

Thermal analyses were focused on the effect of mechanical degradation on the structure of polymer matrix and NPs. The presence of NPs in the dust particles was indirectly confirmed by changes in polymer melting and crystallization behavior comparing to the polymer dust without NPs.

The aim of this study was to investigate the effect of different fillers (nanofillers and fibres) during machining processs and low energy impact into reinforced composites panels**.** Total **airborne particle concentration and particle geometric mean size** were measured by means of a Scanning Mobility Particle Sizer (SMPS+C) and particles were sampled with an Electrostatic Precipitator (ESP). In contrast with previous task, characterization data obtained are those of airborne NPs, as collected by means of the SMPS. SEM Micrographs complete measurements on particle size distribution.

The results clearly showed that by impacting of **nanoreinforced PU**, NPs are generated especially in the **size range <100nm**. Regarding the **PP nanocomposites**, the results showed the potential impact of machining of nano and fibre reinforced panels, as for unreinforced, just a slight increase of NPs could be measured, but for the nano-reinforced panels a huge increase could be measured.

Aligned with outcomes obtained previously the **integration of nanoclay into the PA matrix** leaded to a **decrease of particles generation**. The geometric mean size vs. time distribution showed also a clear difference between the PA-6/MMT panels. While the PA-6/MMT soon after termination of drilling showed sizes of >200nm, all the others needed longer to reach larger sizes.

Within WP3, a series of **Industrial Scale Dispersion Tests** were defined and performed by the use of equipment and techniques as close as possible to existing and future industrial production ones: on this purpose, a specifically dedicated (and set-up) pilot-plant of an Italian masterbatch producer was used (VIBA). The aim of these tests was to provide subsequent WP (WP4 and WP5) with both relevant information and materials that will be useful for the evaluation of possible **environmental pollutions and health hazards** related to the manufacture, use and disposal of NMs. Such tests involved the dispersion of three classes of charges and two polymeric matrices, obtaining six composite materials to be subsequently processed and analyzed: SiO2/PP, MMT/PP, glass fibre/PP, SiO2/PA, MMT/PA and glass fibre/PA.

All the Industrial Dispersion Tests were accomplished until the production of 2-3 kg of each sample. Subsequently, a second series of industrial scale dispersion tests was performed, by extrusion of four further composite materials: ATH/EVA, ATH/MMT/EVA, MDH/EVA, MDH/MMT/EVA. These materials were selected for the LCA development in WP5.

Apart from these tests, the **effect of matrix and reinforcement material on the energy absorption capabilities of polymer composite structures** was evaluated. The axial crash experiments of glass–reinforced polymeric cones were conducted. The low velocity impact tests were carried out using a high energy drop tower at velocities not exceeding 8 m/s. Furthermore, quasi static compression tests of the conical structures and quasi-static tensile tests were conducted using Instron electro-mechanical machine. The impact event was recorded using a high-speed camera with maximum speed of 1000 frames/sec.

The experiments showed that by changing the matrix and the reinforcement material it is possible to change the micro-mechanism of the crash and therefore control the energy absorption characteristics of the composite.

In this sense, a significant increase in tensile properties (stiffness, strength or elongation to brake), of PA composites was shown. Furthermore, SiO2 and glass-spheres (GS) reinforcements were found to increase the energy absorption capabilities of PA materials, whereas MMT reinforcement caused a decrease in that property. On the other hand, all mechanical properties of PP were decreased after the addition of secondary reinforcement. Bad dispersion of the particles and weak filler-matrix interaction were indicated as a possible reason of that problem.

The last activity of WP3 was focused on the **development of NMs persistence studies**. The aim was to assess the **persistence of dust generated during mechanical abrasion** reproducing wear and tear of polymeric product incorporating NMs.

From all experiments developed, it came out that alteration / aging of dust from polymer incorporating NPs can vary not only as function of the polymer matrix but also the type of incorporated NPs.

In the case of **PP aging / alteration experiments** indicated that Si ions were released from solid materials. Even if Si is initially present in polymers without NMs, it appeared that adding nano-SiO2 and MMT lead to **higher level of Si dissolution**. In all cases, aggregation of dust from mechanical abrasion occurred. Even that, the fractal dimension of the aggregates were low (1.8 to 2.1). It was shown that in the case of PP-MMT the structure of the nanoclay was modified with the release of the ammonium cations from the interlayer space.

In the case of **PA aging / alteration** had very low effects on both properties and structures of the polymer materials. A partial dissolution of incorporated Si NMs occurred and to a larger extend in the case of Nano-SiO2 than MMT. As for PP materials, aging under light lead to the increase of particle size due to aggregation. However in the dark aggregation remained very limited.

In the case of **PU aging / alteration experiments** revealed a slight oxidation of the PU matrix. Aggregation was also observed with mean particle size increasing from 20 to 40-60 microns. But as opposed to PA and PP incorporating Si NMs, PU – SiO2 and PU-MMT were not affected by Si dissolution indicating that NM were not at the surface of fragment produced by mechanical abrasion. This was further evidenced by SEM analysis.

3.4 WP4 – Health Implications of NMs

NPs are potentially more hazardous than their larger counterparts to human health. Very little information is currently available on the release of NPs throughout product life cycle and related health effect. The development of WP4 aimed to assess the **toxicological mechanisms and health impacts**

of the selected NMs, collaborating in establishing reliable and useful *in vitro* methodologies for the regulatory demands of the safety assessment of nanotechnological products.

To accomplish these evaluations, *in vitro* experiments were performed to determine effect of nanosized particles of PU, PA and PP nanocomposites containing glass fibres, glass particles, montmorillonite, silica gel and polymers alone (reference materials) generated within WP3 and collected both in **liquid suspensions and in dry status**. The results of these evaluations are detailed in following sections:

Considering the (1) **raw NPs**, Aerosil® 200 as well as Aerosil® 974 were analyzed for blood serum lipid as well as protein oxidation. A method for blood serum *lipid oxidation determination in vitro* in presence of Silica NPs by measuring contents of the products of the lipid peroxidation - aldehydes reacting with thiobarbituric acid has been used. A method for blood serum *proteins oxidation determination in vitro* in presence of Silica NPs by measuring contents of reduced SH-groups in proteins by reaction with fluorescence probe ThioGlo has been used.

Aerosil® 200 as well as Aerosil® 974 at concentrations 0,1; 1,0; 10; 100 mg/L do not affect (increase or decrease) blood serum lipid and proteins oxidation determined *in vitro*. In contrast, iron containing nanosized particles (used as a control) at concentration 25; 50; 100 mg/L statistically significant increase blood serum lipid oxidation as determined *in vitro*. The same control particles under identical conditions statistically significant decrease contents of reduced SH-groups in proteins which confirms increase of blood serum proteins oxidation *in vitro.*

Aerosil® 200 as well as Aerosil® 974 at concentrations 0,1; 1,0; 10; 100 mg/L do not affect (increase or decrease) gluthatione content in blood serum *in vitro*. In contrast, iron containing nanosized particles (as a control) at concentration 10; 25; 50; 75; 100 mg/L statistically significant decrease gluthatione content in blood serum in vitro.

Therefore, it can be concluded that evaluated samples do not influence blood serum lipid oxidation either protein oxidation and are inert towards the non enzymatic antioxidant gluthatione content in blood serum as determined *in vitro*.

In relation to the results obtained with regards to montmorillonite allow to confirm that Dellite 43B (D43B), Dellite 72T (D72T) as well as Dellite LVF (DLVF) at the investigated concentrations of 100; 200 and 600 mg/L do not effect (increase or decrease) lung bronchoalveolar lavage fluid or blood serum lipids as well as proteins oxidation as determined *in vitro* by TBARS-test and SH-group consumption, respectively. In contrast, iron containing nanosized particles (selected positive control) at concentration 100; 200 and 600 mg/L statistically significant increase lipid and protein oxidation as determined *in vitro* by TBARS-test and SH-group consumption, respectively.

In relation to the (2) **samples collected in liquid suspensions**; tests carried out include TBARS-test for blood serum lipid oxidation; Fluorescence Test for blood serum protein oxidation and Fluorescent Test for the evaluation of gluthatione as non enzymatic antioxidant system. Additionally, lung bronchoalveolar lavage fluid (LBALF) was prepared and the oxidation of LBALF was determined. NPs of PU, PA and PP nanocomposites containing glass fibres, glass particles, montmorillonite, silica gel and polymers alone (reference materials) generated by mechanical means (drilling, crashing) and collected in DDW at the investigated concentrations -50% V/V- do not effect (increase or decrease) lung bronchoalveolar lavage fluid or blood serum lipids as well as proteins oxidation as determined in vitro by TBARS-test, lipid hydroperoxide-test and SH-group consumption, respectively.

In contrast, iron containing nanosized particles (used as a positive control) at concentration 10; 25 and 100 mg/L causes an statistically significant increase in lipid and protein oxidation as determined in vitro by TBARS-test, lipid hydroperoxide-test and SH-group consumption, respectively.

