WHITE BOOK

EXPOSURE TO ENGINEERED NANOMATERIALS AND OCCUPATIONAL HEALTH AND SAFETY EFFECTS

Produced by INAIL,
Department of Occupational Medicine, formerly ISPESL

National Network for the identification of preventive and protective measures related to the occupational exposure to nanomaterials (NanOSH Italia)

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Preface

The Italian National Institute for Occupational Safety and Prevention - ISPESL - has been supporting an analysis of the current health and safety issues related to the workplace nanotechnology development. Nanotechnology applications include health sector, biotechnology, production of clean energy, information and communication, chemistry, electronics, military sectors, agriculture and construction industry. It has been estimated that, by 2020, approximately 20% of all goods manufactured worldwide will involve nanotechnology, although these technologies are emerging and the risks associated to the production and the employment of nanomaterials are mostly unknown .

A substantial imbalance exists between the knowledge of nanotechnology applications and their impact on the human health. The information available concerning the effects of nanotechnologies on health and the nanomaterials risk assessment in the workplace is scarce; systematic approaches to assess exposures to nanomaterials remain unknown and the intensive and richly diversified use of nanomaterials in industry makes it difficult to estimate the number of workers exposed to them.

This knowledge gap calls for the scientific community in the field of Occupational Health and Safety to gather efforts to provide a shared opinion on health and safety of workers employing, handling and producing nanomaterials. A national and international cooperation is crucial to properly assess and manage such emerging risk. In this perspective, ISPESL launched a range of research initiatives in this area, including the creation of an ad-hoc National Network (called "NanOSH Italia") aimed at promoting cooperation and launching integrated research activities within the framework of occupational risk of exposure to nanomaterials through a multidisciplinary approach to risk assessment.

The first output of such cooperation is the publication of this White Book intended to start an important and authoritative debate to outline the necessary policies and ensure the development of nanotechnologies in Italy while respecting the laws of competitiveness and sustainability on the one hand and the reduction of health risks for workers on the other.

The Scientific Coordinator
Sergio Iavicoli M.D., Ph.D.

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Executive summary

1. Nanomaterials definitions

Many aspects of our existence and working life will soon be affected by nanotechnologies, thus fostering innovation. Therefore, it is essential to provide the industry and research with the right tools to develop and employ such technologies in a responsible and sustainable way. Moreover, an efficient evaluation approaches and assessment protocols based on sure and certain standards are also required.
The use of an unambiguous terminology for the description of fundamental concepts, definitions or classifications, acknowledged by the main bodies and institutions in this field, provides a starting point to deal with a new approach to occupational health and safety issues with respect to the emerging risk of nanotechnologies exposure.
The White Book covers the occupational exposure to engineered (or specifically produced) nanomaterials (NM). These have very different chemical and physical features from other environmental particulates that make them hazardous to human health such as dimension, mass, chemical composition, surface area, concentration, aggregation and agglomeration status, water solubility, surface chemistry, morphological structure; nevertheless, to date there is not an unequivocal opinion on the specific correlations between nanomaterials and toxic effects.
Industrial hygiene studies may serve as a starting point for the development of a broad risk assessment approach; however, in order to identify the hygienic limit values for the exposure and for the setting up of adequate prevention and protection systems for workers and the environment, it is fundamental to univocally identify the "right" parameters relating to toxicity aspects of nanomaterials that, according to the studies conducted on the biological interaction, need to be assessed through a multiparametric and metrological approach based on specific exposure evaluation and monitoring techniques at work.

2. Perspectives in the Italian production sectors

Being nanotechnologies involved in a wide array of scientific disciplines and applicative sectors, they can hardly be fit into specific productive and development sectors or end-market.
"The Second Nanotech IT Census of Nanotechnology in Italy", conducted in 2006 by AIRI/Nanotec IT provides a picture of the Italian situation in the field of nanotechnologies. The census identified 180 public research centres and companies actively engaged in nanotech all over the country: 57% in the North, 28% in the centre and the remaining 15% in the South.

A number of initiative have been put in place to improve the use of resources, increase the overall operating efficiency and strengthen the commitment: Centres of Excellence in nanotechnology have been established in different Universities; many research activities, although located in different sites, have been brought together and assigned common objectives; some Technological Districts have given priority to research in nanotech.
The ongoing research and development activities, both at public and private level, involve a wide array of fields such as chemistry and materials (structural and functional), nanoelectronics and photonics, bio(nano)science, medical science and instrumentation. Potential applications involve fundamental productive sectors varying from pharmaceutics to the production of electromedical devices, from cosmetics to electronics and information technology, from transportation to environment and energy production as well as fields relating to small and medium enterprises such as textiles, fashion, shoe and food industry, building materials, advanced mechanics and the protection of cultural goods.
Furthermore, nanoscience and nanotechnologies play an outstanding role in the educational programs in the main Italian Research Entities and Universities providing internationally acknowledged structures and competences.
The National Research Institute (CNR) in concert with the Italian National Health Institute (ISS), the National Institute of Nuclear Physics (INFN), the National Institute of Metrological Research (I.N.RI.M.), the National Institute for Occupational Safety and Prevention (ISPESL) and the National Institute for work-related injury insurance (INAIL) are taking joint action to deal with the cross-sectional aspects of nanotechnologies, such as characterization and metrology of nanomaterials and related risks.
Finally, a recent study conducted by the Italian Association for the Industrial Research (AIRI) in 2009 defines quite clearly the technological development needs for the most innovative part of the industrial system and the Italian advances services. Nanotechnologies have a particularly prominent role in microelectronics and semiconductors manufacturing, chemistry, pharmaceutics, biotechnologies, energy production, environment with a short to medium term development perspectives (usually 3 years).

3. Research needs and mapping

The main funded research initiatives on the impact of nanotechnology on human health, environment and safety launched at national level, as partnerships or project coordination, are included within the EC Framework Research Programmes and projects funded by some Italian Ministries and Regions and evidently play a leading role in research on Occupational Health and Safety.

Since 1984, at the European level, the Framework Programmes (FPs) for Research and Technological Development have been the main financial tool created by the European Commission, the executive body of the European Union, to support and encourage research on technological innovations based on transnational collaboration in the European Research Area. Over the last 25 years, seven FPs have taken place: the FP7, the last one, started in 2007 and will run until 2013.
A number of Italian organizations are getting involved in the funded research projects focused on the impact of nanotechnology on environment, health and safety (EHS) and, as a consequence, on occupational health and safety. Within FP6, the Italian partners were involved in 7 research projects out of 15 addressing the impact of nanotechnology on health and safety, and within FP7, Italian organizations took part in 4 out of 10 projects launched from 2007 until September 2009.
Within the last three FPs (1998-2009), 12 out of a total of 28 funded projects involved Italian partners. All the 19 Italian partnerships are developed by 16 different organizations (some of them participating in more than one of them). Public Research Entities account for 43% of the partnerships, private companies for 38% and non-governmental organizations (NGO) for 19%.
As for the financial plan, more than 50% of the funding for the research on the EHS impact of nanotechnology have been allocated to projects involving at least one Italian partner.
Initiatives funded by some Italian Regions are also worth mentioning: Lombardy Region launched the "Nanoscience for materials and biomedical applications" project and the European Centre of Nanomedicine Foundation (CEN); Piedmont Region launched the "NANOSAFE" and "Cytotoxic and genotoxic damage of nano and micro silica particles: molecular basis and strategies for prevention and inactivation" and "Biocompatible, nanostructured materials for biomedical applications"; Veneto Region has funded 6 projects in concert with the Nanotech Veneto District, launched in 2005.
From 2004 up to 2009, the Ministry of Education, University and research assigned approximately 650.000 Euros to 5 projects addressing the impact of nanomaterials on the human health, the molecular mechanism underlying cellular response, the interactions with biological systems, cytotoxicity and genotoxicity mechanisms, physical and chemical testing and toxicity studies of in vivo and in vitro models.
Finally, the Ministry of Health assigned an amount of 465.000 Euros to the "Nano-OSH Italia" project within the announcement of 2006 strategic health research programme coordinated by ISPESL: this project which is expected to run until 2011 aims at developing an innovative approach for a preventive assessment of workplace exposure to the functionalized carbon nanotubes.

4. Protocols for information gathering and exposure characterization of nanomaterials

A number of professional sectors deal with exposure to nanomaterials (NM) and nanoparticles (NP): from productive sectors to those where particles of nanometric dimensions are occasionally released during specific processes or operational cycles as by-products of thermal and chemical reactions.
The "voluntary" production of nanomaterials for nanotechnologies is realized under a "bottom-up" approach, which is the piercing together of atoms to give rise to materials according to pre-established schemes (through chemical and physical processes) and under a "top-down" approach, as a part of the electronic industry, which consists of breaking down materials and components and usually involves mechanical processes.
However, as already underlined, NPs in the workplaces do not always represent the final product of the technological cycle, because metallic NPs and/or metallic oxides are produced during chemical and physical processes such as combustions, nucleation and condensation, metal manufacturing and refining, high-temperature spray application, soldering, grinding, and carving of metals or alloys.
The characteristics of particles generated during those processes depend on the chemical-physical conditions of the area they take place; however, primary particles usually have a diameter of 10 to 15 nm and coagulate rapidly according to their concentration in the point of origin and end up becoming bigger than NPs.
Potential exposure routes. The primary route of exposure to airborne particles in the workplace is inhalation; NP compounds deposit in different regions of the lung according to their diameter but predominantly in its alveolar, tracheobronchial and extrathoracic region. Once deposited, the destiny of NPs is determined by their biopersistence and potential to translocate to other tissues; though, research is being carried out today to determine the factors that regulate such phenomena and the mechanisms that contribute to the NPs clustering and de-clustering as well as to discover NPs role in the toxic activity following the inhalation. In particular, a high percentage of NPs deposit all along the airways and an increase of their diameter determines the diminishing of their total deposition and an increase of their alveolar component.
Interaction between NPs and biological systems, on the contrary, may vary according to the specific chemical-physical characteristics of NPs whereas the nanofibers deposition is strictly dependant on the type of fiber.
Within such a context, to identify the inhalation reference exposure limit values (still to be established at national and international level), further investigation is required on

the differences and analogies between the biological impacts of discrete NPs and NPs agglomerates/aggregates (containing the same volume of material and undergoing disaggregation and deagglomeration processes after deposition) which settle in the respiratory system. Should exposure to discrete NPs and NPs agglomerates/aggregates produce similar effects on the human health (notwithstanding the dimensions of the deposited particles), such values have to be set considering both discrete NPs and NPs agglomerates/aggregates. Otherwise, differentiated hygienic limit values should be set.
As for the dermal exposure to NPs, studies are investigating the potential penetration of NPs (in particular TiO and ZnO) through intact skin and their harmful potential effects.
Finally, the olfactory system and the gastro-intestinal tract may be affected by a considerable amount of NPs with small aerodynamic diameter. NPs can deposit in the upper airways, in particular in the olfactory mucosa, and be absorbed in the central nervous system through the olfactory nerves or ingested through mucous which incorporates and removes NPs deposited in the respiratory tract, contaminated food and water or oral contact with contaminated hands or surfaces.
Characterization of NMs in inhalation exposure. Considering the different chemical and physical characteristics of NPs (dimensions, morphology, chemical composition, surface area etc.) that may cause potential hazardous effects to human health, a specific critical issue is represented by the metrological aspects of the NMs inhalation exposure characterization. Recent studies aim at identifying the "right" parameters for the definition of NP exposure levels through in-door sampling and analysis techniques. Although hygienic standards have not been identified, the assessment approaches to the evaluation of the NP exposure in occupational settings are regulated by ISO/TR 27628, 2007 and ISO/TR 12885, 2008 indicating best evaluation methods for the inhalation exposure to NPs, nanostructured aerosols and engineered NPs.
Monitoring and characterization techniques now available in this field and covered by the above mentioned regulations permit to evaluate the exposure to nano-sized particles with respect to mass (also associated with their chemical characterization), number concentration, surface area, morphological analysis through a wide array of tools. Though, it is necessary to harmonize the different analysis systems existing today in order to optimize the global process of risk assessment associated with NPs and overcome the difficulties related to the adoption of a single measurement system. Although the mass determination, for example, through static cascade impactors sampling dimensional materials, permits the chemical characterization of particles gathered on substratum (by means of specific analytical off-line techniques) it also requires the use of proper instruments to import data relating to the quantity and surface characteristics as well as to distinguish NP agglomerates/aggregates from the single NPs.

