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Nano-Cathedral

D4.1 – State of art on Nano-technology impact on environment and health in the field of stone preservation

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1 Nanotechnology and nanomaterials

Nanotechnology and nanomaterials will be the keys of technological innovation in European Union (EU) promoting a smart, sustainable and inclusive growth. Nanotechnology has rapidly triggered the development a new generation of smart and innovative products, materials and processes that have created a tremendous growth potential for a large number of industry sectors. However, the marked benefits brought about by engineered (man made) nanomaterials and nanotechnology applications have also created some concerns of their possible effects on human health and safety and environmental burden. Indeed, a few observations on some potentially harmful effects of man made nanomaterials have in some cases overshadowed the dramatic benefits of these materials and their nanotechnology applications. Therefore, the real concern, rather than observations on some potential hazards of exposure to nanomaterials, is the lack of systematic studies on hazards of or exposure to ENM. To deal with this urgent need for sufficient knowledge to allow reliable assessment of the risk associated with nanomaterials, EU has promoted the *EU Nanosafety* Cluster, an initiative to maximize the synergies between the existing FP6 and FP7 projects, addressing all aspects of nanosafety including toxicology, ecotoxicology, exposure assessment, mechanisms of interaction, risk assessment and standardisation.

1.1 Analysis of European projects dealing with the topic

The EU NanoSafety Cluster released in June 2015 the "Compendium of the projects in the Nanosafety cluster", edited by Iseult Lynch, University of Birmingham, United Kingdom. The compendium documents the status of important EU-funded projects on nanomaterial toxicity and exposure monitoring, integrated risk management, research infrastructure and coordination, and support activities as well as regulatory-focussed research on nanosafety. The running (2015) FP7 projects are well into their activities, while the first of the H2020 projects are just preparing to start over the coming months.

The compendium also aims to bring the research community closer together and show them the potential for synergy in their work and it is a means to establish links and communication between them during the actual research phase and well before the publication of their results. It thus focuses on the communication of projects' strategic aims, extensively covers specific work objectives and the methods used in research, and documents human capacities and partnerships.

As such, the compendium supports collaboration on common goals and the joint elaboration of future plans, whilst compromising neither the potential for scientific publication, nor intellectual property rights. In this perspective, the EU NanoSafety Cluster could be a very platform tool for the Nano-Cathedral team, to share information about the nanoparticles and nanomaterials developed and applied in the project.

An overview of the EU-founded ended and running projects dealing with the EU nanosafety cluster research themes is reported in table 1.

Table 1. EU-founded projects dealing with the EU nanosafety cluster research themes.

Overview matrix: Research themes of the NanoSafety Cluster projects

	H2020	FP7 running projects (or just ended)																										FP7-ended	
Project Acronym	NanoFASE	ProSafe	NanoREG II	enanoMapper	FibralSpec	FutureNanoNeeds	GuideNano	MARINA	MembraneNanoPart	Mod-ENP-Tox	NanoDefine	Nanodetector	NanoHeter	NanoMICEX	NanoMILE	NanoOxiMet	NanoPUZZLES	NANoREG	NanoSafePack	NanoSOLUTIONS	NanoValid	PreNanoTox	QualityNano	SANOWORK	SIMWI	SIRENA	SUN	NanoFATE	NanoReTox	NanoSustain	ModNanoTox
Start Year	2015	2015	2015	2013	2014	2013	2013	2011	2013	2013	2013	2012	2013	2012	2013	2013	2013	2013	2011	2013	2011	2013	2011	2012	2013	2013	2013	2010	2008	2010	2011
End Year	2019	2017	2018	2016	2017	2016	2016	2015	2015	2015	2017	2015	2016	2015	2017	2016	2015	2016	2014	2017	2015	2016	2015	2015	2016	2015	2016	2014	2012	2013	2013
Characterisation and measurement of NMs	X					X	X	X			X	X			X					X	X							X		X	
Physico-chemical properties	X		X		X	X	X	X	X	X	X	X	X	X	X	X		X	X	X	X	X	X	X	X		X	X	X	X	
Next generation NMs	X					X	X				X														X		X				
Exposure assessment for humans and the environment	X					X	X	X						X					X		X						X	X		X	
Develop & validate exposure measure / modelling methods	X					X	X	X		X			X	X			X	X	X		X				X	X	X	X			X
Application of measurement / modelling methods on NMs	X					X	X	X					X	X				X	X		X	X	X	X			X	X	X	X	
Environmental Exposure Assessment	X				X	X	X	X					X	X				X	X		X				X	X	X	X		X	X
Interaction of NMs with biological systems	X				X	X		X							X					X	X							X		X	
Interaction with physiological mechanisms	X		X			X		X	X	X					X	X	X	X	X		X	X	X	X				X	X	X	
Toxicokinetics	X		X					X	X	X				X	X		X	X			X			X				X		X	
Inter- /intraspecies variability	X							X							X		X	X	X		X							X	X		
Predictive models	X		X					X	X	X				X	X	X	X	X	X	X	X		X					X	X	X	X
Long term monitoring and assessment	X				X													X										X			
Human Health						X		X												X	X									X	
Development and validation of a testing and assessment strategy			X					X		X							X	X		X	X		X		X				X	X	
Application of a testing and assessment strategy								X						X			X	X	X	X	X			X	X					X	
Coexposure / Toxicology of mixtures						X															X				X						
Ecotoxicology	X					X	X	X							X		X			X	X							X		X	
Development of a testing and assessment strategy	X		X			X	X	X						X	X		X	X		X	X		X		X		X	X	X	X	
Application of testing and assessment strategy	X					X	X	X							X		X	X	X	X	X				X		X	X		X	X
Control measures at workplace								X													X						X	X		X	
Development and validation of methods								X				X						X		X	X						X			X	
Application of methods					X			X				X		X				X	X		X			X						X	
Control banding approach								X						X					X					X							
Preliminary handling guidelines	X							X							X		X			X	X						X			X	
Collection of available and ongoing approaches	X	X	X					X							X		X	X	X	X	X					X	X		X	X	
Evaluation and further development	X	X				X		X							X		X	X	X	X	X			X		X	X		X	X	
Information transfer	X					X									X					X	X							X			
Database generation	X	X	X		X	X	X	X					X		X	X	X	X		X	X	X	X	X		X	X	X	X	X	X
Public dialogue	X	X			X		X	X		X			X				X				X			X				X			
Information to and training of workers, business and employers			X		X	X	X							X					X		X			X		X	X	X		X	
National /international collaboration	X	X			X		X	X					X				X			X	X							X		X	X
Development	X	X			X	X	X	X				X	X		X	X	X	X		X	X	X	X	X	X	X		X	X	X	
Testing	X	X				X	X	X	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		X	X	X	
Validation	X	X	X				X	X	X		X		X		X		X	X	X	X	X	X	X		X	X		X		X	
Standardisation	X	X	X		X			X			X		X		X	X	X	X			X	X	X			X		X			
Assessment activities	X	X					X	X					X	X			X	X	X	X	X	X	X	X				X		X	