Concerning the (3) **samples delivered as dry powders**, after the extraction of the nanozised fraction out, the presence of NPs in the supernatants in several samples was confirmed by TEM. However, this was not confirmed in all of them and the distribution patterns were not uniform (big agglomerates).

Dosimetry should always report mass concentration but the aforementioned protocol does not allow quantifying the quantity of nanosized particles assays are being performed with. For this reason, results obtained –even if positive effects are reported- should be considered cautiously.

According to results obtained it can be summarised:

1. PA nanocomposites containing nanosilica gel (LO_SiO2_PA) generated by drilling and collecting as a dry dust effecting blood serum lipids oxidation *in vitro*.
2. PP nanocomposites containing glass fibers (LO_GNF_PP), silica gel (LO_SiO2_PP), polymers alone (LO_PP) generated by drilling and collected as a dry dust is effecting blood serum nonenzymatic antioxidant – glutathione *in vitro*.
3. No effect was observed for the rest of samples.

The second group of investigations under WP4 were devoted to investigate **toxic effect of NPs on potential cellular targets**. Within this task toxic effects of different NPs have been investigated: Silica and MMT NPs, Dust NPs From Crash and Drilling Tests, and Dust Suspension Produced By Mechanical Abrasion And Aging Procedure.

This work developed can be divided into three different research lines:

1. The study of different cytotoxicity endpoints including cell viability, membrane damage, oxidative stress, and inflammatory effect induced by dust NPs and raw silica NPs *in vitro.*
2. Cytotoxicity evaluation of the selected NPs (raw NPs, samples from mechanical processing and samples from accelerated aging) by American Society for Testing and Materials (ASTM) ASTM E2526-08, including MTT ((3-4,5-Dimethylthiazol-2yetl)-2,5-diphenyltetrazolium bromide)) and Lactate Dehydrogenase (LDH) assays in two cell lines.
3. Study of cellular effect of NP by toxicogenomics.

Regarding the assessment of **silica and MMT NPs** it is worth to highlight that in lung cells (A549), skin keratocytes (HaCAT) and foetalfibroblast (MRC5) cells, all the silica NPs tested at 10-100 µg/ml induced time dependent loss of cell viability as assessed by MTT test, and early increase in intracellular Reactive Oxygen Species (ROS) level and membrane damage. The effects appeared not to be concentration dependent and can be detected for the lowest concentration applied. The bioluminiscense (ATP) and biodreduction capacity (XTT) assays as well as transcriptomic studies also show that silica NPs are toxic in A549 cells in a dose-dependent manner, starting from the concentration 7.5 µg/ml. DLVF, Aerosil 200 and dimethylbenzyl hydrogenated tallow ammonium chloride DMBHT are all cytotoxic in a dose-dependent manner. On the contrary, toxicity of Dellite 43B (D43B – a commercial nanoclay deriving from MMT) and Dellite 72T (D72T - a commercial nanoclay deriving from MMT) is not dose –dependent attesting a different mode of action. In the case of MMT and compared to SiO_2 NPs, CI50 (determined by ATP tests) was the following D43B®: 15 µg/ml < Aerosil 200® (a commercial nanosilica): 60 µg/ml < D72T®:100 µg/ml > DLVF®:500 µg/ml. The toxicity of Dellite® mainly depends on the nature of the cationic organic molecule intercalated in the interlayers of montmorillonite. Particularly CI50 of DMBHT (embedded in MMT 43B) is 2.5 µg/ml.

Transcriptomic study allowed to obtain information about modes of action of these compounds. Study of mode of action showed that **silica NPs enter cells through an endocytosis process** clathrin-dependent and induce overexpression of many proinflammatory interleukins. On the other hand, MMT and DMBHT probably activate and saturate some external receptors resulting in disturbance of G-protein Coupled Receptor signaling Pathway and they induce a more massive inflammation.

The **toxicity of raw NPs** was also assessed in HepG2 and LLCPK1 cell lines. It seems that LLCPK1 cell line is more sensitive to these NPs. In addition, Dellite samples trigger higher level of toxic effects than Aerosil samples at concentrations up to 100ug/ml. Dellite 43B is the sample which triggered more toxic effects.

With regards to the assessment of **dust NPs from crashing and drilling tests**, it is worth mentioning that the dust NPs released from different polymer-composites and after crash and drilling tests exhibit no difference in terms of cytotoxicity as compared with the NPs released from their respective neat polymers in the chosen *in vitro* models. At 25-100 µg/ml, the PA based dust NPs showed toxic potency as determined by MTT assay in A549 cells. In comparison, the PU and PP based NPs showed no effect.

Concerning the assessment of **dust suspension produced by mechanical abrasion and aging procedure**, PU suspension incorporating NPs or not did not exhibit any toxic effects while PP-MMT and PA incorporating NPs revealed some cytotoxic effect. In the case of PP-MMT the release of ammonium used as spacer in the montmorillonite is like to be at the origin of the toxicity.

The analysis of **transcriptome of PA nanocomposites** showed that:

- PA/SiO_2 does not induce any inflammation response contrary to Aerosil 200 Silica NPs, according to their respective expression levels of interleukins. Moreover polymer's uptake is based on macropinocytosis when Aerosil 200 Silica NPs penetrate through clathrin-dependant endocytosis.
- PA/MMT nanocomposite inherits from part of D43B 's toxicity. The high toxicity of nanoclay D43B comes from the cocktail of toxicities of its linker DMBHT and its inorganic structure DLVF, but with a cumulative and synergistic effect, likely due to the stiffness brought by the presence of the linker. This organoclay triggers a drastic response of signal transduction, via interconnected signaling pathways and activates the molecular mechanisms of cancer, especially DNA damage through BRCA1 pathway. The nanocomposite PA/MMT displays the same toxicity through the activation of the same pathways.
- The organo clay D72T seems also to induce a very drastic cellular response, likely for the same reason. Therefore, it would make sense to analyze the transcriptome of cells exposed to PP/MMT (D72T). However this way was not selected because the linker DMDHT displayed less toxicity than DMBHT, and hypothesizing that the final toxicity would be due to the release of the sole linker from the nanocomposite. However our last results let us think that toxicity is due to a release of the organoclay.

- Another question would be to find the minimal concentration of PA/MMT (UV) which does not induce any transcriptomic toxicity, since with tested concentrations (20 µg/mL to 0.075 µg/ mL), toxicity was not dose-dependant.

The study of the evolution of the surface properties of NPs and their relation with the **cell absorption and cytotoxicity potential** was also carried out within WP4. The work accomplished within this activity included the following analysis:

- Chemical composites of silica NPs in water and in culture medium were obtained by Infrared (IR) assay
- Analysis of the effects of storage temperature and duration on the chemistry of silica NPs Absorption of the selected NPs by the cell
- Evolution of surface properties of NPs during biological tests

In order to explore the relationship of **physiochemical characteristics of silica NPs with their toxicity potential**, the chemistry of silica NPs were analysed by IR. Storage conditions were also investigated for possible effect of storage on silica NPs chemistry. It was demonstrated that both Aerosil 200 and Aerosil 974 shared same IR spectra when dispersed in water stored either at 4oC or at RT, suggesting that silica NPs is stable under the two storage temperatures for 1 week. The IR spectra of the two silica NPs in culture medium were slightly different from that in water. The peak at ~1410cm-1, corresponding to the C-H bond, appeared to overlap with the peak 1384 cm-1, a hydrogen bond, which was present in the IR spectra of silica NP dispersed in culture medium but not in water. This change could be due to the adsorption of protein on the surface of silica. It appears that the hydrophobic Aerosil 974 was more reactive with proteins than the hydrophilic silica NP Aerosil 200, as indicated by the peak at ~1410-1384 cm-1 being more prevalent in the IR spectra of Aerosil 974 sample, which could imply the difference in their reactivity with cells.

During the analysis of the reactivity of **raw NPs** within nutritive media, it was detected that their structure is strongly affected in nutritive media. Indeed, it was proved that the cationic ammonium organic compound inserted in the interlayer space of montmorillonite was partly released. This structural information combined with results obtained in WP3 strongly suggest that the **toxicity of MMT is not related to the size of NP, but much more due to the chemical modification** of the clay. For all raw NPs, strong aggregation occurred in nutritive media and then during biological tests.

An internalization of the three studied NPs by the HepG2 cells was observed. Nanosilica and nanoclays were mainly distributed inside the cytoplasm and on the cell membrane, but no NPs were observed in the cell nuclei.

These results for silica NPs coincide with the ones reported by Ling Hu et al relating their work on the cellular endocytosis and exocytosis of silica NPs of diverse sizes in the same cell line. Although it is known that there are several possible uptake pathways for the NPs internalization (endocytosis -fluid-phase endocytosis, adsorptive endocytosis and receptor- mediated endocytosis- phagocytosis and micropinocytosis) according to Ling Hu et al, the mechanism of clathrin mediated endocytosis can be applied universally to the silica NPs with the diameter ranging from 60nm to 600nm.

Nevertheless, bigger aggregates of nanosilica have been found in the development of the project (bigger even for nanoclays).