NP samplers and counters available today, however, need to be adapted in terms of costs, compactness and portability features for a routine workplace application. The assessment and characterization of workplace exposure to nanoaerosols is hindered by the lack of proper "individual" NP counters and, as a consequence, the combined and contemporary use of multiple equipments for *in-situ* measurements and off-line analysis of the most relevant parameters is today the best approach to the assessment of the occupational exposure to nanoparticles. It is, therefore, necessary to identify an appropriate sampling strategy addressing the interpretative limits to estimate the individual NPs exposure based on area samplings. Data collected through static samplers depend on the aerosols characteristics varying according to the source (distance, emissions, multi-source, etc.) as well as to the movements of the air caused by forced ventilation and gas-phase nucleation or accretion of chemical species by coagulation or condensation leading to spatial and temporal variation of nanoaerosol mass and number concentration. Results of static NP samplings, therefore, require a specific assessment if considered with respect to the worker's "individual exposure" and some fundamental aspects must be taken into account to plan a proper monitoring strategy. Through a detailed analysis of the exposure scenario (evaluation of further occupational activities entailing the use of working tools or the presence of smoke or the detection of secondary sources, etc.), also considering the air flow movements (due to forced ventilation, for example) which determine the spatial and temporal variations of aerosols, the sampling areas need to be properly selected in order to ensure a correct interpretation of data with respect to the individual exposure. In particular, due to the different origins of the NP aerosols, an optimization of sampling and analysis techniques must be considered in order to identify the potential involuntary emission sources (by means of sources profiles achieved through Principal Component Analysis) also taking into account external contributions.
As long as an appropriate metrological system for the assessment of NP exposure and professional NP exposure limit values are identified, it is pivotal to set up sampling strategies and evaluation protocols aimed at determining the chemical composition and the dimension distribution of nanoparticles through a multiparametric approach (including the characterizations of impurities due to the potential presence of organic compounds in the engineered NMs imputable to the production process).

5. Effects of engineered nanomaterials on health

Epidemiologic studies and information about toxic effects of nanomaterials (NM) on exposed population are still not available. Most of the studies in this area were conducted *in vitro* or on laboratory animals (primarily mice) and the information con-

cerning the effects on organs and apparati are extrapolated from estimations at a cellular level. Recent studies showed the potential genotoxic, cytotoxic and oxidative effects of NMs at cellular level and the respiratory, dermal, neurotoxic, cardiovascular, immunological effects caused by NM exposure.

Genotoxic and oxidative effects. Studies on NM genotoxicity investigate primarily on carbon nanotube (CNT) and metal oxide particles which may cause DNA damage either directly or indirectly through the induction of oxidative stress. Depending upon the dimensions and aggregation state of nanomaterials, they may be able to penetrate into the cell through passive diffusion and endocytosis, then into the nucleus through diffusion across the nuclear membrane, transport via the nuclear pore complexes or thanks nuclear membrane dissolving during the cell division. Once within the nucleus, NMs may interact with DNA or histonic proteins, thus causing DNA damage. Genotoxic damage may also be caused indirectly by the interaction with other cellular proteins, such as those taking part in the cell divisions processes, through the generation of free oxygen radicals, inflammatory phenomena, alteration of proteins involved in DNA repair.

Cytotoxic effects. Today, numerous studies on cytotoxic effects of NMs are available and they show a wide variability among nanoparticles (NP) in terms of their ability to cause toxic effects. Cytotoxic and apoptotic effects on CNTs have been demonstrated but they presumably depend on the aggregation state, the presence of metal catalyst, functionalization and purity degree, length and diameter. Fullerenes are likely to be less cytotoxic even though the response provided depends on the cell type; about metallic NPs a wide variability of cellular response has been observed according to the type of metal: effects have been observed in silver, copper, zinc, molybdenum and aluminum NPs. As far as quantum dots are concerned, cytotoxic effects vary according to dimensions and type of coating.

Respiratory effects. *In vitro* and *in vivo* studies reported that lungs are the main target organ for NM toxic effects. Most of the studies on potential effect of NMs on the respiratory system cover the CNT issue and their negative effects are likely to be correlated to the NMs toxicity on different cell population, to their ability to cause fibrosis, their "asbestos-like" activity, bioaccumulation and potentially low biodegradation levels of such NMs. In particular, some correlations have been observed between the pathogenic properties of multi-walled CNTs (MWCNTs) and those of asbestos fibers with respect to their ability to cause inflammations and oxidative stress. Comprehensively, *in vitro* studies suggest that engineered NMs may produce cytotoxic effects on the observed biological systems, in particular on bronchial and pulmonary cells and alveolar macrophages. *In vivo* studies highlight how NM exposure may induce an acute pulmonary inflammatory response, granulomatous and emphy-

semic lesions, collagen deposition with a consequent interstitial fibrosis and the induction of a significant amount of oxidative stress. Nevertheless, toxicity varies considerably among NMs and this complexity makes generalizations impossible. Further studies are therefore required to define NMs toxicity in the respiratory system.

Dermal effects. At the dermal level, there is evidence that NPs may cause local irritative action on keratinocytes and partially penetrate skin's corneous layer and the epidermis thus entering the systemic circulation: however, the data available to date are too scarce to allow definitive conclusions. Currently, much information is provided by the pharmaceutical industry that investigated the effects of zinc oxide (ZnO) and titanium dioxide (TiO_2) NPs used in skin protection creams. Important data concerning the human health are not yet available, even though a number of cosmetic products, especially sunscreens, have contained NPs since 1997. More systematic research is then required to define the dermal risk of NP exposure.

Effect on the Central Nervous System. *In vitro* and *in vivo* studies showed the ability of NPs to induce neurotoxic effects. In particular, significant neurotoxic effects have been observed on human and animal neuronal and glial cells and in different types of animals exposed to NPs. The main mechanism through which NMs show their toxicity, including within the Central Nervous System (CNS), is the induction of a relevant oxidative stress. Moreover, NPs might alter the integrity of the emato-encephalic barrier (EEB) and modulate the expression of a number of genes involved in apoptosis and inflammatory response.

More research is expected on the neurotoxic effects of engineered NMs, other than metallic NMs, such as carbon NMs and quantum dots.

Cardiovascular effects. With respect to the cardiovascular apparatus, atheroma, arterial thrombosis and platelet aggregation are induced in mice and rats following exposure to CNTs. Further studies assessed the potential effects of CNTs on the systemic inflammation which is considered to be by now one of the main predisposing factor for the development of atherosclerosis, and highlighted a significant activation of the systemic inflammatory parameters and of biomarkers of neutrophil activation.

It is mandatory, before drawing to definitive conclusions on the potential cardiovascular effects of the engineered NPs, to conduct further studies aimed at reflecting faithfully the exposure conditions expected in the workplace and the environment .

Immunological effects. Scarce data, primarily derived from *in vitro* studies, are available to date on the effects of NMs on the immune system. These suggest that NPs, once in the systemic circle, may interact with proteins circulating or deposited on the cell surface thus determining the exposure of usually unexposed aminoacidic residues (cryptic epitopes) and the potential autoimmune response. One more potential of damage mechanisms is represented by the interference with opsonizations and,

therefore, with the clearance of extraneous materials (i.e. microorganisms) usually removed through such process. Available data are insufficient to form an opinion on the toxicity of engineered NPs on the immune system even in consideration of the used high doses.
Overall, the available studies show a wide variability among cytotoxic and genotoxic effects depending upon the specific characteristics of NMs which need to be taken into consideration.
Moreover, as high amount of NMs are employed in the studies, further research is expected to be conducted on the potential exposure to the most used NMs and at lower concentrations.
Finally, *in vivo* studies involving organs and apparati demonstrate that the respiratory, nervous and cardiovascular systems appear to be more affected even though data are still insufficient to provide clear answers on the effects of engineered NMs on the human health.

6. Risk assessment and risk management

Nanomaterials (NMs) may determine new risks of exposure for an ever growing number of workers, due to their widespread use in the industrial field. In this view, NMs have been included in the community strategy on occupational health and safety as a topic to address within the framework of new and emerging risk identification. Moreover, the European Commission has developed an action plan for the implementation of a safe, integrated and responsible approach to nanotechnologies. More information is, therefore, required for an effective management of the exposure risk. To reach such goal, further knowledge is needed on the approach to adopt to the assessment of NM exposure risk and on the prevention and protection measures required to ensure proper risk management and reduction.
For NMs, it is possible to adopt the approach already used for the risk assessment of workers being exposed to chemical substances. In particular, the "risk assessment" is to be considered as the collection of the knowledge-based and operational procedures required to assess health and safety risks of workers associated with the exposure to NMs, according with specific work activity.
Risk assessment is a complex and iterative operation which necessarily requires, in every environment or workplace, the identification of sources of the occupational exposure to NMs, the identification of subsequent NM exposure risks with respect to the specific work activity and the assessment of NM exposure risks in relation to the welfare protection policies identified.
According to the precautionary principle, it is necessary to minimize the exposure

and this can be achieved by reducing the duration of exposure and/or the number of people exposed, but also by reducing the concentrations of NMs through the implementation of efficient prevention and protection measures. These should, in order of priority, eliminate NMs, replace them with non-hazardous materials, isolate/confine/disgregate exposure sources, identify technical measures to capture, limit and expel NMs, to modify the work organization and to employ the PPDs as an integration of such technical measures.
Finally, the implementation of a series of good practices (through, for example, the control banding approach to the risk management programme) can help to minimize workers' exposure to NMs.

7. Prospects for policies and communication strategies

The regulation of production and employment of nanotechnologies and, in particular, of the so called engineered nanomaterials (NMs) is necessary to reduce the risks for Environment, Health and Safety (EHS) and to manage the Ethical, Legal and Social Implications (ELSI) of nanotechnologies.
Currently, the definition of a satisfactory regulation system is hindered by uncertainties in the characterization of NMs and their impact on EHS; the development of new approaches to assess the risk and the exposure values for nanomaterials is far from easy task.
Some key issues are still the focus of an intense debate among institutions and stakeholders, due to:
- the wide variety of materials and applications;
- the unique features and behaviours of nanomaterials (at nanoscale);
- the lack of shared technical regulations on materials nomenclature and metrology;
- the proprietary nature of information;

the need for communication and comparisons among disciplines.
Therefore, regulation in this field depends substantially and basically on the knowledge development.
Furthermore, another issue to be addressed concerns the normative instruments for the implementation of the regulation; they can be:

- the legislative route (*hard regulation*) entailing the creation of binding rules and a sanction system providing an incentive to abide by them. This route, however, is hampered by the above mentioned knowledge-related uncertainties and by the difficulty to find the proper consensus for the definition (and approval of binding rules) of equilibrium points to meet protection requirements and, at the same time, not to compromise research and development (and the potential benefits). The

analysis shows that, generally, the regulation in the field of nanotechnologies is obtained through the adaptation (with technical specifications, guidelines etc.) and integration of the existing regulatory framework for the production, use and marketing of chemical substances and/or related to occupational safety and health and environmental protection.

Moreover, with respect to the regulation of activities involving the use of NMs, the applicability of the precautionary principle and the identification of the subject intended to assure the non-hazardousness of products (either the producer or public authorities) are some of the important issues still being discussed.

- the development of voluntary measures (*self regulation*) whose validity is acknowledged by stakeholders to identify contact points among all interests at stake. However, when promoted at institutional level, the efficacy of the voluntary measure is often invalidated by a limited spontaneous adhesion. With respect to this kind of measures, the analysis conducted enabled to identify three levels of intervention:
  - Monitoring Systems whose aim is to improve the knowledge of operators involved in the regulating the relevance and dissemination of NMs as well as their production modalities;
  - Codes of conduct aiming at defining values, principles and guidelines which can help a safe and responsible development of nanotechnologies;
  - Risk Management Systems, generally developed at the industrial level, for a safer management, handling and use of engineered NMs.
- the development of technical rules (*Standards*) currently intended to define, outline, specify, assess and characterize NMs and nanoproducts and, primarily, designed to develop binding or voluntary regulation levels. Sometimes performance standards and risk management approaches can be developed as a regulatory tool for specific activities which can be voluntarily used by operators to attest the accuracy of their own performances.