Note that 3 projects just started within the Horizon 2020 framework (NanoFASE, ProSafe and NanoREG II), 4 project (within the FP7 framework) just ended (NanoFATE, NanoReTox, NanoSustain, ModNanoTox), while most of them (25 projects) are still running within the FP7 framework (eNanoMapper, FibralSpec, FutureNanoNeeds, GuideNano, MARINA, MembraneNanoPart, Mod-ENP-Tox, NanoDefine, Nanodetector, NanoHeter, NanoMICEX, NanoMILE, NanOxiMet, NanoPUZZLES, NANoREG, NanoSafePack, NanoSOLUTIONS, NanoValid, PreNanoTox, QualityNano, SANOWORK, SIINN, SIRENA, SUN).

In addition, other older projects dealing with the toxicity of nanomaterials, in particular inorganic nanoparticles, tested in vivo and in different natural environments, have been carried out in the framework of FP7 (INLIVETOX, ENPRA, ENNSATOX, MARINA, NEPHH, HINAMOX, NANEX, NANODEVICE, NANOFATE, NANOHouse, NANOimmune, NONOSH, nanopolytox, NanoSustain, NeuroNano).

The full description of the listed projects is beyond the objective of the present deliverable. However, a few results and the conclusions of those projects dealing with the toxicity and the environmental impact of inorganic nanoparticles potentially applicable within the Nano-Cathedral project, such as SiO_2, metal oxide, metal hydroxides, and carbonates are of interest, and could represent a significant starting point to address the specific Nano-Cathedral issues.

In this perspective, FutureNanoNeeds, MARINA, NANEX, NEPHH could give, among the others, the most useful starting platform to develop the specific knowledge related to the inorganic nanoparticles investigated in the NanoCathedral project.

1.1.1 INLIVETOX (2009-2012)

a. Summary: objectives and results

In vitro testing offers a potential solution to the challenge of how to ensure that as nano-particles (Nano-particles) are developed and used, any unintended consequences of exposure to humans are minimised. The InLiveTox project has significantly advanced the capability of in vitro testing in particular of Nano-particles.

The InLiveTox project focused on the impact of NP exposure via ingestion, on the vascular endothelium, liver and gastrointestinal tract (GI). Exposure via ingestion is particularly relevant due to the inclusion of Nano-particles in food, food packaging and in oral medicines.

b. Conclusions relevant for Nano-Cathedral

The effect of Nano-particles depends much on their dimension. The studied systems were based on TiO_2, Ag and Au. This project can be important for establishing the impact of Nano-particles onto human health, as it deals with ingestion.

1.1.2 ENPRA (2009-2012)

a. Summary: objectives and results

ENPRA utilised the latest advances within in vitro, in vivo and in silico approaches to nanotechnology environment, health & safety (EHS) research to realise its aims about finding methods for evaluating risks in the use of Nano-particles. ENPRA has provided a database, standardised tools and critical mathematical models for accurate assessment of the risks of exposure to selected ENano-particles. The risk assessment framework will have important impact on workplace, consumer and environmental safety, as well as the development of future standards and regulatory policies.

b. Conclusions relevant for Nano-Cathedral

The effects of four ENano-particles (titanium dioxide (TiO_2), zinc oxide (ZnO), silver (Ag) and multi-wall carbon nanotubes (MWCNTs)) was considered. All the Nano-particles were reported to be toxic in vivo tests, but the toxicity of TiO_2 is quite lower than that of ZnO and MWCNTs.

1.1.3 ENNSATOX (2009-2012)

a. Summary: objectives and results

The aim of the project was to study and relate the structure and functionality of well characterised engineered nanoparticles, such as zinc oxide (ZnO), titanium dioxide (TiO_2), and silicon dioxide (SiO_2), to their biological activity in the aquatic environment, taking into account the impact of the nanoparticles on environmental systems from their initial release to uptake by organisms. The activity of the nanoparticles was studied in a series of biological models of increasing complexity from single cells to fish. The project also conducted parallel environmental studies to examine the behaviour of the nanoparticles in natural waters and how they modify the particles' chemical reactivity, physical form and biological activity. A comprehensive model was finally developed to describe the environmental system and predict the effects of nanoparticles.

b. Conclusions relevant for Nano-Cathedral

The most interesting results and achievements of the project can be summarized as by following: 1) the full characterisation of commercial and in-house synthesised SiO_2, ZnO and TiO_2 dispersions prior to, during and following their employment in toxicology testing; 2) the intercalibration of toxicology results from effects of SiO_2, ZnO and TiO_2 on successively more complex levels of biological organisation from in vitro models of cell membrane and cell cultures to multicellular organisms of crustaceans and fish. This returned a full understanding of the toxicity mechanisms involved at the molecular level; 3) the full understanding of the behaviour of dispersions of SiO_2, ZnO and TiO_2 with respect to charge and surface chemical characteristics in model environmental aquatic systems; and 4). a comprehensive predictive mathematical model which describes the behaviour of SiO_2, ZnO and TiO_2 following discharge into the aqueous environment and their subsequent impact on model organisms in the environment including their uptake by these organisms.