Nowadays, the maximum size for internalization of NPs is still in debate. Although some authors indicate a maximum size for internalization by non-phagocyte cells (e.g 1 um latex beads are not taken by mouse melanoma), gold nanowires that have a length of several micrometers and a 200 nm diameter have been observed inside fibroblasts and HeLa cells .Moreover, some authors have reported that some NPs aggregate during the uptake process, forming clusters inside the cells and it could also happen here.

The impact of these NPs was assayed in terms of cell cytotoxicity in task 4.3 by means of MTT and LDH release assay, revealing that Aerosil 200 and Dellite 72T had little influence on cytotoxicity of these cells even at the highest evaluated concentration. These results suggest that uptake of these two silica particles brings rather low toxicity to HepG2 cells. Nevertheless, Dellite 43B triggered a higher toxic response to this cell line.

The possible exocytosis of the NPs from the cells was beyond the scope of this task, but, it seems that it probably happened. The NPs can be degraded by the action of enzymes or can be packaged in vesicles inside the cells, fuse with the plasma membrane and finally can go out from the cells. This effect was observed by Ling Hu et al for silica NPs who described a prominent clearance of the particles during the first six hours, revealing that smaller particles are more easily cleaned. In the assays developed in this task, exposure time was extended up to 24 hours and, so, it is probable that initially uptaken NPs were gone out at the end of the assay.

This can explain the fact that during the project execution, although a washing procedure to remove non introduced (or non strongly attached to the membrane) NPs was set up, silica NPs (Aerosil 200) were observed in the medium after the 24 hours exposition.

Finally, the **study of genotoxical effects of selected NPs** was developed. The determination of the Genotoxic potential of NP and NMs is of high importance due to the fact that in most cases DNA damages are strongly involved in many human diseases including cancer. Three genotoxic tests were performed: (i) the Ames Test was used to detect gene mutations, (ii) the Comet Assay to detect primary DNA damage, and the (iii) Micronucleus Assay was used to reveal chromosome mutations.

Experiments were performed on **raw NMs** (aerosils ®, delites®), on dust suspension produced by **mechanical processing** (crash tests or drilling) and on dust produced by **mechanical abrasion**, sieved and submitted to aging experiments.

In the case of **dust produced by mechanical processing** the determination of the initial concentration was extremely challenging. Different techniques (destructive or not) were implemented to determine the concentration of particles within the suspensions, finding Huge discrepancies between techniques. Considering these difficulties, the preparation of the set of samples provided after abrasion and aging experiments was modified. The initial quantity of dust was weighted in dry state. Higher quantities were produced to increase the mass and then increase the accuracy on the mass measurements.

In terms of methodology NEPHH results tend to suggest that the use of the AMES test can be questionable in the case of NMs. Indeed and even if this test has recently been selected as one of the two assays recommended by the UK Expert Advisory Committee on Mutagenicity; most papers published recently challenged the fact that the bacterial cell uptake might be much smaller than for eukaryotic cells. In the case of the NEPHH project's samples, only 3 samples out 26 tested exhibit a positive mutagenic effect (raw NM Aerosil 200®, PA/SiO2 after mechanical abrasion and aging). This result is not in perfect agreement with genotoxic results based on eycaryotic cells (comet and micronucleus assay).

Based on Comet Assay (primary DNA lesion) and micronucleus (chromosome damages) assays the highest genotoxic potential was found for D43B®. String DNA primary lesion appeared at concentration higher than 2.5 mg/l and chromosome damages appeared at concentrations higher than 1.25 mg/l. This result is perfect agreement with all cytotoxic results . From all NEPHH results the toxicity seems to be more likely related to the presence of DMBHT (dimethylbenzyl hydrogenated tallow ammonium chloride) incorporated in the interlayer of the clays. It has been clearly showed that D43B® and D72T ® are modified while introduced in nutritive media.

D72T® is also genotoxic but to a lower extend compared to D43B®. Then aerosil were showed to exhibit effect on chromosomes at concentrations higher than 25 mg/l.

In the case of **dust from mechanical processing** (crash test and drilling chambers) **no genotoxic potential was found**. However, since a strong issue concerning accurate concentration of provided suspensions still remains, it is most probable that the tested concentration were lower than supposed. This could explain the low genotoxicity.

Dust produced by mechanical abrasion and submitted to aging experiments were tested genotoxic based Comet ad micronucleus assays. Dust from PA/MMT is by far the most genotoxic NMs. Significant primary DNA lesions can be observed for concentrations ≥ 6,25 mg/l. Except PP (DNA lesions occurred at concentrations ≥3.125 mg/l) all other PA and PP dust tested did not exhibit DNA lesion after 2 hours of exposure. For longer exposure time (24h) chromosomes damages can be observed for all PA and PP samples at concentrations ≥ 3.125 mg/l. However the most genotoxic potential is observed for PA/MMT altered dust.

3.5 WP5 – Environmental Implications of NMs

The main objective WP5, running in parallel with WP4, was the **assessment of the environmental implications of NMs compared to conventional materials**. This analysis also intends to provide a baseline Life Cycle Assessment -LCA- of the alternative NMs. This evaluation involved the study of the persistence, bioaccumulation, toxicity and ecotoxicity of selected NMs and, the analysis of their risks and hazards in different abiotic media.

The first activity developed within WP5 was focused on the study of the bioaccumulation **and biodegradation of NMs**. In this sense, the **effect of nano by-products on bacteria** was assessed not only at the individual level but also on bacterial biofilms and on the plants. Most of the NMs tested already contained bacteria However, these contaminants did not impair the experiments performed with *E.coli*, whose results are shown in the following points:

1. The harmfulness of D43B and Aerosil 200 depends on the nature of the polymer in which they are added to, and likely the stability of the polymer, and the accessibility of the nanocomposites at the surface of the NMs.
2. Dark-aged PA is safe to E. coli. Addition of D43B and Aerosil 200 to PA increases its toxicity. UV and UVA rays adversely modulate the toxicity of PA-based NMs. As nanocomposites are available at the surface of the NMs, releases of nanocomposites or compounds from the polymer could account for the toxicity.

3. Dark-aged PP alone is toxic. Addition of Aerosil 974 and D72T to PP decreases the toxicity to E. coli. As the nanocomposites are accessible at the surface of NMs, they could participate in the reduction of PP toxicity. PP seems to contain some compounds with photocatalytic sensivity, since aging of PP-based NMs under UV or incubation under UVA decreases the toxicity.
4. Finally PU and PU-containing D43B and Aerosil 200 are nontoxic to *E. coli*, which is consistent with the fact that NPs are not released and not accessible.

For most samples, whether incubations with cells occurred under UVA or in the dark, the **toxicity of PP- and PA-based materials occurs from the size fraction above 0,05 µm**. This result means that toxicity is not related to molecules (such as Si or ammonium ions) released from nanomaterial aging. PP contains chemical compounds in the fraction <0,05µm whose toxicity is disabled by UV and UVA or MMT additive. In PA/SiO_2, UVA also alleviate the toxicity of some chemicals in the fraction <0,05µm.

Effect of nano by-products on bacteria was assessed not only at the individual level but also on bacterial biofilms.In this sense, the susceptibility of biofilms to NMs was tested using a modified procedure of high-throughput MBEC™ assay. The results showed that UV and dark aged PA/MMT significantly destabilized E. coli biofilms after 4h incubation. This effect was enhanced after 24h for UV aged PA/MMT that showed toxic to planktonic cells shed from the biofilm. This result was consistent with the toxicity of UV aged PA/MMT found for planktonic cells. Interestingly, dark aged PA-SiO2 increased the amount of biofilm formed on the peglids but does not alter planktonic cells growth.

PP based NMs slightly inhibited biofilm formation. Except UV and dark aged PP/MMT, all NMs boosted the growth of planktonic cells after 24h. PP based NMs improved bacterial growth, but did not favor biofilm formation, which does not mean that bacteria would not form biofilms on surfaces covered with such NMs.

The next activity developed within WP5 was focused on the **ecotoxicological assessment of the samples** produced in the framework of the project: raw NPs, samples from mechanical processing and samples from accelerated aging.

In order to evaluate the ecotoxicity at the individual level *Daphnia magna* immobilisitaion test was carried out. These organisms are used as bioindicators because they filter large quantities of water, and therefore, they have a great potential to be affected by the ingestion of pollutants.

It was observed that from all the raw analysed materials, only MMT D43B elicit a positive response at the analysed concentration. For the other raw materials, ecotoxicity was not detected up to 200mg/l. Although many positive results were observed for samples from mechanical processing, it was suggested that it could be due to the sticking of the samples to the outer skeleton of Daphnia that drecreases their swimming capability.