  The effectiveness of standards, not included in the binding regulatory framework, mainly depends upon the authority of the issuing organization and their scientific validity widely acknowledged by stakeholders. The study carried out demonstrates that numerous strategies have been launched at national and international level to set standards for nanotechnology.

Communication has a key role in the occupational risk management of handling and use of nanomaterials. An appropriate communication is pivotal to ensure efficient behaviours of workers in terms of prevention and to avoid unfounded scientific alarmism and promote employers' proactivity. Communication is key to strengthen

existing rules and to fill some gaps of the regulatory framework, through the promotion of voluntary and efficient behaviours among workers and the adoption of self-regulatory codes among employers.
An appropriate communication should be:

- Credible: the sources must be authoritative and reliable for recipients. In the specific field on nanotechnologies, where the current state of knowledge is somehow still insufficient, the risk of contradiction among sources must be considered.
- Correct: the message must be complete (not characterized by omissions aimed at producing a persuasive effect), objective and supported by a scientific feedback. In this respect, communication in nanotechnologies is another peculiar problems due to the lack, in some cases, of certain data related to their impacts and to the uncertainties in the risk assessment. Conveying incorrect messages may irreparably compromise the credibility of a source.
- Clear: that is easily and fully comprehensible to recipients. In nanotechnologies, this means to overcome the complex knowledge transfer difficulties and avoid a technical terminology which is not immediately intelligible to non-experts. It is important to bear in mind that an excessive simplification of the message could compromise correctness.

Moreover, an analysis of the information channels and of the message recipients must be conducted to develop an efficient communication strategy. Even though there are not precise rules to choose the most adequate instruments to reach goals, it is important to take into account the relevant characteristics of subjects or groups involved in order to reach everyone in the most efficient way. It is essential to gain a deep knowledge of the potential message recipients (their perception and comprehension of the issues being addressed, their expectations, their states of knowledge, etc.) in order to provide them, through the use of the most efficient information channels, with accurate and easily accessible sources of information.
Nevertheless, bi-directional communication, involving directly the recipients and the direct interaction with the bearers of knowledge (and viewpoints) so as to give an overall picture of the issues being dealt with, appears to be the more efficient instrument to address the theme of risks associated with the use and development of nanotechnologies which might very likely create skepticism, distrust and indifference.
Lastly, one more aspect to take into account concerns the way the message is put forward which influences the receptivity of the audience to the issues faced. In this case, it can be useful to focus on message framing in persuasive communications and contextualize the message by adapting it to the actual use of nanotechnologies instead of a highly specific laboratory situation.

Introduction

In the field of nanotechnology, the gap between technological progress and research on Occupational Health and Safety is still huge. Studies on the health effects and nanomaterial (NM) exposure risk analysis are scarce and there are not validated approaches to the workplace risk assessment. Therefore, due to the imbalance between a scarce knowledge of NM exposure risks and the exponential spreading of nanotechnologies over the next years, it is necessary to develop a research that focuses on the risk analysis for workers exposure and highlights critical issues and the needs of occupational health and safety policies on the development of nanotechnologies with a view to directing efforts towards a responsible and sustainable approach to their use.

In 2008, the Italian National Institute for Occupational Safety and Prevention - ISPESL - launched the "National Network for the identification of preventive and protective measures related to the occupational exposure to nanomaterials" (NanOSH Italia) aimed to:

- enhance and consolidate at national level the cooperation in the research activities on the occupational NM exposure risks, through a survey of funding needs, priorities and possibilities;
- develop a multidisciplinary approach to risk assessment through the promotion of integrated research activities;
- identify adequate instruments for the promotion of communication and knowledge transfers in the field.

The Network is made up of ISPESL researchers involved in the field of occupational health and safety with regard to NMs and representatives from Universities and Agencies that have proved sensitive to the issue, at national level.

First output of this work is the White Book on occupational exposure to engineered NMs; its main objectives are to define the state of the art of Italian research in this field, and to start debate over the impact of nanotechnology on the human health and safety in the workplace and over regulatory perspectives with a closer involvement of social partners.

The work was carried out in two separate phases; in the first phase the plan and schedule of this paper, as well as the key topics to be addressed and the expert subgroups have been defined; the White Book has been published after editorial review. In the second phase, a process for identifying national stakeholders playing an active role in nanotechnologies with different approach to the matter has started. The stakeholders involvement in a consultation process allows to obtain contributions from institutions, business, research and economics which take part

in responsible and sustainable development of nanotechnology. This White book, through knowledge and opinion acquisition, provides a national level review of perspectives and issues related to the development of nanotechnologies and occupational risks.

chapter 1

Nanomaterials definitions

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1.1 Nanomaterials definition: sizes and structures

Nanotechnologies will soon impact every facet of our lives and our work organization, thus opening the way to outstanding innovations in the field of communication, health, manufacturing, materials and knowledge-based technologies. Most of the authorities in this field are therefore convinced that providing the industry and research the proper tools for the development and application of such technologies is essential. Moreover, it is fundamental that legislators and regulatory agencies involved in the environment and health protection are provided with efficient evaluation approaches and assessment protocols based on undoubtedly certain standards (ISO, 2010).
Some key concepts need to be defined in order to illustrate the scope of this book. First of all, "Nanosciences" is the output of a cooperation among physics, chemistry, biology, biotechnology, materials science and engineering for studying phenomena and manipulation of materials at atomic, molecular scales, where properties differ significantly from those at a larger scale. (Royal Society, 2004; IRGC, 2006).
In this publication, the definition of nanotechnologies is provided by the *National Nanotechnology Initiative* (NNI, 2006 a,b,c), the Research and Development program established by the Federal Government of the United States to coordinate all efforts across the fields of nanoscale sciences, engineering and technology. According to such definition, the term "Nanotechnology" can be associated with:

1. the development of research and technology at atomic, molecular and macromolecular level, in a range of the nanoscale varying from 1 to 100 nm;
2. the design and application of structures, devices and systems which are innovative in size;

3. the ability to control and manipulate the matter at the atomic scale. This definition has been included in the nanotechnology reference documents by the American *Environmental Protection Agency* (EPA) and by the *National Institute of Occupational Health and Safety* (NIOSH) (EPA, 2007; NIOSH, 2009).

Some definitions include nanomaterials (NMs) that are larger than 100 nm (www.lanl.gov/mst/nano/definition.html), other identify NMs as "having structured components with at least one dimension less than 100 nm" (Royal Society, 2004) and including nanoparticles (NPs) with all three external dimensions in the size range from 1nm to 100 nm (ISO, 2010).
This publication refers to NMs as those intentionally produced in the laboratory or industrial settings. These "engineered" nanoscale materials would exclude the broad range of naturally occurring particles (from forest fires or biological particles, etc.) and the unintentionally released by anthropogenic products (from diesel engines, power plants, etc.), although their sizes fall in the 1-100 nm range.
Engineered nanoscale materials can be further subdivided according to the production process they undergo: they can be made by either "top down" or "bottom up" techniques (Royal Society, 2004). Top-down processing involves cutting or milling of a larger single sample of material to obtain the nanoscale material in the desired configuration, while bottom-up approaches assemble smaller subunits to obtain the larger nanoscale material through processes such as the chemical synthesis. Many top-down applications, such as the lithographic processes used to manufacture computer chips have been used for years, while other bottom-up approaches, such as production of carbon nanotubes are relatively new. The specific technique used to produce a nanoscale material could influence the human health risk associated with that material (Thomas K and Sayre P, 2005).
The conceptual underpinnings of nanotechnologies were first laid out in 1959 by the physicist Richard Feynman in his lecture, "There's plenty of room at the bottom" (Feynman R, 1959). He explored the possibility of manipulating material at the scale of individual atoms and molecules, imagining the whole of the Encyclopaedia Britannica written on the head of a pin and foreseeing the increasing ability to examine and control matter at the nanoscale. The term "nanotechnology" was not used until 1974, when Norio Taniguchi, a researcher at the University of Tokyo, used it to refer to the ability to build engineering materials precisely at the nanometer level. The primary driving force for miniaturization at that time came from the electronics industry, which aimed to develop tools to create smaller (and therefore faster and more complex) electronic devices on silicon chips. Furthermore, at IBM in the United States, a technique called electron beam lithography was used to cre-

ate nanostructures and devices as small as 40 to 70 nm in the early 1970s (Royal Society, 2004).
Since the beginning of the new millennium, the development of nanotechnologies has gained an exponential trend, underpinned by the number of commercialized products and by the funds allocated to research and development in this field.
Four overlapping generations of new nanotechnology products and processes (called below "nanoproducts") have been identified by Roco et al. (Roco MC, 2004; Roco MC, 2006; Renn O and Roco MC, 2006) which had potential for development in 2000-2020.
Each generation of products is marked by the creation of commercial prototypes:

1. Passive nanostructures (2001): output of the primary research on nanostructured materials and instruments for the assessment and control of nanometric processes, such as NPs, NMs and carbon nanotubes.
2. Active nanostructures (2005): energy storage and conversion devices and systems; instruments for molecular medicine and food system; nanoelectronics, nanoscale 3D instruments and manufacturing.
3. Systems of nanosystems (2010): heterogeneous nanostructures and engineered supramolecular systems, such as artificial tissues and sensorial systems; quantum interactions for nanoscale systems; nanoscale electromechanical systems; cell therapy with nanodevices.
4. Molecular nanosystems (2015): atomic manipulation for the designing of new atomic and supramolecular systems; dynamics at the single molecule level; molecular machines; design of big and heterogeneous molecular systems; controlled interaction between light and matter with relevance to energy conversion.

Today, this trend seems to be accelerating, thus anticipating the start up process for each of the above mentioned prototypes (Iavicoli S et al, 2009).
Nanotechnologies, more than other fields, require the integration of different sciences with engineering and technical competences and disciplines. Their applications will impact every facet of the human life (communication, health, work, mobility, house, leisure, energy, alimentation) and will bring about social , economic and ecological changes (IRGC, 2006).
However, properties making NMs unique from a chemical, physical and biological viewpoint, if compared to larger particles of the same material, may cause a risk for health and environment. Materials reduced to the nanoscale exhibit properties that influence their physical, chemical and biological behaviours with environment and human health impacts that are still far from a precise interpretation.
Along with nanotechnologies, which deliberately produce and employ these specific

chemical structures, a wide range of productive sectors and work/life environments where NMs may represent a source of risk (NM manufacturing, disposal or recycling, or the formulation and employment of products containing NMs,) can be considered for drawing up preventive measures.

1.1.1 Hygienic-industrial definitions

Talking about microparticles and NPs, it is necessary to draw on an important physicochemical concept and apply it to the industrial hygiene which is deeply involved in the aerosol particle sampling with fractionation of dimensional classes. Granulometric fractions of airborne dusts are classified according to the particle "aerodynamic diameter", defined as "the diameter of a sphere with the density of 1 g/cm^3 that has the same settling velocity at the same temperature, pressure and humidity".
The UNI-EN 481:1994 standard "Workplace atmospheres - Granulometric fraction definitions for measurement of airborne particles" defines clearly the three inhalable, thoracic and respirable dust fractions and provides technical specification on the necessary sampling instruments. (HSE, 2004).
On the contrary, talking about "fine dusts", "ultrafine particles", the definition of their dimensional range is not provided in literature. In 1998, ultrafine dust were defined by Preining as having a diameter below 100 nm (Preining O, 1998). Similarly, the Royal Society put forward a definition of nanotechnology according to which NPs have a diameter smaller than 100 nm (Royal Society, 2004).
The International Standard Organization defines a nanoparticle as "a particle having a diameter sufficiently small for chemical and physical properties […]. A nominal, but not exclusive, NP tip diameter is of 40 nm". Furthermore, the standard defines two important classes of airborne particles (ISO, 2007):

1. *agglomerate*: group of particles held together by relatively weak forces, including Van der Waals forces, electrostatic forces and surface tension;
2. *aggregate:* heterogeneous particle in which the various components are held together by relatively strong forces and thus not easily broken apart.