1.1.4 MARINA (2011-2015)

a. Summary: objectives and results

The aim of MARINA was to develop and validate the Risk Management Methods for Nanomaterials. Therefore, MARINA addressed the four central themes for the Risk Assessment and Management of Nanomaterials: Materials, Exposure, Hazard, and Risk. MARINA developed beyond state-of-the-art referential tools from each of these themes and integrate them into a Risk Management Toolbox and Strategy for both human and environmental health. Indeed, there was a need to evaluate and develop specific reference methods for all the fundamental steps in managing the potential risk of ENM. The methods had to be integrated in an overarching, coherent strategy for regulators and industry to adapt them. Thus, a safe and environmentally responsible nanotechnology is expected to safeguard current and future global investments and will be the key to the sustainability of this industry.

b. Conclusions relevant for Nano-Cathedral

A complete and updated Risk Management Methods for Nanomaterials was developed within the MARINA project, and can be a useful reference and starting point for all the nanomaterials developed/applied within the Nano-Cathedral project

1.1.5 NEPHH (2009-2012)

a. Summary: objectives and results

The NEPHH project took a further step forward in current researches by analysing, besides primary nanomaterials (or nanoparticles), secondary and tertiary products derived from nanocomposites which incorporate siliceous particles of a nanometric nature. The project had a global focus, considering all stages of the life cycle of the selected nanomaterials, from their production to their elimination, including their final use. The main objective of the project was to identify and rate important forms of Nanotechnology related environmental pollution and health hazards that could have resulted from activities involved in the life cycle

of Silicon-based polymer nanocomposites currently used in a variety of industrial sectors and also to suggest means that might reduce or eliminate these impacts.

b. Conclusions relevant for Nano-Cathedral

A state of the art assessment of the safety issues related to a selection of nanomaterials was developed within the project. In particular, all the stages of the life cycle of the selected nanomaterials, mainly inorganic fillers form polymeric materials, from their production to their elimination, including their final use were assessed.

1.1.6 HINAMOX (2009-2012)

a. Summary: objectives and results

The work of the HINAMOX consortium focused on **metal and metal oxide Nano-particles as potentially dangerous to biological organisms**. Metal oxide and metal Nano-particles are widely used in various industrial processes and common products. Some examples of these are TiO_2 and ZnO as catalysts and UV protectors, CuO in anti-fouling paints, Al_2O_3 as a surface protector, CeO_2 in polishing, indium-tin oxides forming anti-electrostatic coatings and various rare earth oxides in electronics manufacturing. The above mentioned industrial applications highlight the technological and economical importance of these Nano-particles spanning the chemical industry, cosmetic industry, paint industry, electronics manufacturing industry and waste treatment

b. Conclusions relevant for Nano-Cathedral

The focus on metal oxides is particularly relevant for Nano-cathedral, since formulations containing metal oxides will be used and studied for the specific applications of interest. Moreover, specific products such as antifouling paints and surface protector were investigated in the HINAMOX project.

1.1.7 NANEX (2009-2010)

a. Summary: objectives and results

The aim of the NANEX project was to develop a catalogue of generic and specific (occupational, consumer and environmental release) exposure scenarios for nanomaterials (MNMs) taking account of the entire lifecycle of these materials. NANEX collected and review available exposure information, focusing on three very relevant MNMs: (1) high aspect ratio nanomaterials- HARNs) (e.g. carbon nanotubes); (2) mass-produced nanomaterials (e.g. ZnO, TiO_2, carbon black); and (3) specialised nanomaterials that are currently only produced on a small scale (e.g. Ag). The exposure information included both quantitative (measurement results) and qualitative contextual exposure information (risk management measures). We also reviewed the applicability of existing models for occupational and consumer exposure assessment and for environmental release from these scenarios. The consortium carried out a small number of specific case illustrations and a gap analysis of the available knowledge and data

b. Conclusions relevant for Nano-Cathedral

The focus on oxides (ZnO, TiO_2) is particularly important for Nano-Cathedral, since formulations containing metal oxides will be used and studied for the specific applications of interest.

1.1.8 NANODEVICE (2009-2013)

a. Summary: objectives and results

Here the main project goal was to develop innovative concepts and reliable methods for characterizing engineered nanoparticles (ENP) in workplace air with novel, portable and easy-to-use devices suitable for workplaces. Additional research objectives were (1) identification of relevant physico-chemical properties and metrics of airborne ENP; establishment of reference materials; (2) exploring the association between physico-chemical and toxicological properties of ENP; (3) analyzing industrial processes as a source of ENP in

workplace air; (4) developing methods for calibration and testing of the novel devices in real and simulated exposure situations; and (5) dissemination of the research results to promote the safe use of ENP through guidance, standards and education, implementing of safety objectives in ENP production and handling, and promotion of safety related collaboration through an internationally nanosafety platform

b. Conclusions relevant for Nano-Cathedral
The polluting effects of the dispersion of nanoparticles used for coatings and paints in the environment is of interest for the Nano-Cathedral project