Besides the *E.Coli* and *Daphia magna* assays, the effects of NMsby-product on plants were also assessed. The test was based on an OECD TG 227 Terrestrial Plant Test: Vegetative Vigour Test. Sterilized seeds of *Arabidopsis thaliana* (ecotype Columbia) were sown on Hoagland/2 medium solidified with Phytagel (7% w/v). Axenic plants were grown in a phytotron for 17 days in controlled conditions, 22 °C with 8 h of light (120 mmol.m-2.s-1) and 19 °C for 16 h light-off. 5 µl of NMs suspensions (100 mg/l) was deposited on leaves (at least 3 to 5 leaves by shoot, three to four plantlets). As control, drinking water and salicylic acid 5mM were used. The plants were incubated for 48h and the symptoms (chlorosis, necrosis, wilting…) were annotated. As expected, salicylic acid produced necrosis of leaves. No symptoms of wilting or necrosis of leaves was observed for NMS. NMs display no impact on plant leaves, regarding stress symptoms such as chlorosis, wilting and necrosis.

The **environmental performance of NMs** was assessed by the **LCA methodology.** The development of an LCA is necessarily related to a product or system with a known application and use: similar applications with different manufacturing processes and/ or compositions are compared in order to evaluate which of them posses a lower risk towards the environment.

Within NEPHH Project, laboratory nanoreinforced composites and their non-reinforced counterparts were manufactured, in principle, at least for lightweight structures. Preliminary findings in the frame of present task revealed that the functionality and possible applications of NEPHH non reinforced composites (PU, PA-6, PP) versus the nanoreinforced alternatives was not comparable because the application was not the same.

In order to evaluate the most suitable option, and considering that MMT was being incorporated into wire coatings for an increased resistance to fire, a comparative study was carried out to compare **nanoclay-reinforced polymer wire coatings** with conventional ones. While the conventional wire coatings contain standard halogen free retardants, in reinforced coatings MMT is incorporated into electric cable linings as a rheological agent for an increased resistance to fire. In addition, a reduced load of standard halogen free retardants is obtained. The synergistic effect of the montmorillonite on traditional flame retardant additives (by the formation of a three-dimensional char network) can lead to a revolution in wire production. The application of nanoclays contributes also to anti-dripping effect and flexibility increase. Some producers have already started commercializing wire with nanotechnology-based coating; in the short term the use of nanoclay in wire coating production will probably reach a significant market share replacing traditional formulations.

Two scenarios were considered in order to make the environmental comparison:

- *Conventional composite for wire coating*, as baseline without addition of nanocompounds (mineral flame retardants like ATH or MDH in a polymer matrix)
- *Nanoreinforced composite for wire coating* (the nanoclay-reinforced wire coating, where the montmorillonite replaces a low percentage of the mineral flame retardant).

Following, a life cycle inventory data was developed for the different stages involved; (1) Raw materials obtention, (2) Materials processing, (3) Product Manufacturing and (4) End of Life stage. To conduct the LCA, the boundaries of the study were established, including the following unit processes:

- Nanoclay Production
- Thermoplastic Material and Mineral Flame Retardants Production
- Cable Coating Manufacturing by Extrusion
- Different End of Life scenarios (Recycling, Incineration and Landfill Disposal)

The method selected for carrying out the impact assessment was Ecoindicator 99 (EI 99H/H); standard Eco-indicators are numbers that express the total potential environmental load of a product or process in dimensionless figure, i.e, Ecopoints (Pt.) The total environmental loads obtained were:

- EVA/ATH 0,3426 Pt.
- EVA/ATH/MMT 0,3428 Pt.

- EVA/MDH 0,3340 Pt.
- EVA/MDH/MMT 0,3349 Pt.

As a conclusion, it was observed that, whereas nanoreinforced composites showed and increased fire retardance (tested by the standard protocol UL94), the addition of NMs seems to have no significant relevance in the environmental assessment.

However, the lack of nano-specific characterization factors for NMs and emission rates associated to the different life cycle stages –mainly in the extrusion and end of life phases, where accidental combustions can take place- still remains a challenge for realistic life cycle assessment modelling.

Furthermore, it is worth highlighting that the "Damage to Resources" category is the main affected in all studied materials and specifically "fossil fuels" category presents the higher contribution to the total environmental burden score (50% - 58%). The total or partial replacement of the fossil fuel use in the whole life-cycle of the product will reduce considerably the potential environmental impact.

In order to support and adapt inventory data related to the end of life stage (it was assumed that the 12% of the final product was disposed to landfill, and the 88% was sent to an energy recovery plant), some experimental test were accomplished. Leaching tests were carried out to support the inventory data, but also to select the landfill category in which the wire coatings should be disposed at the end of their lifetime. These leaching tests results allowed to conclude that every studied materials could be accepted in municipal landfill and the presence of those materials as waste did not significantly changed the environment in a controlled landfill. The results showed that the product systems which had more aluminium in the leachated were EVA/ATH and EVA/MDH/MMT, with 0,32 mg/l and 0,11 mg/l respectively. Regarding the magnesium content, as it was expected, EVA/MDH and EVA/MDH/MMT systems had higher values (1,57 mg/l and 1,03 mg/l, respectively). It is worth to highlight that silicon content was, in all cases, lower than 0,05mg/l. Finally, EVA/ATH product system was the one with the highest Dissolved Organic Carbon (DOC) value (2,2 mg/l).

In order to support the data inventory related to incineration stage, some combustion experiments were carried out. The results showed that improved flame retardancy was observed for nanocomposite (EVA/ATH/MMT) compared to composite (EVA/ATH), mainly thanks to the formation of a reinforced protective layer (char) due to the incorporation of MMT. This effect was validated from:

- **the mass balance**: for the nanocomposite EVA/ATH/MMT, the residual mass was significantly greater (2,001 g) than for the composite EVA/ATH (0,997 g). This suggests the presence of unburnt or partially burnt polymer in the residue that means that material has failed to totally react with the available oxygen.
- **gas emission results**:. the decrease in CO_2 yield (from 99 mg/g of EVA/ATH sample to 49 mg/g of EVA/ATH/MMT sample) was accompanied by an increase in CO yield (from 18 mg/g of EVA/ATH sample to 27 mg/g of EVA/ATH/MMT sample).

But this improvement was not observed for the consumer products. However, it should be underlined that consumer products have shown a significant reduction in rate of thermal decomposition (from mass balance, and residual C contents in ashes) compared to materials in pre-market stage.

After the evaluation of the environmental performance of NMs, the next step pursues to contribute to an effective **environmental decision making according to Ecological Risk Assessment (EcoRA).**

Within this task three different studies have been performed with the objective of using and assessing different EcoRA approaches:

- EcoRA of traditional and nanoclay-reinforced polymer wire coatings focused on, different end of life (EOL) scenarios, by means of matrix model
- Risk and hazard analysis of FGC in different abiotic media, by means of tree of failures technique
- Stage-by-stage ecological exposure and toxicity assessment of nanosilica and MMT incorporated within polymers, by means of matrix model

Firstly, a **qualitative ecological risk assessment of wire coating** (with and without nanoclays) focused on the EOL scenarios (recycling, energy recovery and landfill) was carried out. In accordance with NEPHH project's aim, traditional wire coatings were compared with nanoenabled coatings, which contain nanoclays (MMT). Although a qualitative approach (based on the assessment of the likelihood and the consequences) was selected, some incineration and leaching tests were carried out to provide with a quantitative basis for the assessment. This approach was selected to carry on with the strategy defined within the task of LCA which dealt with the environmental impacts related to the whole life cycle of different cable coatings.

According to the results obtained, the **most risky situations** are related to accidental conditions: (i) a failure in the extruder in the recycling process, (ii) a bug of the purification system in the incineration with energy recovery and (iii) an accident caused by human error in the landfill disposal.

Since the consequences and behavior of nanowaste are still not fully known, and in order to work under a conservative perspective, the consequence category selected for nanowaste was one level higher than for conventional waste. Therefore, the risk level of nanowaste in all scenarios (recycling, energy recovery and landfill) is equal or higher than the risk level of the waste free of NMs, but never lower.

Secondly, a Risk and Hazard Analysis of the FGC production technology based on quenched cullet was carried out. To evaluate the possibility of failures at FGC production a logic-graphical technique called 'tree of failures' was used. In analyzing the 'tree of failures' such combinations of the equipment failures as external exposures were used, which result in emergency situations.

Depending on the type of the final product (granules, crushed stone, slabs) different flow sheets, equipment and process conditions are used. It was stated that both parametric and functional failures were possible. From the 'tree of failures' analysis at technological faults when producing granulated FGC, it was stated that the main cause of these faults were parametric failures; a parametric failure is possible in case of the disturbance of optimum heat curing of the mixture. In producing FGC using a 'continuous belt', the main cause of technological faults were functional failures; a functional failure can occur at the blending system breakdown.

As a whole, the 'tree of failure' analysis showed a high manufacturability and ecological safety of FGC production. The technology developed can be recommended as safe for a large-scale industrial introduction. Research developments related to this technology provide both the safety methods and high-quality products.

Lastly, based on results of the previous WPs a simplified life cycle of polymers was designed to compare stage-by-stage exposure and toxicity of nanosilica and MMT incorporated within polymers.

From the estimations nanosilica incorporated into polymer seemed to provide less risk than modified MMT. In the case of nanosilica/polymer material use stage exhibits the highest risk, as a result ofthe combination of medium exposure and medium genotoxicity levels.