These definitions are particularly important for the toxicological properties and their measurement (sampling and analysis).
Particles with an aerodynamic diameter smaller than 0.5 μm should be classified according to their "diffusion diameter", that is the "diameter of a sphere with the same diffusion coefficient at the same temperature, pressure and humidity". NPs belong to the second class and, therefore, diffusion process may contribute to keep particles airborne.
The MAK Commission of Germany (maximum admissible concentrations of chemical substances in the air at workplaces that, in the light of current knowledge, do not

affect workers' health or cause undeserved pain - MAK Commission: commission of the hazardous substances in the workplace) finally considers agglomerates to develop the following definition of ultrafine particle: " Ultrafine Particles (NPs) […] include aggregates and agglomerates as parts of dusts and fumes. Their primary particles have a diffusion diameter smaller than 100 nm" (BIA, 203; HSE, 2004).

1.1.2 Nanoparticles in occupational and industrial hygiene

Many NMs used in nanotechnologies consist of NPs or fibrous materials that are initially produced as aerosols or colloidal suspensions. The Organization for Economic Co-operation and Development (OECD, 2008) has subdivided most of NMs produced today, or about to enter the market, into the following types:

- *Fullerenes (C_{60}):* any molecule composed entirely of carbon, in the form of a hollow sphere or cage. The most known of fullerenes is the C_{60} which consists of 60 carbon atoms, arranged to form a sphere made up of pentagon or hexagon panels.
- *Carbon Nanotubes (CNT)*: nanotubes may be regarded as a rolled up graphite sheets closed at one end. They may be single-walled (SWCNTs) or multi-walled (MWCNTs) depending on the number of coaxial layers they are made up. Because of their dimensions (length/diameter ratio), they fall under the category of fibers, they are highly electrostatic and appear agglomerated in beams or filaments with a diameter of approximately 20 to 50 nm. As the productive process involves the use of metallic catalysts, the final product may contain iron, nickel and cobalt.
- *Metallic and metal-oxide materials* such as:
  - Silver and iron NPs
  - Titanium and silicon dioxides
  - Aluminium, cerium and zinc oxides
- *Carbon black*
- *Polystyrene*
- *Dendrimers*: nanoscale synthetic polymers built up from branched units (from the Greek, *déndron* - tree). The surfaces of dendrimers are characterized by several chain terminals which can be adapted enabling specific chemical functions (their use, for example, as catalysts or drug vectors due to the inner cavities in their 3D structure)
- *Nanoclays*: NPs of layered mineral clays.

In addition to the above reported NMs, it is worth mentioning:

- *Nanodots*: nanoscale crystalline structures made from cadmium, selenium, tellurium and sulphur; their nominal diameter is of the order of some nanometres;

they can be found suspended in a vehiculated agent or englobed in a solid (polystyrene, polyurethane, polycarbonate, silicium).
- *Carbon nanofoam*: it is the fifth known allotropic form of carbon, and consists of a cluster assembly of carbon atoms with a diameter of 6-9 nm, casually linked in a fabric-like structure. It is an extremely light, porous semiconductor solid that exhibits magnetic properties and contains impurities such as iron and nickel.
- *Quantum dots*: crystalline NPs with specific size-dependent properties due to the effects of the quantum confinement on the electrons (ISO, 2009).

1.2 Chemicophysical characteristics and chemical properties of nanomaterials

The research in this field is investigating on whether NM and NP exposure represents a risk to workers' health and to what extent the chemicophysical and chemical properties may influence such risk. Different studies demonstrated that the presence of NMs and aerosols in various workplaces, either intentionally produced and manipulated or involuntary released during particular physicochemical processes, may represent potential risks to workers' health and safety, on the basis of experimental evidences supporting a correlation between exposure and diseases affecting, in particular, the respiratory tract and the immune and nervous systems (Marconi A, 2006; Ostiguy C et al, 2006).
Studies, mainly conducted on animals or *in vitro*, have highlighted the possibility of nephrotoxic, genotoxic and reproductive effects, granulomas and tumoural reactions in lungs (Oberdoster G et al, 1994; Borm P et al, 2004) and translocation to other tissues or organs (Oberdoster G et al, 2002; Oberdoster G et al, 2004) depending on the different physicochemical characteristics of the NPs. However, due to the limited number of studies, to the short period of exposure, to the different composition of tested NPs and to the often unusual exposure routes in workplaces, additional researches are required to assess the risk associated with inhalation and dermal exposure of workers to NP.
Particular attention must be focused on the metrological aspects as, although different parameters (such as dimensions, mass, chemical composition, surface area, concentration, aggregation and agglomeration state, water solubility, surface chemistry and morphological structure) may contribute to the hazardous interactions of NPs with the human body, there is not a univocal opinion on how to relate specific toxic effects of NP with one or more of such parameters.
A univocal identification of the "right" parameters for the toxicity evaluation of NPs is an integral component in setting proper occupational and exposure levels for NPs

and implementing adequate prevention and protection systems through a multiparametrical and metrological approach, also supported by the studies on the NP biological interactions, which involves the use of specific indoor sampling and analysis techniques.

The physicochemical characterization of ultrafine particulate is extremely important to distinguish naturally occurring airborne NPs or those occasionally released as by-products of thermal or chemical reactions taking place during pressing processes (He C et al, 2007; Kagi N et al, 2007) or, more in general, during combustions, from the engineered NPs and nanoaerosols intentionally produced and/or handled in industry. These latter, in fact, exhibit different physicochemical properties from the other environmental particulates (Oberdorster G et al 2005a). Although many physicochemical properties of the engineered NPs house in the nucleus, the surface and the shell are also endowed with properties of great interest as they are the points through which NPs come in contact with organisms (Christian P et al, 2008).

As for dimensions, it has been demonstrated that they deeply influence the deposition of NPs (in particular, in the respiratory tract at the alveolar level) which is considerably high for those particles with a diameter smaller than 100 nm (Oberdorster G et al, 2005a; ICRP 1994; ICRP, 2002; Bailey M, 1994). Also, NPs may move into the cells through the membrane and translocate, via diffusion, into other parts of the organism (Oberdorster G et al, 2005a; BeruBe K, 2007; Card JW et al, 2008) eluding alveolar macrophages and penetrating into the pulmonary interstice, although this has not yet been demonstrated in humans (Oberdorster G et al, 2005a; BeruBe K, 2007; Card JW et al, 2008, Oberdorster G et al, 2005b).

Shrinkage in size may create discontinuous crystal planes that increase the number of structural defects and disrupt the well structured electronic configuration of the material (Nel A et al, 2006).

The aggregation/agglomeration states may exert a major influence on deposition, local toxicity and toxic kinetics of NPs, due to significant variations of the diameter (wider in aggregates) and of the reduction of the surface area occupied by NPs (Tsuji et al, 2006; Borm PJ et al, 2006); hence, the behaviours of large NP aggregates may be compared to that of the *in vivo* ultrafine particles (Tsuji et al, 2006; Borm PJ et al, 2006).

Aggregation/agglomeration depends upon the inner features and number concentration of NPs but also upon the properties of the mean they are contained (pH, ionic force, organic material dissolved in the mean) (Christian P et al, 2008). The volume occupied by particles and the mass decrease with dimensions but, consequently, the surface area per unit mass, as well as the potential for biological interactions, increase (Oberdorster G et al, 2005; Borm PJ et al, 2006; Warheit DB, 2008). As the particle

reduces its dimensions, in fact, the percentage of atoms localized on the surface increases depending upon the percentage of atoms occupying the rest of the volume (Oberdorster G et al, 2005b; Nel A et al, 2006; Warheit DB, 2008 Warheit DB et al, 2008; Nel AE et al, 2009). This may exert influence on both charge surface composition and catalytic activity and may determine an increase in the number of potential reactive groups on the cell surface (Card JW et al, 2008; Nel A et al, 2006; Warheit DB, 2008; Warheit DB et al, 2008; Nel AE et al, 2009).
Hence, reactive groups may, supposedly, modify the biological activity of NPs and may be crucial for the definition of their toxicity. For the same chemical composition, therefore, the surface area per unit mass is shown to be an extremely relevant parameter for evaluating NPs toxicity (Oberdorster G et al, 2005b).
Surface reactivity is correlated with the chemical composition of the particle itself (presence of reactive groups on the surface), surface charge (deeply influencing the deposition of particles at the pulmonary level), catalytic activity, absorption and desorption capacities of molecules, imperfections in crystals and impurities (Oberdorster G et al, 2005a; Yang W et al, 2008; Nel AE et al, 2009; Aillon KL et al, 2009).
Also the porosity contributes to a significant increase in the total surface area which is to be added to the geometric surface area (Powers KW et al, 2006a). In some cases, an increased surface reactivity (and a consequent increased biological activity) produces positive effects (such as, for example, antioxidant activity, vehiculation and release of therapeutic substances, due to a large penetration capacity of NPs), in other cases, toxic effects may appear (such as induction of oxidative stress and cytotoxicity) (Oberdorster G et al, 2005b; Nel AE et al, 2006; Yang W et al, 2008; Limbach et al, 2007), and sometimes positive and toxic effects may appear simultaneously (Oberdorster G et al, 2005b; Yang W et al, 2008). Finally, surface reactivity is fundamental to define interactions between NPs and biological macromolecules (proteins, elements of the cytoskeleton; collagen, membrane structures, receptors, DNA, etc.). In most cases, therefore, NPs exhibit the same inflammatory and cytotoxic potential as larger particles having the same chemical composition.
Dimensions and surface chemistry/reactivity influence also toxicokinetics of particles after their deposition in the alveolar regions (Oberdorster G et al, 2005b; Powers KW et al, 2006a; Balbus JM et al, 2007).
More in detail, some authors focused their attention on chemical composition, surface charge and surface energy: although uncertainty exists with regard to the correlation between the nuclear chemistry and NP toxicity, the chemical composition of the surface may exert an influence on aggregation/agglomeration, biomolecules uptake and, therefore, *in vivo* dissolution, distribution and biopersistence. Similarly, the net surface charge is essential to explain not only the interaction between NPs and

biological molecules but also their capacity to penetrate cell membranes. Cell membranes are negatively charged at physiological pH values, which allows positively charged NPs to penetrate, through electrostatic interactions (Nel AE et al, 2009; Elder A et al, 2009). Furthermore, it has been assumed that a cationic surface may predispose to an uncontrolled cationic transport across the lysosomal membrane with consequent cytotoxicity (Xia T et al, 2009). Finally, low energy (hydrophobic) surfaces are absorbed, in an unspecific way, and may favour protein unfolding. Moreover, due to their surfactant-like properties, they are able to disorganize lipid components of cell membranes with an increased epithelial penetration. Conversely, high energy (hydrophilic) surfaces, in particular those with a low negative or neutral charge, bear the same affinities as proteins and reduce the cell accumulation (Nel AE et al, 2009; Elder A et al, 2009). NPs binding to proteins may generate more mobile complexes that can enter tissue sites that would normally be inaccessible. Denaturalization and degradation of proteins deposited on the surface of NPs may cause functional and structural alterations and possibly lead to a complete or partial inhibition of existing enzyme activity. The toxicity of NPs is significantly correlated with the hydrophobic or hydrophilic, lipophilic or lipophobic, catalytically active or passive character of NPs (Nel AE et al, 2006; Nel AE et al, 2009; Dutta D et al, 2007).

In many cases, specific coatings may be used to modify NPs surface properties, reduce their reactivity, prevent aggregation or agglomeration, favour dispersion and keep the main properties unaltered. However, translocation of particles from the respiratory tract to the systemic circulation can be accelerated by altering the distribution of NPs in the human body (Warheit DB et al, 2208; Carlotti ME et al, 2009; Clift MJ et al, 2008; Gupta AK et al, 2007; Leonov AP et al, 2008; Mancini MC et al, 2008; Nakano K et al, 2009; Okassa LN et al, 2007; Ryman-Rasmussen JP et al, 2007).

Solubility or biopersistence (durability) aspects have been shown to exert an influence both on the identification of the target organ for NPs, on the clearance mechanisms of particles and toxicokinetics.