1.1.9 NANOFATE (2009-2014)

a. Summary: objectives and results
NanoFATE was conceived to fill knowledge and methodological gaps currently impeding sound assessment of environmental risks posed by engineered nanoparticles (ENano-particles). The project vision returend the assessment of environmental ENP fate and risk in selected high-volume products for which recycling is not an option, namely: fuel additive, personal care and antibacterial products. Two market ENano-particles from each product (CeO_2, ZnO, Ag of varying size, surface and core chemistries) were followed through their post-production life cycles - from environmental entry as “spent product”, through waste treatment to their final fates and potential toxic effects. In this way the consortium tested the applicability of current fate and risk assessment methods and identified improvements required for early stage assessment of ENano-particles

b. Conclusions relevant for Nano-Cathedral
The polluting effects of the dispersion of nanoparticles, expecially metal oxides, used for coatings and paints in the environment is of interest for the Nano-Cathedral project

1.1.10 NANO HOUSE (2010-2013)

a. Summary: objectives and results
The nine partners involved in Nano House project investigated and generated missing data on the potential exposure levels and the hazard due to this chronic exposure for 2 nanoparticle types: nano silver (Ag) and nano titanium dioxide (TiO_2) contained in indoor and outdoor coatings and paints. Both direct and indirect exposures (through the environment to human: vegetables, drinking water) were considered.

b. Conclusions relevant for Nano-Cathedral
The polluting effects due to the release in the environment of nanoparticles used for outdoor coatings and paints, metal oxides in particular, is of interest for the Nano-Cathedral project.

1.1.11 NANOSH (2006-2009)

a. Summary: objectives and results
The NANOSH project focused on occupational exposure to nanoparticles and their health effects. One goal of the research was to characterise the levels of exposure to specific engineered nanoparticles. Exposure levels were evaluated both under laboratory conditions and during the manufacture of the particles. The particles were characterised with respect to their morphology and particle-size distribution, surface activity, and potential for agglomerate formation. The overall goal of the project was to delineate the health effects of selected nano-sized particles relevant to the occupational environment. The health effects studied included genotoxicity, pulmonary inflammatory responses, and effects on the vasculature. The information gathered together with the state-of-the-art technology utilised in these studies increases our knowledge on nanoparticles and help to create a reliable basis for the evaluation of possible health risks associated with these new materials.

b. Conclusions relevant for Nano-Cathedral
The impact on health of the dispersion of nanoparticles used for coatings and paints is of interest for the Nano-Cathedral project.

1.1.12 NANOPOLYTOX

a. Summary: objectives and results
The main objective of NANOPOLYTOX was the monitoring of the life cycle of three families of nanomaterials (carbon nanotubes, nanoclays and metal oxide nanoparticles) when embedded in selected polymeric hosts. The project will include monitoring of the chemical and physical properties of the nanomaterials and their toxicity from the synthesis, processing, aging, and recycling to their disposal, covering their migration and/or release during their life cycle. The theoretical analysis of the data obtained during the project will lead to the development of predictive models to assess the biological and environmental fate of the studied nanomaterials. Moreover, the overall human health and environmental impact were assessed by LCIA analysis, specifically designed for nanomaterials

b. Conclusions relevant for Nano-Cathedral
The effects on the environment and on the human health of the dispersion of nanoparticles used for coatings and paints having a polymeric matrix is of interest for the Nano-Cathedral project.

1.1.13 NANOSUSTAIN

a. Summary: objectives and results
The ultimate goal of NanoSustain was to explore, examine and develop new solutions for the sustainable design, use, re-use, recycling and final treatment and/or disposal of specific nanomaterials and associated products. The research strategy was based on a comprehensive hazard characterization and impact assessment of selected environmentally and economically relevant materials products.

b. Conclusions relevant for Nano-Cathedral
The effects on the environment and on the human health of the dispersion of nanoparticles used for coatings and paints is of interest for the Nano-Cathedral project.

1.1.14 NEURONANO

a. Summary: objectives and results
The project focused on understanding the origin of reactive oxidative species and protein aggregation and mis-folding phenomena in the presence of nanoparticles. NeuroNano draws together a unique team, several of whom have pioneered the preliminary results in this field, and supplements them with the necessary skills and facilities required to address these questions. A knowledge-based approach was carried out, for it probes the questions in the deepest manner, isolating each separate element of the nanoparticle's physic-chemical qualities that control fibrillation and oxidative stress, and access to the brain, determining their consequences separately. Indeed, nanoparticles *may* reach the brain – less than 40 nm particles can potentially pass through the blood-brain barrier. Nanoparticles *may* induce oxidative stress in living systems. Oxidative stress from ambient or combustion particles contribute to cell damage, including DNA damage. The large surface area of nanoparticles means that they can modulate the fate of protein fibrillation in solution. Whether this has significance *in vivo* is not yet determined.

b. Conclusions relevant for Nano-Cathedral
The effects on human health of the dispersion of nanoparticles used for coatings and paints is of interest for the Nano-Cathedral project.

2 Nanoparticles in stone material treatments

2.1 Risks related to health and environment of consolidants

In consolidations the most important materials are Trietoxysilane (TEOS) and similar compounds, often used in consolidation. In the present project the use of this kind of consolidant togheter with nano-silica, nano-calcite (nano-calcium carbonate) or/and other nano-particles, such as zirconia, hydroxyapatite and allumina will be considered.

Trietoxysilanes and its derivatives are compounds forming a solid structure thanks to sol-gel chemistry. Hence its morphology after the reaction is not yet nano-structured. Hence no concern due to nano-dimension can originate from it. As it is a consolidated compond with many uses in several sectors, safety datasheets are available and the procedure for applying it is well known. Toxicity tests onto TEOS were carried out about two decades ago[1].