In the case of polymers incorporating modified MMT risk scores were higher. The higher score compared to nanosilica/polymer was related to a higher danger. Indeed it was clear that the molecules used to modify MMT (ammonium molecules) were responsible for the high cyto an genotoxicity values. In this case, at least three life cycle stages obtained high risk score: organic modification of MMT, production of polymer composite and use of polymers.

The results showed that the use of ammonium molecules as MMT modifier induces an increase of danger and risk compared to non-modified MMT, as well as compared to nanosilica. Therefore, it is important to design new modified clays by using other non-toxic positively charged molecules or organo-metallic compounds.

It can be concluded that the qualitative EcoRA methodologies used (tree of failure and likelihood and consequences matrices) are fully applicable to NMs. However the lack of information related to the exposure, ecotoxicity and behavior of NMs entails several difficulties and shortcomings, such as the uncertainty related to the assumptions and estimations needed. In addition, there is still a long way to go to achieve all the information required to accurately characterize the exposure and effects of NMs, and specially to accomplish a complete quantitative EcoRA (through the calculation of the PEC, Predicted Environmental Concentration and PNEC, Predicted No Effect Concentration).

3.6 WP6 – Guides for Minimisation of Health and Environmental Impacts of NMs

NEPHH's WP6 aimed at developing guidelines for the assessment of the environmental and health implications of NMs in order to define the appropriate measures and minimize the exposure of workers and environment to NMs. For this purpose, two Guides have been elaborated, one tittled "Guide of actuation for people working on nanomaterials" focused on the most appropriate procedures to reduce the potential points of exposure to NMs of workers in their workplaces, and other tittled "Guidelines for responsible management of waste nanomaterials" aimed at establishing the standards and procedures for the safety and responsible managent of nanowaste.

1- The main objective of the "Guide of actuation for people working on nanomaterials" is to provide an overview of the potential hazards that the use of NMs in manufacturing and processing activities can produce in workers, as well as a compilation of measures and practices that should be taken to minimise workplace exposure and associated risks. The Guide is expected to serve as a reference to raise awareness of the Occupational Safety and Health practices in laboratories where engineered NMs are produced or processed, what will improve the safety in the workplace of the workers and researchers.

Since there is presently no enough quantitative data for the development of a Quantitative Risk Assessment, an integrated Qualitative Risk Assessment has been accomplished using the Control banding approach based on the combination of toxicity and exposure. In spite of this, and with the aim of providing the Guide with actualized data, results obtained within the WP4 related to health implications of the selected engineered NMs (toxic effects on potential target cells, genotoxicological effects and adverse health effects generated by the by-products in several life cycle stages) have been employed as an added value for the Guide.

The Guide shows special attention to **control measures**, as in the absence of adequate toxicity and behaviour knowledge, and the absence of specific standards, strict control measures are fundamental to minimise as possible the risk to workers. In this regards, the three main risk control categories have been fully analysed and described: (i) Engineering Techniques (design, elimination/substitution, isolation/confinement, ventilation, etc.), (ii) Administrative measures (information/training, work procedures, cleaning and equipment, personal hygiene, work periods, etc.) and (iii) Personal Protective Equipment (respiratory, skin and eye protection). Although these three risk control categories are arranged by decreasing effectiveness degree, the reality is that their implementation in the workplaces is just the opposite. Engineering techniques are normally more effective than administrative measures and PPE, because they are independent of the worker´s behavior and prevent the possibility of contact between the pollutant and the worker. Administrative measures must complement engineering techniques when they are not achievable or cannot completely control the risk factors, but must never substitute engineering techniques. Finally, PPE should only be used as a last resort when the engineering techniques and administrative measures are unsatisfactory to protect the workers. However, as it was mentioned before, the common practice in most of the laboratories is just the contrary, being the use of PPE the control measures more extended in most of the workplaces due to diverse reasons such as lower costs, simple and easy to use, etc.

In addition, the Guide also include a complete **Emergency Plan** to handle sudden unexpected situations, a guideline to reduce fire and explosion risk, an Occupational Health Surveillance with the aim to protect the health of employees by early detection of adverse changes or disease, and several practical guidelines necessary to handle in the workplace in order to offer laboratory researchers complete information on the necessary safe work practices to be followed when working with engineered NMs in research facilities.

After the completion of the Guide elaboration, two major necessities have been identified: on the one hand, the urgent need of further information concerning the exposure potential and adverse effects of NMs to humans and environment, in order to be able to develop a Quantitative Risk Assessment, and on the other hand, the indispensable demand of a specific regulatory context to standardize all the information gathered worldwide. It must be considered that there is still a long way to go to achieving these two necessities, so the encouragement of all market players to collaborate is essential in this point.

2- Regarding to the second Guide, tittled “Guidelines for responsible management of waste NMs”, the main goal of this publication is to outline guidelines for **safety and responsible final treatment and disposal procedures for the management of NMs**. The target audience of this guide are industrial stakeholders, primarily those linked to plastic sector, as well as regulators and public administrations.

In particular, the guide has been focused on nanocomposites, as they are the NMs in which NEPHH Project is based. The relevance of NEPHH’s contribution to the present guidelines is that the envisaged approach goes one step beyond the waste management protocols for free NMs (“free” stands for not embedded in a given matrix, polymers in the particular case of NEPHH) but also addresses the specific waste management needs of final products incorporating nanotechnology.

The guide includes a detailed description of the nanocomposites of interest and their main applications. The regulatory framework has been analyzed, and traditional waste management systems have been thoroughly studied. Furthermore, risks related to introducing waste streams containing NMs into traditional waste management systems have been assessed. Finally, current nanowaste management procedures, as well as nanowaste classification initiatives, have been identified, and several potential improvements have been proposed.

The lack of information is surely the most significant conclusion. In this sense, it is noteworthy the limited knowledge regarding the efficiency of the current waste management systems to remove the nanowaste. Since nanotechnology incorporating products are widely spread in the global market, more efforts should be focused on assessing the suitability of waste management systems for nanowaste treatment and also on the declaration of use and incorporation of nano. Risk reduction and mitigation in industrial environments should be handled with a three pronged approach: identification of the hazard, knowledge of the potential adverse effects, measurement and control of the exposure.

Furthermore, there is a legal vacuum or loophole regarding how nanowaste must be managed and treated. Although most paper and guides published up to date recommend disposing the nanowaste as hazardous waste, policy maker should face up to this issue and establish some compulsory measures. Moreover, since not all the nanowaste may have the same toxicological and exposure potential a standard nanowaste classification should be defined. British Standard Institute published a nanowaste definition that includes pure NMs as well as solid matrices with NMs that are friable or have a nanostructure loosely attached to the surface. The performance of the pure NMs will probably differ considerably from the performance of the solid matrices (such as polymeric matrices) with NMs. Regulation, therefore, should be based not only in the toxicological characteristics of the nanowaste, but also in its exposure potential. In this regard, the nanowaste classification proposed by Ndeke Musee, and described in the section “Other initiatives for nanowaste classification” of this guide, could be an useful starting point.

A future legal framework should also deal with the need of identifying nanotechnology incorporating in order to select the most suitable end of life scenario and treatment for each nanoproduct after its lifetime. Also quantitative life cycle analysis of NMs toxicity should be done. All the information should be transferred into standars and guidelines, harmonized on a European and international level in different areas such as exposure measurement or toxicological testing. This may therefore need the identification of nanoproduct to be made compulsory for the producer.

4 Potential Impact and main Dissemination and Exploitation Activities

4.1 Potential Impact of the Project

Nanotechnology is considered as the most promising approach towards moving forward the technological sciences beyond the limits that are currently restricting further progress due to inherent structural supramolecular material constraints. The experience gained during the development of NEPHH Project results in the possibility of establishment of understanding, manipulation and uses the structure/functions of (macro) molecules on the nanometre scale to help construct the foundations on which on **NMs toxicology and ecotoxicology** will be built.

Furthermore, the obtained results will help developing a **suitable methodology for testing NMs impact on human toxicology and ecotoxicology in a Life Cycle approach**. This fact will have a great impact and added value to the EU and indeed world community, as NEPHH's results can contribute to a better understanding of the mechanism of interaction between the NMs and the molecular, cellular and organ levels. The development of specific *in vitro* assays to predict human responses to NMs exposures can contribute also to the safety assessment of nanotechnology products.

Apart from that, the selected materials, **Silicon based polymer nanocomposites** constitute a new field of research and development in which great hopes have been placed for significant social and environmental benefits linked to a greater economic development. Generated knowledge contributes to better understanding the human health and environmental impacts of the selected NMs. These targeted materials represent an innovative selection supplementing ongoing investigations and setting a basis for future ones.

With regards to the potential impact in the **scientific community**, the project provides with valuable quantitative data on NMs toxicology and ecotoxicology, which could be the basis for meeting regulatory requirements. It is expected that these data provided could not only supplement ongoing investigations, but also set a basis for future ones. In this sense, NEPHH project has entailed a meaningful step forward on the assessment of the health and environmental implications of NMs throughout their life cycle.