In addition, toxic substances in highly soluble NPs, such as some metals, may cause a direct exposure to metallic ions with consequent toxic effects (Card JW et al, 2008; Borm PJ et al, 2006, Balbus JM et al, 2007; Borm P et al, 2006).

Degradability is also considered: non-biodegradable NMs may accumulate in organs and cells and cause long-term biological alterations. Besides, biodegradable NMs may induce an unexpected toxicity due to toxic degradation products (Aillon KL et al, 2009).

Another fundamental parameter for the toxicity of NPs is their shape. Although most is still unclear about the effects of the shape and porosity on toxicity, it is known that porosity influences deposition and absorption of NPs in the human body.

The same does not hold true for fibrous materials (Oberdorster G et al, 2005; Oberdorster G et al, 2005b). In particular, exposure to fibers increases the risk of fibrosis and lung cancer after prolonged exposure and the major parameters for evaluating NP toxicity are doses, size and biopersistence. The penetration of fibers in lungs is in indirect ratio to the diameter. This is particularly true for some NPs of great industrial interest, such as nanotubes. Animal studies demonstrated that the exposure to nanotubes produces the same effects on the lungs as other known toxic fibers (i.e. asbestos), even if part of these effects are supposed to be induced by metal impurities (Al_2O_3, Fe_2O_3, TiO_2) contained in the nanotubes as a consequence of manufacturing process (Oberdorster G et al, 2005; Oberdorster G et al, 2005b).

Finally, some authors report the main physicochemical characteristics influencing the toxicity of NPs (Tab. 1.1) and provide, for some cases, the minimum data for the relevance of a toxicity study *in vivo* and *in vitro* studies. Many authors agree with parameters reported in the table and cataloging, in some cases, the major physicochemical features according to their classification (Aillon KL et al, 2009; Ju-Nam Y and Lead JR, 2008; Lewinski N et al, 2008; Shulte PA et al, 2009). Others provided schemes indicating correlations among NP physicochemical properties and their toxicity (Nel A et al, 2006; Nel A et al, 2009; Xia T et al, 2009; Fischer HC and Chan WCW, 2007).

On the basis of some studies (Aillon KL et al, 2009; Murdok RC et al, 2008), the European Agency for Safety and Health at Work (EU-OSHA), in its literature review, reports the following NP features: shape, surface area, surface chemistry, composition, homogenous composition between nucleus and surface, heterogeneous composition distribution, solubility, charge (in biological fluids), crystalline structure, porosity, changes in size and structure after exposure, stimuli-responsive behaviours, functional response to local environment.

In relation with these features, although no unique physicochemical parameter exists for characterizing nanostructured particles, EU-OSHA has proposed to consider the following parameters before performing toxicology studies: size, distribution, specific surface area, crystalline structure, surface reactivity, surface composition, purity (EU-OSHA, 2009).

Also considering the tests suggested by Murdock et al, 2008 (Murdock RC et al, 2008), EU-OSHA proposed other potential parameters for the characterization of NPs: shape, zeta potential, solubility and hydrophobic potential (EU-OSHA, 2009). It is therefore evident that a complete characterization of NPs requires sophisticated techniques which still need to be completely optimized (Murdock RC et al, 2008; Ju-Nam y and Lead JR, 2008; Hassellov M et al, 2008).

With regard to identification and characterization of the specific chemical composi-

tion of NMs, it is fundamental to underline some relevant aspects of toxicological and ecological risk assessment.
A complete characterization of NMs requires that, apart from the structure and composition, further features (molecular weight, boiling and freezing points, vapor pressure, octanol-water partition coefficient, water solubility, reactivity, stability) need to be considered; information on the formulation and preparation of NMs, as well as performance and applications, are shown to be relevant in understanding the degree of purity and variability of the product, as well as the performance and use perspectives.
Due to the diversity and complexity of NMs, the chemical identification and characterization are considered particularly relevant. A specific typology of NMs can be manufactured through different processes, each one creating by-products bearing different chemical and physicochemical properties and, as a consequence, potentially different toxicological and ecological properties (Oberdorster G et al, 2005).
The above mentioned chemical properties could be fundamental in determining a potential hazardousness associated with a given NM, although, size and dimension distribution of particles, the surface/volume ratio, shape, electronic and surface features, dispersion/agglomeration status and conductivity play a relevant role (Powers KW et al, 2006b).
It is, therefore, essential to have more information on the properties of the observed NM whose complete characterization will enable an appropriate assessment of the exposure risk and, as a result, the drawing up of prevention and protection measures for the human health and the environment impacts.
Tab. 1.2 provides a brief summary of NMs as emerging contaminants, including their physical and chemical properties according to the *U.S. Environmental Protection Agency (EPA) Federal Facilities Restoration and Reuse Office* (FFRRO) (EPA, 2009).

Table 1.1 - Basic physicochemical features of NPs associated with toxicity.	
• particle size and size distribution (wet state) and surface area (dry state) in the relevant media being utilized depending upon the route of exposure • crystal structure/crystallinity • aggregation status in the relevant media • composition/surface coatings • surface reactivity • method of nanomaterial synthesis and/or preparation including post synthetic modifications • purity of the sample	Warheit 2008, Murdock eta l 2008 Card JW et al, 2008
• size • shape • chemical composition • crystallinity • surface properties (area, porosity, charge, surface alterations, coatings) • agglomeration and aggregation status • biopersistency • absorbed doses	Oberdorster G et al, 2005 a,b
• particle amount and dimensional distribution • dose in the target organ • surface treatments • aggregation/agglomeration levels • surface charge • particle shape and/or electrostatic attraction potential; • method of synthesis (liquid or gaseous, post-synthetic modifications)	Tsuji et al, 2006
• particle size and dimension distribution • shape • surface area • redox and potential properties • purity/presence of contaminants • different catalytic activity from ROS generation	Balbus et al, 2007
• particle size and amount • surface dose • surface coatings • agglomerating/aggregating capacity • surface charge • method of synthesis	Borm et al, 2006
• aggregation status • mass concentration and elemental composition • particle amount • shape • particle size and dimensional distribution • solubility • speciation (metals) • surface area and porosity • surface charge • surface chemistry	Tiede et al, 2008

Table 1.1 - Basic physicochemical features of NPs associated with toxicity.	
• particle size • shape • charge • aggregation status • surface irregularities • hydrophobicity • presence of coatings or surface functional groups	Xia et al, 2009

Table 1.2 - Chemical properties and use of NMs[1]

Types of nanomaterial (NMs)	Examples	Chemical properties	Application
Carbon NMs (natural or engineered)	Fullerenes / Buckyballs / (C_{60}, C_{20}, C_{70}); Carbon nanotubes; nanodiamonds; nanowires	Stable, limited reactivity, completely made of carbon, high in antioxidants	Biomedical applications, supercondenser, sensors, photovoltaics
Metal oxides (natural or engineered)	TiO_2, Zn_0, CeO_2	High reactivity, photovoltaic properties	Photocatalyst, pigments, drug vehiculation, diagnostics, UV sunscreen protector, diesel fuel additive
Zero-valent metal (engineered)	Nanoscale Zero-Valent Iron (nZVI), Emulsified Zero-Valent Iron (EZVI), Bimetallic Nanoscale Particles (BNPs). BNPs include elemental iron and metal catalysts (such as Au, Ni, Pa or Pt)	High surface reactivity. Common materials used in the manufacturing include (Fe [III]) or ferrous (Fe [II]) sodium salts with sodium borohydride	Waters, sediment and soil applications for the reduction of contaminants such as nitrates, trichloroethylene or tetrachloroethylene
Quantum dots (engineered)	CdSe, CdTe and ZnSe quantum dots	Packed semiconductors whose excitons are confined in all three spatial dimensions. Possible metal structures include: CdSe, CdTe, ZnSe, InAs or PbSe for the nucleus and CdS or ZnS for the shell	Medical diagnostics, photovoltaics, telecommunications and sensors
Dendrimers (engineered)	Hyper-brunched polymers; dendrigaft polymers and dendrons	Highly brunched; multifunctional polymers	Drug delivery, chemical sensors, modifies electrodes and DNA transfer agents
Composite NMs (engineered)	Characterized by two different NMs or by NMs combined with nanoclays, they can also include NMs combined with synthetic polymers and resins	Multifunctional components; catalytic features	Potential application in drug delivery, optimization of the mechanical and flame retardant properties
Silver NMs (engineered)	Colloidal silver, silver wires, nanosilver dust and polymeric silver	High surface reactivity, strong antimicrobial properties	Medical applications, water purification, antimicrobiotics. Contained in many commercial products

[1] From EPA, 2009. Emerging contaminants-Nanomaterials Fact Sheet. EPA 505-F-09-011.

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chapter 2

Perspectives in the Italian production sectors

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2.1 Production sectors, use and development of nanotechnologies

At the beginning of the 21st Century, nanotechnologies are almost unanimously seen as one of the key drivers for technological development. The collective term "Nanotechnology" refers, in fact, to all knowledge-based techniques and processes derived from the materials science, quantum physics, supramolecular chemistry and molecular biology that allow to observe, measure and manipulate the matter on an atomic and molecular scale (nanotechnologies deal with molecular structures comprised between 1 and 100 nanometres). For the first time, nanoscale matter can be manipulated and, thanks to its specific properties and behaviours, it is possible to get from it materials and devices with hugely improved and completely new features. Nanotechnologies represent a new way of producing and, due to their general purpose features, are able to cause deep technological changes in many production sectors and into the way we live our life.

Over the last 8-9 years, in the most important countries there has been an exponential increase in the focus on worldwide emerging technologies. At first, public funding represented the driver of nanotechnologies, however, over the last years, the involvement of the private sector has gradually increased, in particular in the United States and East Asia. Currently, the worldwide estimated cost for nanotech research and development is more than 13 billion dollars, evenly divided between public and private sectors (Lux Research, 2008).

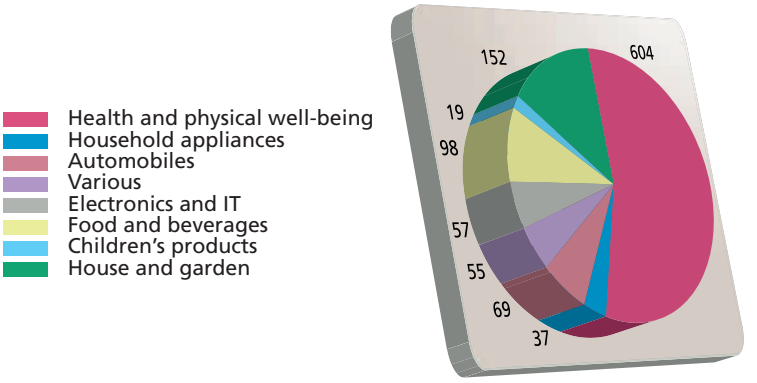
All over the world, more than 2.000 companies are estimated to be involved in this field, 70% of which are represented by SMEs; nonetheless, big worldwide brands are investing more and more resources in nanotechnologies, persuaded that they

play a strategic role in enhancing and defending their own competitive and technological position.

2.1.1 The nanotechnology market

According to a recent US surveys, approximately 1.000 nano-related products have already reached the market (Fig. 2.1).

a) Application areas



Application areas	Examples
Health and physical well-being	Cosmetics, clothing, sport, body care, suntan creams etc.
Household appliances	Batteries, air-conditioners, refrigerators, washing machines, purifiers, etc.
Automobiles	Accessories and finishes, sealants, car polishers, catalysts, tyres, etc.
Various	Coatings and surface treatments
Electronics and IT	Processors, hard-disk, earphones, chips, displays, antibacterial cleaners for PCs, paper, photography, etc.
Food and beverages	Kitchenware, food containers, mineral and vitamin supplements, frying oil regenerators, etc.
Children's products	Antibacterial fluffy toys and feeding bottles and toys, etc.
House and garden	Construction materials and coatings, household products, purifiers, antibacterial products for pets, anti scratch paintings, etc.