On the other hand the nano-structured consolidants, less investigated and known in the field of cultural heritage protection, deserve to be considered. In the treatment of stone material, they are applied onto stone surface and they should penetrate in stone porosities to increase its resistance. In the pores the nano-particles are then strongly linked to the stone host material or to other consolidants (such as TEOS) if they are used in combination. The migration from the stone porosities is then not easy. The impact on exposure to human body and also to environment (air or soil) can be extremely low. However, it is correct to know the actual state of art related to the single consolidant treatments that will be considered in the Nano-cathedral project.

Nano-silica is one of the most popular nanomaterials that are produced on an industrial scale as additives to cosmetics, drugs, printer toners, varnishes, and food. In addition, nanosilica is being developed in biomedical and biotechnological applications such as cancer therapy, DNA transfection, drug delivery, and enzyme immobilization. Nano-silica might lead to multiple organ damage[2]. Inhalation of nanosilica causes pulmonary inflammation, myocardial ischemic damage, and increase in fibrinogen concentration and blood viscosity. Nano-silica exposure also results in DNA damage, size-dependent hydroxyl radicals generation, and lung fibrogenesis in rats. Nano-silica could be preferentially distributed in liver, leading to liver injury. The results obtained by Niu et al. indicated that nanosilica and polyacrylate/nanosilica have a similar toxicity. The liver is an important organ for detoxification in the body and plays a vital role in the metabolism of nanomaterials, specifically nanosilica. It has been shown that tail vein injection of nanosilica resulted in significant accumulation of nanoparticles in the liver and resulted in liver injury.

Nano-Hydroxyapatite (HA) is another interesting material for formulating new consolidants. Hydroxyapatite in fact is a material present in animal bones, hence it is kept into account in biomedical applications because of its good biocompatibility. A state of art about the assessment of its toxicity is reported in the opinion of the Scientific Committee on Consumer Safety of the European Commission of 16 october 2015. The document represents an answer to an applicant that asked if nano-HA could be used in oral cosmetic. This study evidenced that there are not enough available data about the possibility of using nano-HA in oral cosmetic. However, it is clear from literature survey that nano-hydroxyapatite in needle form is of concern in relation to potential toxicity. Therefore, needle-shaped nano-hydroxyapatite should not be used in cosmetic products. In particular the shape-dependent effects of nano-HA on cytotoxicity, inflammatory cytokine expression and particle–cell association was demonstrated by Zhao et al.[3]. It must be noticed that the needle shape is not proper for stone consolidation since it can prevent the effective filling of porosity.

Nowadays, manufactured nano-particles of aluminum oxide (*nano-alumina*) are used in many fields. The aim of Zhang et al.[4] study was to compare the toxicity of nano- and micro- particles of alumina for detecting particle size related toxicity, and to compare the toxicity of nano-alumina and nano-carbon with the same particle size for determining chemical composition related toxicity. The present study revealed that nano-

particles of alumina were much toxic than micro-alumina particles, indicating a particle size related toxicity; moreover, they were much more toxic than nano-carbon particles as well, manifesting a chemical related toxicity. The mechanism might be concerned with the involvement of the lysosomes. In conclusion, toxicity of nano-alumina is a combination of the toxic effects of its particle size and chemical composition, due to its low but not negligible solubility. The purpose of this study [5] was to follow-up the distribution, lethality percentile doses (LD_s) and bioaccumulation of aluminium oxide nanoparticles (Al_2O_3-Nano-particles, average diameter 9.83 ± 1.61 nm) in some tissues of male albino rats, and to evaluate its genotoxicity to the brain tissues, during acute and sublethal experiments. The LD_s of Al_2O_3-Nano-particles, including median lethal dose (LD_{50}), were estimated after intraperitoneal injection. The computed LD_{50} at 24 and 48 h were 15.10 and 12.88 g/kg body weight (b.w.), respectively. For acute experiments, the bioaccumulation of aluminium (Al) in the brain, liver, kidneys, intestine and spleen was estimated after 48 h of injection with a single acute dose (3.9, 6.4 and 8.5 g/kg b.w.), while for sublethal experiments it was after 1, 3, 7, 14 and 28 days of injection with 1.3 g/kg b.w. once in 2 days. Multi-way analysis of variance affirmed that Al uptake, in acute experiments, was significantly affected by the injected doses, organs (brain, liver, kidneys, intestine and spleen) and their interactions, while for sublethal experiments an altogether effect based on time (1, 3, 7, 14, 28 days), doses (0 and 1.3 g), organs and their interactions was reported. In addition, Al accumulated in the brain, liver, kidney, intestine and spleen of rats administered with Al_2O_3-Nano-particles were significantly higher than the corresponding controls, during acute and sublethal experiments. The uptake of Al by the spleen of rats injected with acute doses was greater than that accumulated by kidney>brain>intestine>liver, whereas the brain of rats injected with sublethal dose accumulated lesser amount of Al followed by the kidney<intestine<spleen<liver. Bioaccumulation of Al, in all studied tissues, was positively correlated with the injected doses (in acute term) and the experimental periods (in sublethal term). In in vivo tests (comet), the results showed significant increase in DNA percentage damage in the brain cells. The obtained results indicate that bioaccumulation of Al was associated with significantly increased levels of comet parameters that depended on the doses and the experimental periods. In conclusion, Al has a high affinity to get accumulated in tissues to a level that is able to induce genotoxicity. Therefore, bioaccumulation is time, dose and organ dependant.