Furthermore, all the toxicological and ecotoxicological results obtained should boost and contribute to the definition of a **specific regulatory framework for NMs**. On the one hand, the project has provided evidence enough for the appropriate regulation regarding the prevention of exposure of human to NMs (from manufacturers or handlers, to the general population). On the other hand, the project has also contributed to the standardization and validation of sampling and test methods for NMs. Although this sampling protocol has been developed for the NMs of interest within NEPHH (silicon based nanocomposites), the protocol is fully applicable to NPs embedded in any other solid matrices.

In this sense, the improvements generated by the NEPHH project can be summarized in the following points:

- Better understanding about the **mechanisms of toxicity** of nanosize dust and appropriate understanding of nanotoxicity, in particular for the determination of **acceptable exposure limits**.
- Providing the European Community with evidence for the appropriate regulation regarding the prevention of exposure of the general population and both manufacturers and handlers.

- Generating of knowledge, by producing the information of the behavior of the NPs in biotic media, being those data useful to understand the environmental toxicological outstanding issues, risks and hazards.
- Contributing to the **standardization and validation of test methods for NMs**, by the developing of a document containing the sampling procedures implemented during the course of the project. This document includes the testing strategies envisaged for the effective production of the samples, their maintenance and storage conditions, their identification and labeling.
- Contributing to other RTD projects. In fact, during the project, the consortium has collaborated with other projects through Nanosafety Cluster and NANOLCA dissemination activity, making a clustering effort to disseminate information about the work accomplished and the results obtained.

Regarding the **social impacts**, NEPHH Project has contributed to the evaluation of protection methods for occupational exposure and the establishment of procedures to improve them, resulting in an important improvement of working conditions. The development of a Guide for the minimisation of health impacts of ENs for exposed workers is expected to have a significant effect in the exposure rate of workers, thus minimizing the potential negative effects of such exposure and health risks.

As mentioned above, the project has produced a guide which is intended to provide an overview of the potential hazards of NMs for workers, mainly for nanocomposites manufacturing and processing. The guide includes the measures that can be taken to minimise workplace exposure and associated risks, and intends to provide the guidelines for a safe and cost-effective minimization of exposure of workers and general public. The development of this guide, together with the Guide for the minimization of environmental impacts of engineered NMs will promote an **integrated and reliable approach for the assessment of safety and environmental impact of nanotechnology based products**.

This fact is remarkable, since it will have a significant effect in the exposure rate of workers, minimisating the potential negative effects of such exposure and health risks.

In addition, the toxicological/ecotoxicological results, together with the guide should lead to a relevant improvement of the working conditions, and therefore, to the minimization of the potential negative effects of such exposure and health risks. Within NEPHH a safety protocol, which includes the assessment of the protection methods for occupational protection and the establishment of the procedures to improve them, has been developed.

Concerning the **potential impacts in the environment**, NEPHH Project contributes to the sustainable development, as the project has carried out an adaptation of a LCA for NMs in order to promote the responsible management of their impacts. By the integration of the information collected during the project execution in a LCA approach, a sustainable and responsible production of Si based NMs will be promoted, as it will allow designing improved N&N based products thus minimising environmental impacts in manufacturing stages. Doing complete LCA on newly developed products, and considering all the ecological as well as the socio-economic components, will help to ensure growth and the competiviveness of the nanotechnology sector.

Other environmental impacts can be summarized as follows:

- Assessment of the potential of NMs to damage the environment, or human health through the environment: the eco-toxicological profile of by-products (dust produced) released from commercial nanoproducts and nanocomposites during their life cycle has been addressed during the project execution. Effects on bacteria, invertebrates (Daphnia) and plants have been studied.

- **Sustainable use of NMs** as the accidental releases can be analysed minimising the pollution focuses at all stages of the Life Cycle, thus it will contribute to the decrease of the risk of environmental accidents.
- **Better Waste Management** through the evaluation of recovery, reuse and recycling alternatives based on the evaluation of current disposal procedures and the development of guidelines for the responsible management of nanowaste. This fact will also impact in the reduction of the waste production, and therefore in the minimization of the potential damages to biodiversity.
- The results of the project will contribute to boost the environmental applications of NPs, encouraging the "green research", by providing the **eco-toxicological profile of by-products** released by nanoproducts during their Life Cycle.

Results achieved within NEPHH do not only have important social and environmental impacts; in fact, this knowledge should also be used as a **cost saving strategy**. The cost saving strategy emerging from the improvement of the working conditions which entails the minimization of the costs related to the healthcare of the workers and the absence to their work posts and therefore the reduction of the sanitary remediation expenses will also succeed in a bigger scale. The Public Health System will reduce the costs related to the treatment of health issues related to NMs, due to the minimization of the exposure to NMs of both, workers and general public.

Besides, a better understanding of the potential toxicological and ecotoxicological effects of NMs could entail a relevant minimization of the environmental costs or environmental remediation expenses. In this sense, the "Guidelines for responsible management of waste nanomaterials", publication developed within NEPHH, includes the assessment of the suitability of the current waste management system for the treatment of nanowaste, and the proposal of several potential improvements.

Apart from those issues that will impact in the competitiveness of the nanotechnology sector, the project will also contribute to the successful promotion of nanotechnology as it has improved the knowledge regarding the safety of N&N, what has a direct effect on the successful promotion of nanotechnology by gaining the public support when assuring the safety of relevant new NMs.

4.2 Dissemination Activities. Exploitation of Results

The main objective of the dissemination activities has been to involve different target groups and to create the conditions to share knowledge, experiences and working methods and acquire, ensuring the openness towards all stakeholders.

Several dissemination activities have been developed in order to guarantee that the project related information is known by all the interested stakeholders involved in issues related to the **health, safety and environmental impact of NMs,** and in order to facilitate the interactivity between all of them. The target audience of these dissemination activities has been, apart from project consortia, scientific community, Industry, Standardisation bodies, and Regulatory administrations

The main dissemination activities carried out can be summarized as follows:

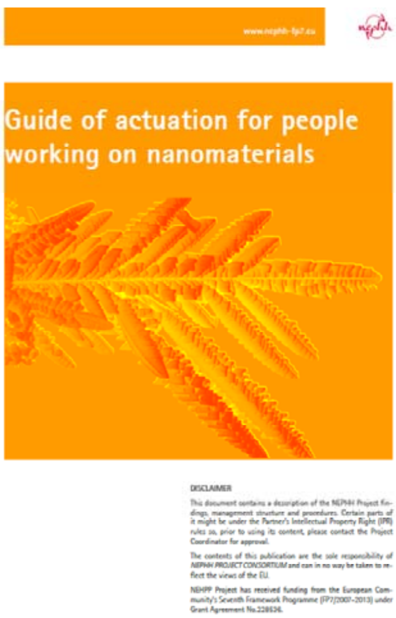
- **Dissemination material**:

Different dissemination materials were designed and widely distributed along the duration of the project. At the beginning of the project, a brochure containing the elementary information of the project was made up.

Another of the first dissemination activities entailed the implementation of the project website (www.nephh-fp7.eu/), which has been used as a powerful dissemination platform. On the one hand, it is worth to highlight that the dissemination bulletins prepared within by the Technological Surveillance System are accessible in project website, making them available to the entire scientific world. On the other hand, 6 newsletters were produced and released, including information related to the project progress, relevant news, links with other RTD projects, events, etc. In order to disseminate these newsletters the identification of target groups was accomplished and a database containing more than 1.200 records was created.

Besides the dissemination activities included other relevant documents, such as *Guide for the minimization of health and environmental impacts of NMs* and G*uide of actuation for people working on NMs* and *Guidelines for responsible management of waste NMs* , which are considered of public interest, and therefore, are freely accessible in project website.

The first guide is expected to serve as a reference to raise awareness of the Occupational Safety and Health practices that will be followed with NMs utilised or generated in laboratories. The content of the guideline is composed of an exposure and effects assessment, as well as, a **prevention programme** containing the necessary control measures and several issues with the aim to offer researchers complete information on the necessary safe work practices to be followed when working with NMs.



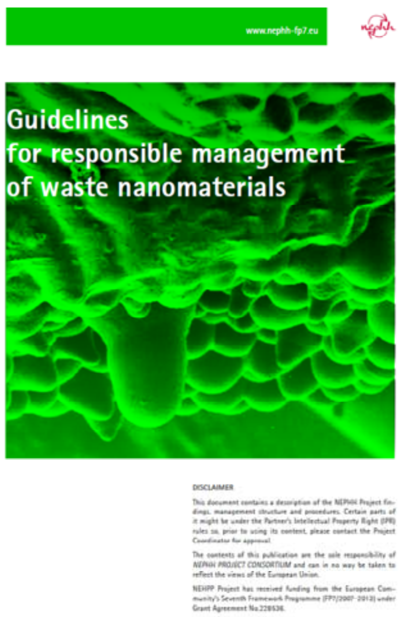


Figure 6- **Guides for the minimization of health and environmental impacts of NMs**

The second guide, related to **nanowaste management**, includes a detailed description of the nanocomposites of interest, as well as their main applications. Besides, the regulatory framework and traditional waste management systems are thoroughly studied. Besides, the risks related to introducing waste streams containing NMs into traditional waste management systems are assessed. Finally, current nanowaste management procedures as well as nanowaste classification initiatives are identified, and several potential improvements are proposed.