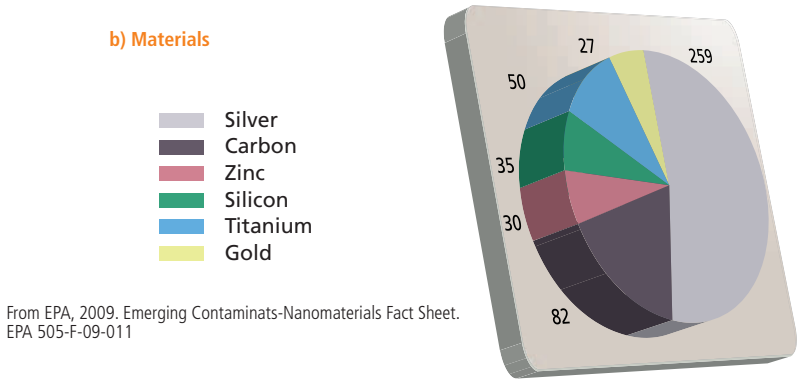


Figure 2.1 - Consumer goods deriving from nanotechnologies worldwide divided by a) applications and b) materials.

The market value of nano-based products was estimated at approximately 147 billion dollars in 2007 and about 310 billion in 2008; Lux Research, however, estimates that this value is forecasted to grow to 3.000 billion dollars from 2015 onwards. (Fig. 2.2). Sectors which are expected to benefit from the use and dissemination of nanotechnologies are:

- nanoelectronics/photonics;
- materials (functional or structural) whose applications may range from surface treatments to tools, from textile sector to clothing industry, building, etc.;
- health care (nanobiotechnologies, nanomedicine).

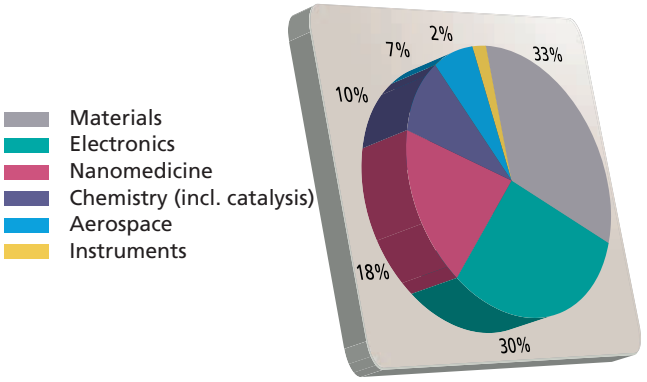


Figure 2.2 - Estimate of the worldwide nanoproduct market by 2015, referring to a global nano market estimated to reach approximately one trillion US dollars by 2015 - National Science Foundation, USA.

However, nanotechnologies may represent extremely innovative and appropriate solutions in terms of responsible development also in other important sectors such as energy, transport and environment.

2.1.2 Development perspective in Italy

Being nanotechnologies involved in a wide array of scientific disciplines and applicative sectors, they can hardly be fit into specific productive, applicative and development sectors.
On the basis of a recent study conducted by the Italian Association for the Industrial Research (AIRI), the 7th edition of the report entitled "Tecnologie Prioritarie per l'Industria" (Priority Technologies for the Industry") has been issued and, albeit not exhaustive, it provides enough information about the technological development gaps of the most innovative part of the industrial system and the Italian advances services[2] (AIRI, 2009).
Nanotechnologies are involved in 8 out of 10 sectors observed in the study[3], and in terms of short-to-medium-term development, they play a relevant role in the following areas:

Microelectronics and semiconductors

1. "Nano on Micro" (integration of nanomaterials on micro-finished sensors and biochip platforms).
2. Optoelectronic and photonic component technologies (nanotechnologies for high-level of optical component and of a new generation of sensors).

Chemistry

1. Nanomaterials for chemical catalysis (nanomaterials for solid catalyst, for catalyst membranes, of high-efficiency and sustainable gas purification and storage).
2. Food packaging (nanomaterials able to extend the shelf-live, sensors for the monitoring of the preservation of the packaged contents).
3. Concrete-based formulas for the construction industry (new potential technological discontinuity due to nanoscale control of the structure of matter).

Pharmaceutics and biotechnologies

1. Medical applications of nanotechnologies (drug delivery systems, nanomaterials

[1] About 110 AIRI industrial partners account for more than 50% of R&D costs of the Italian industry.
[2] The 10 sectors addressed in the report "Tecnologie Prioritarie per l'Industria" ("Priority Technologies for the Industry") are: Information Technologies and Telecommunications, Microelectronics and Semiconductors, Energy, Chemistry, Pharmaceutics & Biotechnologies, Environment, Transportations, Aeronautics, Space, Capital goods and Mechanics.

for medical devices, biosensors, nanoscalpel, new *in vivo* imaging diagnostic systems).
2. Transportation system (phospholipid particles containing molecules with a pharmacological activity for targeted drug delivery).

Energy
1. Innovative technologies for solar energy development (third-generation photovoltaic technologies: semiconductor crystals; nanoscale network for organic solar cells)
2. Hydrogen storage technologies (solid storage in alloys and innovative intermetallic compounds, nanostructured oxides, etc.).

Environment
1. New technologies for water treatment and reuse (development of new zeolite-like nanoporous materials).
2. Systems for pollution reduction and quality air control (sensitive nanostructured materials, sensors, catalysts).

2.2 Map of research industries and laboratories

2.2.1 Nanotechnologies in the Italian context

Over the last years, a constant increase has been made in nanotechnology which is a reality today. "The Second Nanotech IT Census of Nanotechnology in Italy", conducted in 2006 by AIRI/Nanotec IT (a third edition is nowadays under progress), highlighted that approximately 200 organizations are involved in Research and Development (R&D) activities in this field; 57% of them in the public sector and the remaining 43% belong to the private sector. If compared to the first census carried out in 2004, the number of private organizations has almost doubled and in the third edition it will surely escalate further. The survey was conducted at national level and Fig. 2.3 provides the number of private and public organizations, for each Italian regions, involved in the last Census. The highest concentration has been registered in the central and northern parts of the country, where Lombardy ranks first and accounts for 20% of all organizations and 30% of operators reported by the Census. It is worth mentioning, however, that also southern Italy, although to a lesser extent than the North, plays a leading role and boasts a high level of competences and equipments and, often, a good critical mass.

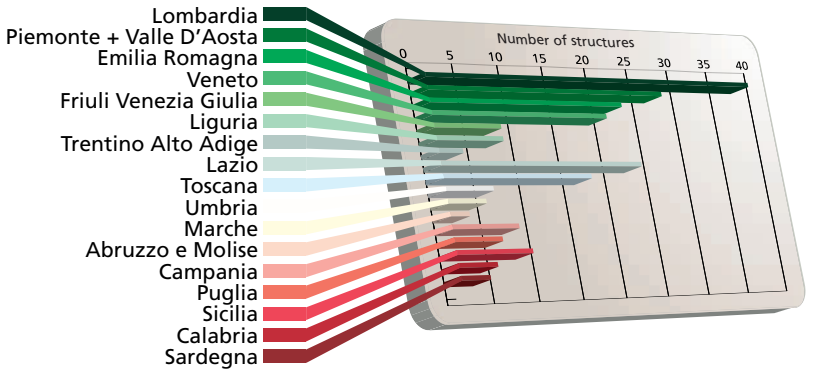


Figure 2.3 - Geographical distribution of research structures active in nanotechnology.

Over the last years, a number of initiatives have been launched aimed at improving the use of resources, enhancing the overall operational efficiency and strengthening the commitment. Centres of excellence in Nanotechnology (Tab. 2.1) have been established in various Universities, under the auspices of the Italian Ministry of Education, Universities and Research (MIUR), and in some of them 2nd level degree, Master's degree and PhD courses in nanotechnologies have been activated. In some Universities, the research activities, although located in different sites, have been brought together and assigned common objectives.

Table 2.1 - Centres of excellence in Nanotechnology.

• Center for Nanostructured Surfaces and Interfaces (NIS)- University of Torino
• Laboratory of Electrochemical Miniaturised Technologies for Analysis and Research (LATEMAR)- Polytechnic of Torino
• Center of Engineering of Nanostructured Materials and Surfaces (NEMAS)- Polytechnic of Milano
• Interdisciplinary Centre for Materials and Nanostructured Interfaces (CIMAINA)- University of Milano
• Center for Preparation, Development and Characterization of Nanostructured Materials and Surfaces (CENMAT)
• Center for Nanostructured Innovative Materials for Chemical, Physical and Biomedical Application (CEMIN)
• Center for the Preparation and Treatment of Organic Material at Nano

Some Technological Districts established in some Italian regions with the support of MIUR have placed nanotechnologies as one of priority areas in their activities (Tab. 2.2) in order to enable the technological development in specific advanced sectors. Veneto Nanotech, focused exclusively on nanotechnologies, was established in 2005;

located in the same district, a nanofabrication laboratory - NanoFab - was built in 2005 and in 2007 the European Centre for the Sustainable Impact of Nanotechnology (ECSIN) was founded.

Table 2.2 - Technological districts involved in nanotechnology.

Region	Area/s of research	Managing company
Veneto	Nanotechnology applied to materials	Veneto Nanotech S.c.p.a
Friuli-Venezia Giulia	Nano-biotechnology	Centre for Molecular Biomedicine CBM S.c.r.l.
Campania	Polymeric and composite materials	IMAST S.c.a.r.l.
Puglia	Nanoscience, bioscience, infoscience	DHitech S.c.a.r.l.
Umbria	Special metal materials, micro and nanotechnologies, mechatronics	DTU - Umbria Region

There is a very broad array of research activities and the fields of study involving organizations and public research centres are substantially the same. Special attention is focused on materials (structural or functional), nanoelectronics and photonics, bioscience, medical field and instruments. As already mentioned, potential applications of nanotechnologies include important productive compartments ranging from healthcare to electronics, from ICT to transports, from environment to energy but also include more traditional sectors related to the "made in Italy" concept such as textile sector, fashion and footwear industry, food packaging, construction, advanced mechanics and cultural goods.
More attention has been paid to the responsible development of nanotechnologies which is a key enabler of their success. Currently three Working Groups are involved in the area: INAIL, formerly ISPESL, Working Group, with the publication of the present White book, UNI Working Group - Nanotechnologies, in line with ISO TC 229 activities - and INAIL Working Group "Emerging Risks in Nanotechnologies".

2.2.2 Leading actors

A. *Public Institutions*
Currently, Nanotechnologies are a priority in the agenda of the main public research organizations (CNR/INFM, INSTM, INFM, ENEA) and Universities. These play a key role in the development and promotion of nanotechnologies in the Country and, as already mentioned, account for approximately 57% of the organizations included in the Census.

The *Italian National Research Council* (CNR), into which *National Institute for the Physics of Matter* merged in 2004, is the main public research institution in Italy. This commitment goes hand in hand with the launch of initiatives aimed at optimizing the use of resources and, starting form 2006, most of the CNR initiatives on nanotechnologies refer to two newly created departments: *Department of Materials and Devices* and the *Department for the Molecular Design*[3].
The institutes most involved in nanotechnologies are the Institute for the *Study of Nanostructed Materials* (CNR-ISMN) of Rome, Bologna and Palermo, the *National Enterprise for nanoScience and nano Technology* (CNR-NEST) of Pisa, the *National Nanotechnology Lab* (CNR-NNL) of Lecce, the *NanoStrucures and bioSystems at Surfaces* (CNR-S3) of Modena, and the *Advanced Technology and Nanoscience National Laboratory (*CNR-TASC) of Trieste.
The *Italian Interuniversity Consortium on Materials Science and Technology* (INSTM) coordinates Research Units located in 44 Italian Universities and is mainly focused on chemical sciences. In 2004, 9 Reference Centers (INSTM-RC) have been established within the Consortium in order to streamline its activity. These Centers often connect research units located in different Universities. Nanotechnology represents the primary (and sometimes exclusive) objective of their research[4].
Furthermore, the Census has pinpointed more than 40 University structures involved in nanotechnologies not linked to INSTM and CNR. These structures, as shown in Fig. 2.4, represent the 35% of the total and their activities focus on physics, material science, engineering (in particular electronic engineering) biotechnology/bioengineering, chemistry, pharmaceutical sciences and, in a limited number of cases, mechanics and environment.

[3] http://www.cnr.it/istituti/Perareetematiche_eng.html.
[4] http://87.241.56.172/test_new_version/index.php?targetpage=include-ricerca-laboratory.php.