Zirconia is an advanced ceramic material quite stable and resistant. Because of its good biocompatibility, high hardness and strength, zirconia is often used as orthopedic implants, such as femoral head component in hip implants and dental implants. Karunakaran et al. studied the effect of *nano-zirconia* on soil[6]. They focused on the ecotoxicological behaviour of bulk and nano ZrO_2 (Zirconia) and TiO_2 (Titania) particles on PGPR (plant growth promoting rhizobacteria), soil and its nutrient contents. The microbial susceptibility study showed that nano TiO_2 had 13 +/- 0.9 mm (B. megaterium), 15 +/- 0.2 mm (*P. fluorescens*), 16 +/- 0.2 mm (*A. vinelandii*) and 12 +/- 0.3 mm (B. brevis) zones of inhibition. However, nano and bulk ZrO_2 particles were non-toxic to PGPR. In addition, it was found that toxicity varied depending on the medium of reaction. The soil study showed that nano TiO_2 was highly toxic, whereas bulk TiO_2 was less toxic towards soil bacterial populations at 1000 mg L(-1). In contrast, nano and bulk ZrO_2 were found to be inert at 1000 mg L(-1). The observed zeta potential and hydrophobicity of TiO_2 particles can be related to its higher toxicity in parallel with particle size. It was observed that nano TiO_2 decreases the microbial population as well as nutrient level of the soil but not zirconia. The authors showed that the mechanism of toxicity depends on size, hydrophobic potential and zeta potential of the metal oxide particles. Arefian et al.[7] studied the effects of ZrO_2 Nano-particles on the liver and kidney tissues as well as the activities in liver and kidney enzymes in the male rats. This study is done on 40 rats, in 4 groups which includes one control and 3 experimental groups that are monitored daily. The control group was fed with a Saline Solution and 3 other with 1ml/day nanoparticle by different doses (50, 25, 100 ppm) intra-peritoneally. After a 1 week period, several parameters were measured. Post-treatment tissue level of malondialdehyde (MDA) as well as the activities of catalase, glutathione peroxidase and superoxide dismutase were measured in the liver. The statistical raw data was then analyzed by a proper software. The significant difference ($p<0.05$) in the levels of foregoing factors was obtained by the application of the maximum density of ZrO_2 Nano-particles (100ppm)

in comparison with the control groups. The rats only when exposed to a high dosage of nanoparticles reported a significant increase in MDA concentration level while significant decreases were observed in other parameters. In the rats which were exposed to high dosage of nanoparticles, the liver enzyme concentration was also significantly increased.

2.2 Risks related to health and environment of protective treatments

Nano-particles kept into account in the Nano-cathedral project for formulating protective treatments were nano-TiO_2, nano-ZnO, nano-Ag and hydrophobins.

The inorganic Nano-particles were much investigated because they are employed in many different sector, such as biomedical and cosmetic, where the contact with body is more important than in the case of protective treatment. In the case of protective treatments, the Nano-particles are present in the liquid formulation which is applied onto stone surface in the presence of a polymeric substrate. During the drying of the treatment the polymeric matrices forms a continuous layer on the stone surface and englobes Nano-particles. The exposure of body or the release on environment of these Nano-particles is thus possible only if the polymer matrix is destroyed completely after aging and degradation, but this conditions can be achieved with difficulty in environment. In fact, in dependence on polymeric structure and its interaction with environment, the degradation of polymer can last centuries.

Zinc oxide based nano-particles and also Copper based nano-particles were compared with titania, and zirconia in Lanone et al [8] work (developed in the framework of Nanosafe2 project, funded by EC). This work indicated that the toxicity induced by nanomaterials depended on chemical composition, size and surface area. In this work it was possible to distinguish Cu- and Zn-based manufactured nanoparticles as nanomaterials with a potential critical use with respect to other treatments.

The principal concern associated with the release of nanomaterials is how their smaller particle size may alter the materials transport, fate, and potential toxicity to aquatic organisms compared to larger particles. For *ZnO*, no size related effects were in principle observed on toxicity to aquatic organisms [9]; rather, the most likely impact of ZnO [10] (nanoparticulate or bulk) resulted from dissolution presumably as Zn^{2+} or small inorganic complexes which are highly toxic to a range of aquatic organisms. This process is not limited to aquatic systems but may equally apply to soil environments, where pH strongly affects the dissolution.

The study of Gupta et al. [11] was focused to optimize the safe application of ZnO-Nano-particles by assessing multiple toxicity points and in vitro distribution in nanoparticle-exposed to *C. elegans*. The authors concluded that in the tests the activity of worms was not adversely affected with exposure to 50 and 100 nm ZnO-Nano-particles ≤1.0 g/l, while small-sized particles 10 nm ≥0.7g/l affects them at large as shown by an increase in the expression of two genes. Distributional pattern of ZnO-Nano-particles reveals that the intestine is the major target tissues for NP toxicity. The application of 50 and 100 nm ZnO-Nano-particles may be safe to the environment in comparison to 10 nm.

The action of ZnO is mediated by its dissolution and Zn^{2+} is the anti-bacterial species responsible for the anti-bacterial activity of ZnO. The formation of clumps or clusters of primary particles (resulting in increased hydrodynamic size and reduced specific surface area) may hinder dissolution by reducing the average equilibrium solubility of the particle system and by introducing kinetic hindrance.