- **Dissemination events**:

Besides the dissemination materials, NEPHH consortium has actively taken part in many conferences, symposiums and workshops. In this sense more than 20 posters have been designed and exhibited in different events. These poster dealt with different topics such as: characterization of engineering thermoplastic nanostructures released during mechanical fracture, safety assessment of novel polymer-silicon composites from LCA perspective, toxicological in vitro evaluation of silicon based NMs, structural and thermal properties of PP and PA6 nanocomposites with MMT or nanosilica, LCA of the application of nanoclays in wire coating, etc.

Furthermore, more than 35 presentations have been made in different events such as NanoImpactNET Conference, HiPerNano 2010 Conference, German-Russian Forum “Nanophotonics and NMs”, ImagineNANO, NanoSuatain Training Workshop, etc.

As a result of the participation in some events, such as Nanostruc 2012, several papers have been published and disseminated:

- Giacomelli, M.; Pielichowski, K. and Leszczyńska, A. *Thermoplastic polymer nanocomposites with montmorillonite-Lab vs industrial scale fabrication*. International Conference on Structural Nano Composites (NANOSTRUC 2012)
- Tellaetxe, A.; Blázquez, M.; Arteche, A.; Egizabal, A.; Ermini, V.; Rose, J.; Chaurand, P. and Unzueta, I. *Life cycle assessment of the application of nanoclays in wire coating*. International Conference on Structural Nano Composites (NANOSTRUC 2012)
- Sachse, S.; Silva, F.; Irfan, A.; Zhu, H.; Pielichowski, K.; Leszczynska, A.; Blazquez, M.; Kazmina, O.; Kuzmenko, O. and Njuguna, J. *Physical characteristics of nanoparticles emitted during drilling of silica based polyamide 6 nanocomposites*. International Conference on Structural Nano Composites (NANOSTRUC 2012)

The **First International Workshop**, organized by GZE with the cooperation of the rest of members of the NEPHH Consortium, was subtitled "Industry and nanomaterials: Benefits and risks" and held on November, 18th within the "Nano Future Expo – 3rd International Exhibition on Nanotechnologies", with the aim to promote its visibility and, hence, impact among different industrial stakeholders.

The Event was organized in a double "structure", with a preliminary series of oral presentations held by highly qualified invited speakers, as well as a successive poster session with relevant scientific outcomes presentations and an open networking activity.

Figure 7- **Workshop flyer**

It is worth to point out that the main industrial sectors showing such interest were the plastics and chemical products producers, users and/or developers, companies performing research and development of/on advanced materials and companies from the textile sector.

In addition, coordinators from three different FP7 funded projects (LEITAT, EKOTEK and CIC BioMagune) in the **area of nanosafety**, including NEPHH project, organized the **International Symposium on Safety Issues of Nanomaterials along their Life Cycle** (**NANOLCA**) in 2011 and 2012.

The original idea was born in a series of meetings in the frame of the NanoSafety Cluster and in a workshop in Milano's Fair trade organized by NEPHH, where HINAMOX and NANOPOLYTOX kindly agreed to participate as speakers.

The aim of the first edition in 2011 was to discuss the **human and environmental impacts of nanomaterials along their life cycle**, from their production through their processing, use, and end of life (recycling and/or disposal). Therefore, the tools and methodologies proposed for the risk assessment (RA) and Life cycle assessment (LCA) of NMs will be one of the main topics of discussion in this symposium.

The Second edition of NANOLCA, in 2012, a Symposium organized by the coordinators of four European FP7 Projects: HINAMOX, NANOPOLYTOX, NEPHH, ENPRA and the Joint Research Centre (JRC) of the European Commission, was targeted at Nanosafety issues, including regulatory concerns along the life cycle of nanometer scale materials. The symposium intended to:

- Present to a broad audience the latest results and progress of the European FP7 Projects HINAMOX, NANOPOLYTOX, NEPHH and ENPRA
- Present the state of the art and recent developments in the legislation and regulations in the EU and the world concerning NMs
- Share knowledge and experiences about the critical issues specific for the risk assessmentand LCA of NMs in a regulatory context
- Identify needs and challenges for policy making and regulation of nanotechnology based materials
- Trigger discussions and networking among experts in the different fields of nanosafety

- **Scientific publication**:

6 scientific papers have been published by prestigious journals:

- Kazmina, O.V. "Effect of the component composition and oxidation – reduction characteristics of mixes on foaming of pyroplastic silicate pastes". Glass and Ceramics. Volume 67, (2010).

The effect of the composition of mix based on different silica raw material and the oxidation-reduction characteristic of the components on the foaming process in obtaining FGC materials is examined. It is established that foam-forming mixes belonging to the transitional oxidation-reduction group, whose oxidation coefficient lies in the range 25 – 100 are optimal for foaming. The oxidative and reducing mixes are characterized by a low foaming ratio and high degree of nonuniformity, which requires adjusting the composition by changing the ratio of the reducer oxidizer.

- Njuguna, J.; Sachse, S.; IRFAN, A. and ZHU, H. “Morphology studies of nanodust generated from polyurethane/nanoclay nanofoams following mechanical fracture”. Journal of Nanostructured Polymers and Nanocomposites. Volume 7, (2011).

Nanoparticles have been generated by impacting PU/MMT nanocomposites via drop weight impact testing. The released particles were sampled and extracted in a suspension and studied through microscopy and DLS techniques. The results have shown that two types of dust particles were generated during impact testing of the nanofoams. Single elliptical MMT layers (350x 120 nm) and NPs of composite material PU/MMT could be found in the suspension. The results clearly showed that the NM which was integrated in the polymeric matrix could be re-founded in the fracture of the nanofoam, but furthermore a hybrid particle of PU/MMT could be detected. These results illustrate new insight into NP behaviour and advice on a new dimension for NM risk assessment.

- Kazmina, O.V.; Vereshchagin, V.I. and Semukhin, B.S. “Structure and strength of foam glass crystalline materials produced from a glass granulate”. Glass Physics and Chemistry. Volume 37, (2011).

The microstructure of FGC materials has been investigated using the EDX, Auger electron and IR spectroscopy, high-resolution SEM, and X-ray diffraction analysis. The strength of the materials is determined and proves to be higher than that for the foam glass prepared from a glass cullet. This is a consequence of the structural effect associated with the formation of nanostructural units in the glassy matrix.

- Njuguna, J. “NPs released from structural nano-enhanced product following mechanical loading at low velocity impacts: a case study on structural polyurethane nanoreinforced foams”. 4th International Seminar on modern polymeric materials for environmental applicationsl. Volume 4, (2010).

In this study, NPs have been generated by impacting PU/MMT nanocomposites via low velocity impact testing. Airborne and fracture particles were sampled and characterised in various steps and with diverse techniques. The results showed that the NM which was integrated in the polymeric matrix could be re-founded in the fracture of the nanofoam, but furthermore a hybrid particle of PU/MMT could be detected. The generation of nanodust by nanocopmosites, while undergoing a mechanical process have been reported in individual studies.

- Sachse, S.; Silva, F.; Zhu, H.; Irfan, A.; Leszczyńska, A.; Pielichowski, K.; Ermini, V.; Blazquez, M.; Kuzmenko, O. and Njuguna, J. “The effect of nanoclay on dust generation during drilling of PA6 nanocomposites”. Journal of Nanomaterials. Volume 2012, (2012).

This paper looks into the influence of nanoclay on mechanical drilling of PA-6 composites, in terms of dust generation. With the help of real-time characterization, submicrometer-sized particles (5.6-512 nm) size distribution and number concentration emitted from PA-6/nanoclay composites during mechanical drilling is studied. Total particle concentration for the PA-6/nanoclay composites was 20,000 cm-3, while unreinforced panel measured a total concentration of approximately 400,000 cm-3. While the airborne particle concentration for the PA-6/ nanoclay composites was 20 times lower than for the PA-6 matrix, the concentration of deposited NPs doubled for the nanocomposite. The results clearly shows that more particles in the size range between 175-350 nm are generated, during drilling of the nanocomposites and this particles deposit in a shorter time. It is likely that the presence of nanoclay in some way retains the formation of high quantity of airborne particles and promotes particle deposition.

– Kazmina, O.V. "The influence of the crystalline phae interpore patition on the strength of glass crystalline foam". Izvestiya of HEIs Physics. Volume 54, (2011).

The paper shows the mechanical strength influenced by the number and the size of crystalline phase particles of glassceramic foamed material which present in the amorphous matrix of the interpore partition. It has been stated that the material with the particle concentration of 5-7% and the size of 90 nm has maximum strength. In forming nanoscale spheroid foamed material in the amorphous matrix, its strength reaches 4 MPa.