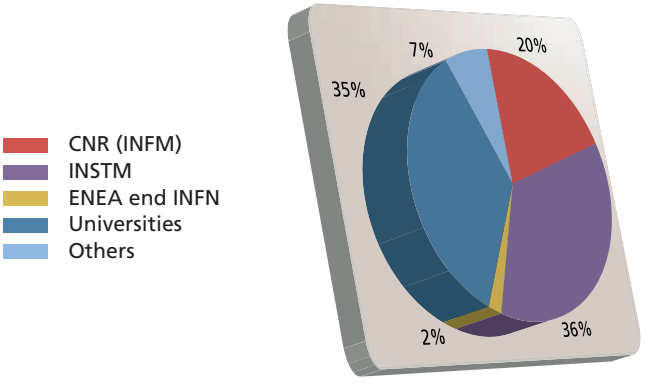


Figure 2.4 - Distribution (%) of structures within the Institutions observed.

Within the *Italian Institute of Technology* (IIT) a "*Nanobiotech facility*" has been activated to foster research on bionanotechnology. The Institute is part of research laboratory network on nanotechnology, including the "*National Nanotechnology Lab*" (CNR-NNL) and the "*National Enterprise for nanoScience and nanoTechnology*" (CNR-NEST), *Scuola Superiore Sant'Anna* di Pisa, active in the areas of nanotechnology, nanomedicine, smart materials, energy production and storage.
The *National Institute of Nuclear Physics* (INFN) and the *National Body for Energy, Environment and New Technologies* (ENEA) are also involved in nanotechnology R&D even though, at present, with a lower commitment than the above-mentioned institutions. At ENEA the R&D activity is carried out within the Department of Advanced Physical Technologies and New Materials (FIM), while INFN conducts its activity at Frascati National Laboratories (LNF).
More research centres and national agencies such as the Italian Institute for *Occupational Safety and Prevention* (ISPESL), the *Italian Health Institute* (ISS), the Italian *Workers' Compensation Authority* (INAIL), and the *National Institute of Metrological Research* (INRIM) are also involved in cross-sectional aspects of nanotechnologies such as metrology, characterization of nanomaterials and their potential risks.

B. *Industry*
During the past few years the number of Italian organizations dealing with nanotechnology has steadily increased. The last update of the Census have identified 86 companies active in this field: a strong increase from the 1st Census in 2004, when only 20 private enterprises were identified.
As shown in Fig. 2.5, SMEs, which account for most of the increase, represent about 70% of the total. Quite many of them are micro (less than 10 operators), often spin-off and start-up (more than one third of SMEs fall into this category).

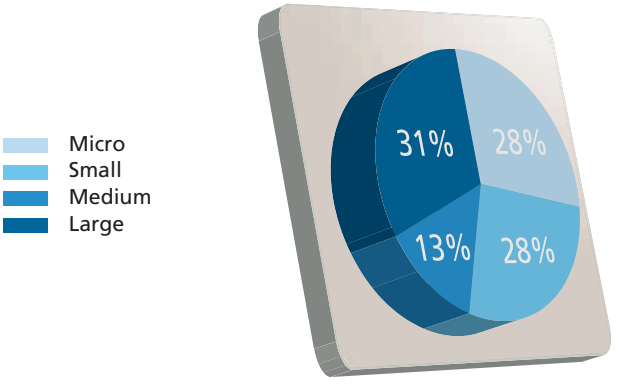


Figure 2.5 - Number (%) of structures referring to enterprises dimension.

Quantitatively the effort is concentrated within large organizations. They include well-known National players such as *ENI* (energy, catalysis); *FIAT Research Centre-CRF*, Brembo, *Pirelli* (automotive); *Bracco Imaging, Fidia Advanced Biopolymers* (biomed); *Colorobbia* (materials); *Center for Material Science-CSM* (materials); *CTG-Italcementi e Mapei* (construction); *Finmeccanica Group* (aerospace, defense) which has organized its nanotech activities into the *Nanomaterials and Nanotechnology Focus Group* bringing together a number of its companies (*Alenia Aeronautica, MBDA, Thales Alenia Space, Elsag Datamat, Selex Sistemi Integrati, Selex Communications*); *Basell Polyolefins, Mascioni, Saati* (textiles); *Saes Getters* (vacuum technology); *STMicroelectronics, Numonyx* (semiconductors).

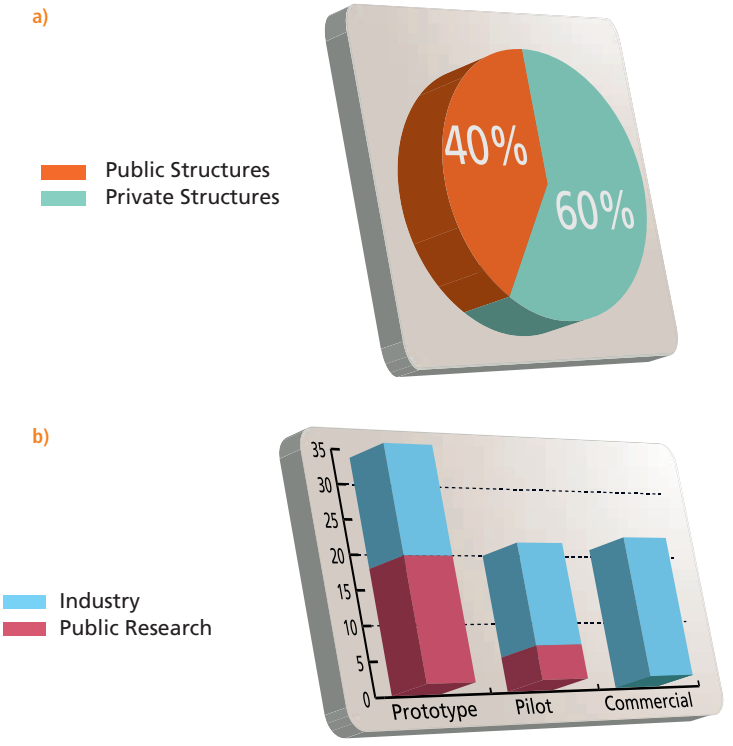


Figure 2.6 - a) Number of organizations (%) divided by typology; b) number of organizations catalogued according to the stage of production.

SMEs, however, have also a key role in disseminating the applications of this emerging technology. To name a few, we can mention: *Ape Research, Avago Technologies, Eontych, Organic Spintronics, Silicon Biosystems, Microla, BilCare Technologies* (Instrumentation, sensors); *MBN, Xenia materials* (nanomaterials); *Grado Zero Espace, SmarTex, MecTex* (textiles), *Nanosurfaces, Kenosistec, Plasma Solutions* (surface treatments); *Finceramica, Tethis, Xeptagen, Nanovector; Mavisud, Cyanagen* (biomed), *Centro Ricerche Plast Optica* (lighting), *Trustech* (technology services).
Large enterprises are normally more focused on their core business than SMEs which aim, first of all, at exploring a wide array of potential applications and widen their offer, exploiting the multisectoral nature of nanotechnology. SMEs are particularly active in the industrial instrumentation even though 25% of the total number are involved in the medical field.

2.2.3 Nanotechnologies-related application and products

Approximately 35% of organizations observed by the AIRI Census report to develop nanotechnologies-related products at prototype, pilot or commercial level. As illustrated by graphs, private organizations only may boast a commercial production of nano-related products; however, it is interesting to point out that some public organizations are committed to the development of nano-related prototypes.

2.3 Estimated number of potentially exposed workers

The critical issue of any risk assessment process (either emerging or known) is identifying the risk exposure value. In the field of occupational health and safety, this is represented by the estimated working population exposed. Being the exposure to nanomaterials, voluntarily produced or used, an emerging risk upon which the information gap still needs to be fulfilled, it must be referred to, at this stage, as a "potential" exposure.
At international level, some estimates have been produced on the economic impact of nanotechnologies on the industrial production. A report by *Lux Research* estimated that by 2014, 10 millions of employees in the manufacturing sector- 11% of the total in the area - will be involved in the manufacturing of nanotechnological products (Lux Research, 2004); by 2015, NIOSH estimates that the global market of nanotechnology products will employ one million workers in the United States (NIOSH, 2007). A systematic approach to the quantification of potentially exposed workers has been set out in a report by *Health and Safety Executive* (HSE) providing a model to assess workers' exposure in United Kingdom.

Three main activity groups have been identified in the report: nanotechnology-related research and development at the university and organizational level; existing ultrafine manufacturing processes; powder handling processes. All processes where nanoparticles are by-products of other productions (i.e. welding and refinery) are also considered and represent the fourth activity group. Approximately 105.000 workers involved in the first three groups are estimated to be potentially exposed and about 1.000.000 in the forth (HSE, 2004).

On the basis of such approach dividing the potentially exposed professional sectors into four categories, an attempt has been made to assess the number of workers employed in the productive sectors involved in nanotechnologies in the Italian context. The number of worker employed in the economic categories potentially involved in the development of nanotechnologies are estimated to be more than 900.000. This output provides a clear picture of the potential impact of the issue on the Italian working population. (Boccuni et al, 2008).

To identify the actual number of workers exposed, it is necessary to analyze, on a case-by-case basis, all types of technologies used during a process posing potential risks and identify the phases of the working activity where exposure takes place as well as the number of workers exposed; furthermore, the exposure scenario, its frequency, duration and the characteristics of nanomaterials in the workplace need to be assessed.

According to the U.S. Department of Energy, any staff member meeting one or more of the following criteria are to be considered an "engineered nanoparticle worker":

- handles engineered nanoscale particulates that have the potential to become dispersed in the air.
- routinely spends (significant amounts of) time in an area in which engineered nanoparticles have the potential to become dispersed in the air.
- works on equipment that is believed to be contaminated and could release engineered nanoparticles during servicing or maintenance.

Furthermore, given the lack of current understanding about dose-response, this document suggests inclusion of workers whose exposures might be relatively high and those whose exposures might be relatively low. This definition should provide the number of workers exposed and may be redefined as soon as new pieces of information on the health effect are available (US Department of Energy, 2008).

This definition represents the starting point for the next phases of the risk assessment process and for the models to adopt in the occupational risk management. Finally, given the current state of knowledge, the estimate of workers exposed must be made on a case-by-case basis, according to the specific production process being considered.

2.4 Conclusions

In conclusion, the aim of this chapter is to highlight that R&D activity in nanoscience and nanotechnology in the Italian context is quite intense and involves both public research and industry. Public research is still prevailing; however, the commitment of private enterprises is increasing in important industrial sectors and this trend is going to continue.

Nanotechnologies, as already mentioned, may represent a strategic tool for the growth of the Italian high technology sectors and also for more traditional ones. To make this happen, however, it is essential to outline a national strategic view aiming at highlighting priorities and objectives, avoiding fragmentations and redundancies end emphasizing the excellence, streamlining the optimization and the use of resources.

In this view, a "responsible" development would be encouraged and this is key to make sure that the big promises in the field of nanotechnologies will be successfully kept.