Titanium dioxide accounts for 70% of the total production volume of pigments worldwide (International Agency for Research on Cancer, 2006). As a particle bigger than 100 nm, it is widely used to provide whiteness and opacity to products such as paints, plastics, papers, inks, foods, and toothpastes. It is also used in cosmetic and skin care products, and it is present in almost every sunblock, where it helps protect the skin from ultraviolet light. When used as a nanoparticle in sunscreen and cosmetics, TiO_2 has comparable UV protection abilities as the bulk material, but loses the cosmetically undesirable whitening as the particle size is decreased. As a nanoparticle, it is also used in air and water remediation. Although

studies showed that neither micro sized titanium dioxide, used for example in sunscreen, could penetrate the dermal layer of the skin, nor nano sized titanium dioxide, an uptake was still considered possible through skin that might be damaged through disease. Nevertheless, titanium dioxide entering an organism via inhalation or injection, is reported to have the potential to cause oxidative stress to a wide range of cell types not as monodispersed particle but often as aggregates of 800 - 1900 nm. In addition, TiO_2 nanoparticles have been shown to cause pulmonary inflammation, tissue damage, and fibrosis at sufficiently high mass doses.

TiO_2 Nano-particles possess different physicochemical properties compared to TiO_2 microparticles, which would be expected to alter their biological properties [12]. Current understanding on their toxicity largely depends on a limited number of experimental animal or cell culture studies, where extrapolation to human exposures is required. Epidemiological studies thus far have not been able to detect an association between the occupational exposure to TiO_2 particles and an increased risk for cancer. The physicochemical properties of TiO_2 Nano-particles may strongly influence their bioavailability and toxicity. Majority of data imply that TiO_2 anatase Nano-particles are cytotoxic or genotoxic. However, this conclusion was based on studies using TiO_2 anatase Nano-particles only. Under conditions of occupational exposure, inhalation of TiO_2 Nano-particles is normally the principal route for entry into the human body. Pulmonary inflammatory responses and lung cancers are the most important adverse effect observed in experimental animals due to TiO_2 NP exposures. When only using realistic doses are considered, as in the case of some inhalation studies, inflammatory responses are still a prominent effect seen. TiO_2 Nano-particles can be absorbed through the lung into systematic circulation and then distributed in different organs such as liver, kidneys, spleen, or even brain causing localized effects. However, the rate of such translocation is currently uncertain. Some evidence has shown that TiO_2 Nano-particles cannot penetrate the intact skin into the human body. TiO_2 Nano-particles may have the potential to penetrate the blood–brain, blood-testis and blood-placenta barriers. However, the rate of translocation appears low and evidence is lacking which link systemic responses to translocation of particles to systemic sites. Many studies have been conducted in vitro and in vivo to investigate the genotoxicity of TiO_2 Nano-particles but the results are conflicting and doses employed were high. Certain reproductive and developmental toxicities in experimental animals or cell cultures have been observed in a few in vivo and in vitro studies. Whether human exposure to TiO_2 Nano-particles causes reproductive and developmental toxicities is unclear. Animal studies imply that accumulation of TiO_2 Nano-particles in organs or tissues may take place after continuous exposure. Responses to accumulation of TiO_2 Nano-particles in systemic organs need to be better evaluated in further studies. In addition, TiO_2 NP-induced generation of reactive oxygen species (ROS) and alterations in cell signal transduction pathways may play an important role in the etiology of carcinogenesis of TiO_2 Nano-particles at relatively high doses. However, these studies should be repeated at lower doses, relevant to normal occupational or environmental exposure conditions, where particle overload is not an issue. Despite this, the results currently available imply that TiO_2 Nano-particles exhibit greater toxicity than TiO_2 micro-particles. In summary, although TiO_2 Nano-particles have been studied extensively in recent years, there is still much remaining to be elucidated concerning their possible health effects to support risk assessment and management. First, to assure worker and consumer safety, it is urgently important to conduct exposure hazard assessment, which would allow the development of a framework enabling risk management for all commercial TiO_2 Nano-particles. This also includes bio-safety evaluation of TiO_2 nanoparticulate carriers for drug delivery application. Moreover, all future studies on TiO_2 Nano-particles should characterize the physicochemical properties of the Nano-particles, such as size distribution, crystalline structure, surface area, surface coating, etc., as delivered to the biological system. This will allow for better comparison of data from different studies and assist in determination of appropriate dosimetry. Furthermore, long-term animal studies comparing the toxicity and carcinogenicity of TiO_2 microparticles and Nano-particles are especially needed. The focus of these studies must be aimed at both occupational and consumer relevant doses and routes of exposure. Moreover, detailed toxicokinetics studies that include absorption, distribution, metabolism, accumulation, and excretion of TiO_2 Nano-particles through

different exposure routes into the human body are indispensable. In addition, future studies should focus on evaluating systemic responses distinct from the organ of exposure and biomarkers reflecting TiO_2 NP exposure and toxic effects. Finally, the molecular mechanisms by which TiO_2 Nano-particles may cause cancer are unclear. Limited data show that ROS generation and signal alterations of certain cancer related genes may be involved in the carcinogenicity of TiO_2 Nano-particles. Therefore, further investigation is needed to elucidate the molecular mechanisms of carcinogenicity for TiO_2 Nano-particles