NANOSAFETY CLUSTER

It is worth to highlight that NEPHH ProjectConsortium worked on the initial definition of the NANOSAFETY Cluster objectives including a list of milestones and deliverables along with other projects: INLIVETOX, ENPRA, ENNATOX, HINAMOX and PARTICLE RISK. The main objectives of the NanoSafety cluster are:

- To facilitate the formation of a consensus on nanotoxicology in Europe
- To provide a single voice for discussions with external bodies
- To avoid duplicating work and improve efficiency
- To improve the coherence of nanotoxicology studies and harmonize methods
- To provide a forum for discussion, problem solving and planning R&D activities in Europe
- To provide industrial stakeholders and the general public with appropriate knowledge on the risks of NPs and NMs for human health and the environment.

According to the objectives of the NANOSAFETY Cluster Initiative, it is envisaged that participating research entities make the results of their studies available to the other members of the Cluster, providing that no confidentiality issues arise. Reporting to the Cluster has included overviews of:

- NPs investigated and their physicochemical properties (chemistry, crystal structure, size, shape, coating, surface charge, etc.)
- Results on bioavailability of NPs
- Results on biodistribution, accumulation, degradation, uptake and excretion of NPs in living organisms and the environment
- Results on toxicity testing and screening
- Models developed
- Methods and techniques used to obtain project data together with their main limitations.

Task and main actions as derived from NEPHH's commitment with NANOSAFETY Cluster have been classified as follows:

- Contributions as committed in DOW
- Attendance to organized meetings
- Inclusion into working groups and implication of consortium members within the requirements of the NANOSAFETY Cluster
- Links with other projects:

    - Initiatives for cooperation with other FP7 projects at national level
    - Initiative for data sharing with other projects involved in NMs implications under a life cycle perspective
- NANOSAFETY Cluster meetings organization

Apart from the developed dissemination activities, and with regards to the foreground generated within the project, it is worth to point out that a patent has been registered: Patent of the Russian Federation N 2415817 - Mixture for the quenched cullet manufacturing for Foamglass-Crystal materials.

5 Address of the project website and relevant contact details

The address of the project public website is the following:

http://www.nephh-fp7.eu

NEPHH Project Consortium and contact details is presented in the following table:

	Ekotek Ingeniería y Consultoría Medioambiental (Spain)	Juan Antonio Gascón jagascon@ekotek.es
	Cranfield University (United Kingdom)	James Njuguna j.njuguna@Cranfield.ac.uk
Palladin Institute of Biochemistry	Palladin Institute of Biochemistry of the National Academy of Sciences of Ukraine (Ukraine)	Oleksandr Kuzmenko akuzm@hotmail.com
	Cracow University of Technology (Poland) - Department of Chemistry and Technology of Polymers	Krzysztof Pielichowski kpielich@riad.pk.edu.pl
	Tomsk Polytechnic University (Russia)	Svetlana Chursina churs@cc.tpu.edu.ru
	Fundación Tecnalia (Spain)	Ainhoa Eguizabal ainhoa.egizabal@tecnalia.com
	Centre national de la recherché scientifique CNRS-ECCOREV (France)	Jerome Rose rose@cerege.fr
	Grado Zero Espace (Italy)	Marco Giacomelli marco.giacomelli@gzespace.com
	Asociación para la prevención de accidentes (Spain)	Martín Silva Casal msilva@apa.es
	Laviosa Chimica Mineraria (Italy)	Valentina Ermini vermini@laviosa.it

Table 1– List of beneficiaries and contact names

6 Acronyms and Abbreviations

A549	Lung cells
ASTM	American Society for Testing and Materials
ATH	Aluminium Tryhydrate
ATP	Bioluminiscense assay
BRCA1	Breast cancer type 1 susceptibility protein
D43B	Commercial nanoclay deriving from MMT
D72T	Commercial nanoclay deriving from MMT
DLS	Dynamic Light Scattering
DLVF:	Pristine MMT
DMBHT	Dimethylbenzyl hydrogenated tallow ammonium
DNA	Deoxyribonucleic acid
DOC	Dissolved Organic Carbon
DSC	Differential Scanning Calorimetry
EcoRA	Ecological Risk Assessment
EDX	Energy Dispersive X-ray
EEA	European Economic Area
EHS	Environmental Health Safety
EOL	End Of Life
ESP	Electrostatic Precipitator
EVA	Ethylene Vinyl Acetate
FGC	Foamglass Crystal
FTIR	Fourier Transform InfraRed
GS	Glass Spheres
HaCAT	Skin keratinocytes
HepG2	Liver cells
IR	Infrared
LCA	Life Cycle Assessment
LDH	Lactate Dehydrogenase
LLCPK	Porcine kidney cells
MDH	Magnesium hydroxide
MFR	Mass Flow Rate
MMT	Montmorillonite
MRC5	Foetal fibroblast cell
MTT	(3-4,5-Dimethylthiazol-2yetl)-2,5-diphenyltetrazolium bromide)
N&N	Nanosciences and Nanotechnologies
NM	Nanomaterial
NP	Nanoparticle
PA	Polyamide
POLM	Polarized Light optic Microscope
PP	Polypropylene
PPE	Personal Protective Equipment
PU	Polyurethane
PUR	Polyurethane foam
REACH	European Community Regulation on chemicals and their safe use
ROS	Reactive Oxygen Species
RT	Room temperatura
SAXD	Small Angle X-ray Diffraction
SEM	Scanning Electron Microscopy
SMPS	Scanning Mobility Particle Sizer
SN	Nanosight

TEM	Transmission Electron Microscopy
TG	Thermogravimetry
TSS	Technolgical Surveillance System
WAXD	Wide Angle X-ray Diffraction
WP	Work Package
XPS	X-ray Photoelectron Spectroscopy
XTT	Bioreduction capacity assay

7 References

1. COM (2005) 243 final.
2. IP/08/193, 8 February 2008.
3. COM(2008) 366 final.
4. Di Pasqua, A.J.; Sharma, K.K.; Shi Y-L.; Toms, B.B.; Ouellette, W.; Dabrowiak, J.C.; Asefa, T. (2008) *Cytotoxicity of mesoporous silica nanomaterials*. Journal of Inorganic Biochemistry 102. PP 1416–1423.
5. Park, M; Annema, W.; Salvati, A.; Lesniak, A.; Elsaesser, A.; Barnes, C.; McKerr, G.; , Howard, C.V.; Lynch, I.; Dawson, K.A; Piersma, A.H; de Jong, W.H. (2009) *In vitro developmental toxicity test detects inhibition of stem cell differentiation by silica nanoparticles*. Toxicology and Applied Pharmacology 240. PP 108–116.
6. Afsset. (Mars 2010). *Évaluation des risques liés aux nanomatériaux pour la population générale et pour lénvironnement*.
7. Deng, X.; Luan, Q.; Chen, W.; Wang, Y.; Wu, M.; Zhang, H.; Jiao, Z., 2009. *Nanosized zinc oxide particles induce neural stem cell apoptosis*. Nanotechnology 20, 115101.
8. Safe work Australia (November 2009). *Engineered Nanomaterials: A REVIEW OF THE TOXICOLOGY AND HEALTH HAZARDS*. ISBN 978 0 642 32921 9 (Online PDF)
9. Aitken, R.J.; Hankin, S.M.; B Ross, B.; Tran, C.L.; V Stone, V.; Fernandes, T.F.; Donaldson, K.; Duffin, R.; Chaudhry, Q.; Wilkins, T.A.; Wilkins, S.A; Levy, L.S.; Rocks, S.A.; Maynard, A. (March 2009) Report TM/09/01 *EMERGNANO: A review of completed and near completed environment, health and safety research on nanomaterials and nanotechnology*.
10. http://www.who.int/foodsafety/fs_management/No_01_nanotechnology_Feb08_en.pdf
11. Nanoscience and nanotechnologies: opportunities and uncertainties, The Royal Society 2004.
12. COM (2005) 243 final.
13. COM (2005) 243 final.
14. IP/08/193, 8 February 2008.
15. *T.D. Fornes, P. J. Keskkula, D. R. Paul, Polymer, 42, 9929 (2001)*
16. Influences of size of silica particles on the cellular endocitosis, exocitosis and cell activity of HepG2 cells. Ling Ju, Zhengwei Mao, Yuying Zhang, Changyou Gao. Journal of nanoscience letters (2011)
17. Size-dependent internalization of particles via the pathways of clathrin- and caveolae-mediated endocytosis. Rejman J, Oberle V, Zuhorn IS, Hoekstra D Biochem J (2004)
18. Studies of surface-modified gold nanowires inside living cells. Kuo CW, Lai AJ, Wei KH, Chen P. Adv Funct Mater 2007
19. The defined presentation of nanoparticles to cells and their surface controlled uptake. Aliciea Piera Alberola, Joachim O. Rädler. Biomaterials 30 (2009).
20. European convention for the protection of vertebrate animals used for experim. and other scientific purposes // Counf. of Europe, Strasbourg, 1986.-53p.
21. Law of Ukraine No 3447-IV: On the protection of animals from abuse // Bulletin of the Supreme Parliament of Ukraine 27 (2006) 230.