Silver, like titanium, is also a transition metal which occurs mostly in the +1 oxidative state and has the highest electrical and thermal conductivity of all metals. As a precious metal it was and still is used in a wide range of applications like tableware, jewellery and coinage. Due to its physical properties, it is today also used in electrical contacts and conductors, in mirrors and in the catalysis of chemical reactions. Also, silver has been known, for a long time, to possess antimicrobial character, as silver ions are reported to kill bacteria by inhibiting the expression of enzymes and other proteins essential to ATP production. These attributes have resulted in the increased use of silver, in its nanoparticulate form, in wound dressings, water filters, food packaging and even clothing. In 2003, Samsung introduced the first “Silver Sterilization Washing Machine” which claimed that 99.9% of bacteria would be killed, preventing bacteria and mould and suppress the odour and contamination that accompanies bacteria and mould formation. In toxicological studies, it has been reported that silver nanoparticles (15 nm) reduced mitochondrial function drastically and increased membrane leakage in mammalian germline stem cells and that aggregated silver nanoparticles are cytotoxic to alveolar macrophage cells as well as epithelial lung cells. While there are studies showing that silver nanoparticles could be used in bone cement or other implantable devices as antimicrobial agents, other studies show that silver in nanoparticulate form could be toxic for the bone-lining cells and other tissues. Concerns over release of nanoparticles in waste water treatment plants has already been mentioned in several reports (Department for Environment Food and Rural Affairs (DEFRA), 2007, Reijnders, 2006, Scientific Committee on Emerging and Newly-Identified Health Risks (SCENIHR), 2007) with waste water enriched with silver nanoparticles adding to these concerns. Another possible source of silver nanoparticles in the environment is the leaching of particles out of food packages. According to a study, the most likely 26 nanomaterials entering the environment through disposal of food packaging will be clay, and silver nanoparticles (Department for Environment Food and Rural Affairs (DEFRA), 2007). The same study estimates that the particle burden of silver nanoparticles in water would be, with 0.1 µg at 10% market penetration, low compared to other particles like titanium dioxide or latex. However the ubiquitously use of Ag could lead to the selection and growth of resistant bacterial species, difficult to be controlled in the future.

Recently, in Gliga et al [13] work, cells were exposed to citrate coated Ag nano-particles of different primary particle sizes (10, 40 and 75 nm) as well as to 10 nm polyvinylpirrolidone (PVP) coated and 50 nm uncoated Ag nano-particles. The results showed cytotoxicity only of the 10 nm particles independent of surface coating. In contrast, all Ag nano-particles tested caused an increase in overall DNA damage after 24 h assessed by the comet assay, suggesting independent mechanisms for cytotoxicity and DNA damage. However, there was no production of intracellular reactive oxygen species (ROS). The reasons for the higher toxicity of the 10 nm particles were explored by investigating particle agglomeration in cell medium, cellular uptake, intracellular localization and Ag release. Despite different agglomeration patterns, there was no evident difference in the uptake or intracellular localization of the citrate and PVP coated Ag Nano-particles. However, the 10 nm particles released significantly more Ag as Ag^+ cation compared with all other Ag Nano-particles (approx. 24 wt% vs. 4–7 wt%) following 24 h in cell medium. The released fraction in cell medium did not induce any cytotoxicity, thus implying that intracellular Ag release was responsible for the toxicity. In conclusion, small Ag nano-particles (10 nm) are cytotoxic for human lung cells and the toxicity observed is associated with the rate of intracellular Ag release.

Hydrophobins are produced by various fungi. They are present in both patogenic and non-pathogenic fungal species. For example, champignon mushrooms (*Agaricus bisporus*) have a hydrophobin layer on their

caps that gives the surface of the fruiting body its hydrophobic properties and keeps the mushroom from getting wet. After consuming the fruiting body, human organism digests hydrophobins together with the other mushroom components without any negative effects. In pathogenic fungi hydrophins are involved in the interaction of the host and the pathogen as a first recognition site on the fungal surface. However, hydrophins themselves are not involved in damaging the host. It was even shown that hydrophobins on the spore surface of *Aspergillus fumigatus*, one of the most ubiquitous airborne fungal pathogens, prevent recognition of spores by the immune system, thereby reducing allergic reactions to the pathogen. The hydrophobins, used in the project, originate from non-pathogenic organisms *Aspergillus nidulans* and *Trichoderma reesei* and present no risk for human health.

In this project a fusion of hydrophobins with antimicrobial peptides is intended to prevent microbial growth on stone material. Antimicrobial peptides are produced in different organisms, among others in humans. They are the natural defense instruments against microbial infections. Currently, the possibility of using synthetic antimicrobial peptides as alternative for antibiotics in medicine is being investigated. There are studies, showing the effectiveness of antimicrobial peptides in external treatments of wounds infected with antibiotica-resistant bacteria. There is no scientific indication that fusion of antimicrobial peptides with hydrophobins could cause risks related to human health.

3 Conclusions

The research about the impact of nano-particles onto health and environment is ongoing. In the present deliverable a state of art related to this topic is presented in order to assist the researchers in the selection of nano-particles keeping into account these peculiar and fundamental aspects.

The exposure of body or environment to the nano-particles used for stone consolidation of protection is anyway much limited. In fact, in consolidation the nanoparticles are deposited in the stone porosity and are linked to the host stone. Hence their release in environment is quite limited. In stone protection a coating containing nano-particles is deposited on stone surface. The traditional polymer matrix formed onto stone surface can prevent the release of nanoparticles. Moreover, as the degradation of polymeric matrix can be extremely long (centuries), the release of nano-particles should be really limited.

Among the different investigated inorganic nano-particles, the use of ZnO can be critical, as evidenced by the systematic and comparative work performed in the NANOSAFE2 project[8]. However, systematic studies can be found only for titania, silver and zinconia nano-particles, whereas for other nano-particles more research work is required. In the deliverable D4.6 - Report on human and environmental hazard evaluation, that will be delivered at month 28, more detailed and specific studies will be reported related to the nano-particles selected in the project work. In that context a proper updating of the literature survey will be included.

In general the dependence of inorganic nano-particles dimension on health toxicity is reported. If nano-particle dimension is lower than 10 nm usually the toxicity of nano-particles is enhanced. As the nano-particles, especially in consolidation, should be small, in order to fill well the stone porosities, it would be necessary to keep into account that this can represent an issue. Hence it would be necessary to select the dimension that on one hand grant an efficient consolidation, but on the other hand, can be manipulated in safety for operators.

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