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chapter 3

Research needs and mapping

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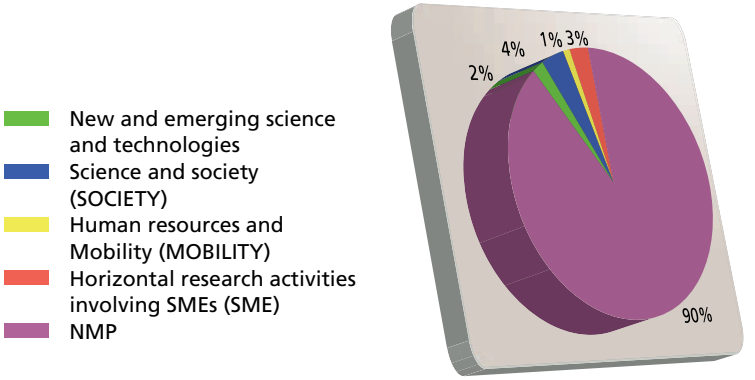
3.1 Research progress within EU Framework Programmes

Since 1984, the Framework Programmes (FPs) for Research and Technological Development have been the main financial tool created by the European Commission, the executive body of the European Union, to support and encourage research on technological innovations based on transnational collaboration in the European Research Area. Over the last 25 years, seven FPs have taken place: the FP7, the last one, started in 2007 and will run until 2013 (EC, 2008a; EC, 2008b).
The first financed research project related to nanotechnologies was the FP3 (1991-1994); however, the FP6 (2002-2006) was the first programme to expressly dedicate a research priority to this issue *("Nanoscience, nanotechnologies, materials & new production technologies" - NMP*). The increased attention paid on nanotechnologies caused not only an increase in the resources but also a wider research area. The research strictly focused on the technological development gradually became more oriented to a sustainable and responsible development also considering the impacts of nanotechnologies on environment, society, health and safety -"*Environmental, Health and Safety (EHS) issues*" as defined by the US Government National Nanotechnology Initiative (NNI, 2008) started by the United State government. These projects play a relevant role in the Occupational Health and Safety research area.
A progress has also been recorded in the approach to research projects: unlike FP5 projects which were focused on the so-called "scattered research" concerning emerging risks, FP6 projects mainly addressed the toxicological aspects and capacity building of nanotechnologies; FP7 projects, instead, address the development of a critical mass and provide potential answers to the key questions related to the devel-

opment of the risk analysis process. These include toxicity issues and health effects, environmental monitoring and assessment models, development of approaches to actual exposure conditions, criteria for health surveillance and implementation of risk communication strategies (Iavicoli et al, 2009).
Tab. 3.1.1 illustrates the funded research projects relating to the Health, Safety and Environmental (HSE) impacts of nanotechnologies (3 projects were launched under FP5, 15 under FP6, and 10 under FP7). In FP6, a total of 1.500 million euro have been allocated to the "Nanoscience and nanotechnologies, Materials and new Production technologies" (NMP) priority, 28 million of which have been used to fund 11 EHS-related research projects (accounting for 2% of the total sum). Over 3 million dollars have also been allocated under other priorities - New and emerging Science and Technology (NEST), Society (SOCIETY), Mobility (MOBILITY) and Small and Medium Enterprises (SME) - to fund 4 more research projects on the impact of nanotechnologies. Finally, more grants (of unknown value) have been allocated to the Work Package on impacts within the CANaPE project (funded for a total of 6,4 million euro). Under the FP7, NMP priority has been funded for a 7-year fund of 3.500 million Euro; about 17 million have been used to fund 7 EHS-related research projects (accounting for 0.4% of the total sum financed so far). Also under FP7, more grants have been allocated for other priorities. To date, 3 more projects have been funded for approximately 4 million euro, under SIS (Science in Society) and Health priorities (see Fig. 3.1.1).

Figure 3.1. - Percentage of funding for projects on EHS impacts of nanotechnologies under FP6 and FP7



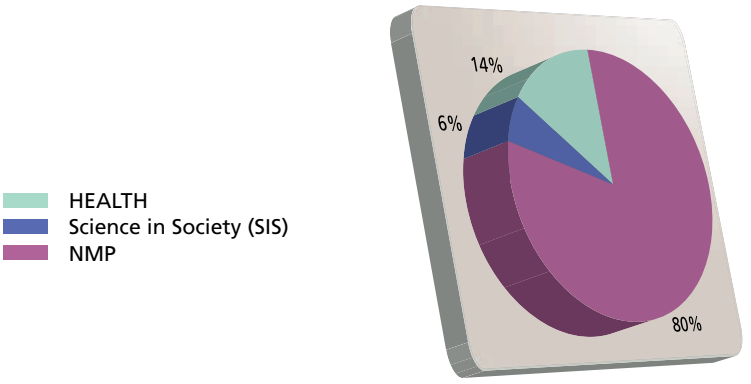


Table 3.1 - Projects on health effects of nanotechnologies under FP5, FP6 and FP7.

FP5 - PROJECTS ON EHS IMPACTS OF NANOTECHNOLOGIES		
Projet	Total amount of funding (Euro)	Total cost of project* (Euro)
NANO-PATHOLOGY	999.937	1.166.049
NANODERM	1.097.994	1.396.888
NANOSAFE	322.787	330.556
TOTAL	**2.420.718**	**2.893.493**
FP6 - Projects on EHS impacts of nanotechnologies		
Projet	Total amount of funding (Euro)	Total cost of project* (Euro)
CELLNANOTOX	2.600.000	3.651.500
DIPNA	2.793.235	4.535.199
IMPART	699.913	699.913
NANOINTERACT	3.300.000	4.616.544
NANOSH	2.400.000	4.000.000
PARTICLE-RISK	799.576	1.120.000
NANOSAFE	6.999.837	12.400.000
NANOTRANSPORT	450.000	450.000
NANOCAP	1.310.000	1.310.000
SAPHIR	8.100.000	15.800.000
NANOTOX	399.894	408.544
NANOTOX 2	180.134	180.134
NANO DIALOGUE	850.000	850.000
EURONANOFORUM2005	300.000	926.997
NANOAIR	1.073.792	1.414.893
TOTAL	**32.256.381**	**52.363.724**

Table 3.1 - Projects on health effects of nanotechnologies under FP5, FP6 and FP7.

FP7 - Projects on EHS impacts of nanotechnologies		
Projet	Total amount of funding (Euro)	Total cost of project* (Euro)
NANOIMPACTNET	2.000.000	3.190.000
NANOMMUNE	3.360.000	4.310.000
NANOPLAT	599.855	792.810
ENRHES	199.938	279.659
NHECD	1.450.000	1.620.000
NANORETOX	3.190.000	5.190.000
FRAMINGNANO*	675.044	742.934
OBSERVATORYNANO*	4.000.000	5.140.000
NANOTEST	2.990.000	3.940.000
NEURONANO	2.498.000	4.783.539
TOTAL	**20.964.837**	**29.985.403**

* include work packages on EHS impacts

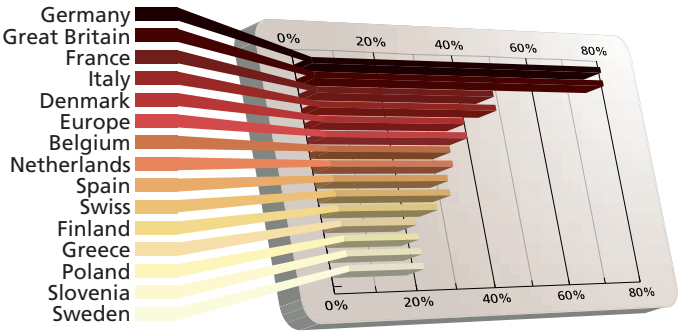
3.1.1 Overview of the Italian participation in financed research projects on EHS impacts of nanotechnologies within the EC Framework Programmes

As stated in the previous paragraph, the first research projects on potential impact of nanoparticles on human health and the environment were funded under FP5 (1998-2002). Three projects were launched and one of them NANO-PATHOLOGY (see Annexes) involved two Italian partners with one of which also coordinating it. The project received approximately 40% of the total grant allocated by FP5 to research projects on the impacts of nanotechnologies as well as 40% of the whole budget (co-funded by EU and partner resources).

Seven out of fifteen FP6 projects on the health and safety impacts of nanotechnologies involve Italian partners. The CANaPE project, in which an Italian partner was involved, is not included in this counting as it has been mainly focused on technological development, even though it included some *marginal* activities on EHS impacts. Projects involving Italian partners are CELLNANOTOX, DIPNA, PARTICLE-RISK, NANOCAP, SAPHIR, NANOTOX and NANO DIALOGUE (see Annexes). In FP6, Italy ranks as the fourth country more involved in the research projects launched with a total of 13 partnerships. As already mentioned, Italy participates in seven projects out of fifteen and two of them are coordinated by Italian institutions.

Projects participated by Italian partners received approximately 40% of the total grant allocated by FP6 for research projects on the impacts of nanotechnologies as well as 40% of the whole budget (co-funded by EU and partner resources).
Within FP7, four research projects on the health and safety impacts of nanotechnologies out of ten, launched in the first two years of activity (up to 2009), involved Italian partners. Projects involving Italian partners are: NANORETOX, FRAMINGNANO, OBSERVATORYNANO, NANOTEST (see Appendix of this Chapter). Four Italian partners (three different organizations, one of which participates in two projects) are involved in the ten funded FP7 research projects addressing EHS impacts of nanotechnologies. Moreover, Italy is represented by 40% of the financed projects. One of the ten FP7 projects is coordinated by an Italian partner. It is interesting to note that projects involving Italian partners collect more than 50% of the funding allocated by FP7 for projects addressing the impacts of nanotechnologies.
Wholly, 12 projects out of 28 funded over the running of last three framework programmes (1998-2009) involve Italian partners. Fig. 3.1.2 shows the countries involved in the projects on EHS impacts of nanotechnologies, according to the amount of projects funded, and illustrates the trend of the Italian participation over the running of the last three FPs.

% Projects on impacts of nanotechnologies over the running of the last three FPs (up to September 2009) and countries involved



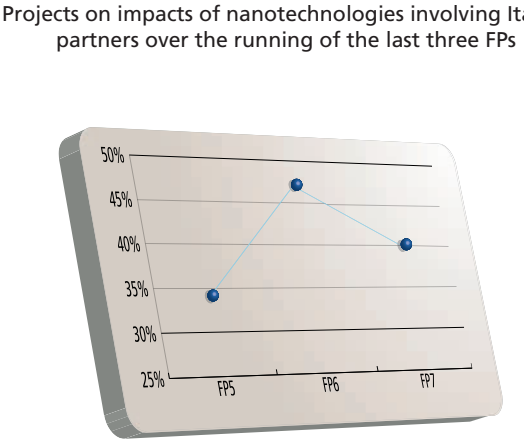


Figure 3.2 - Amount (%) of projects on EHS impacts of nanotechnologies over the running of the last three FPs.

The total Italian partnerships are 19 with 16 organization involved (some of them join more than one project): public research entities and Universities (43%), private organizations (38%) and Non-Governmental Organizations - NGOs (19%) (Fig. 3.1.3).

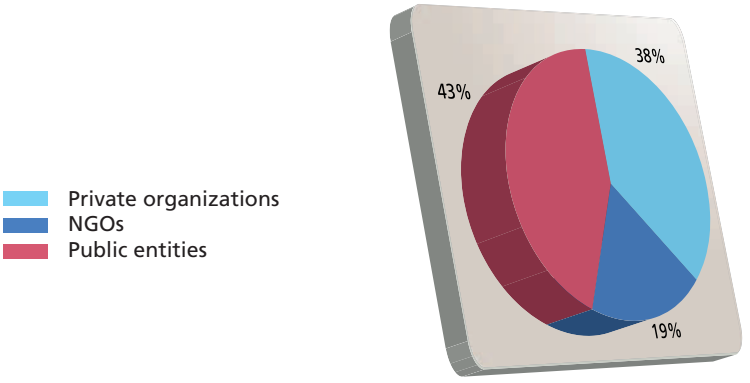
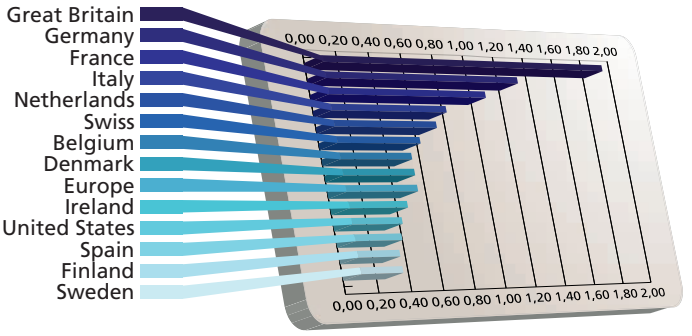


Figure 3.3 - Italian partnership in projects on nanotechnologies EHS impacts, referring to project types, over the running of the last three FPs

In order to provide more information on scope and trends of the Italian participation in the research projects over the running of the last three FPs, a *presence rate* has been elaborated, indicating the partnerships started compared to total number of funded projects over the running of the last three FPs on the EHS impacts of nanotechnologies (Fig. 3.1.4).

Presence rate in projects over the running of the last three FPs, divided by country



Presence rate of Italian partners in projects, divided by Framework Programme

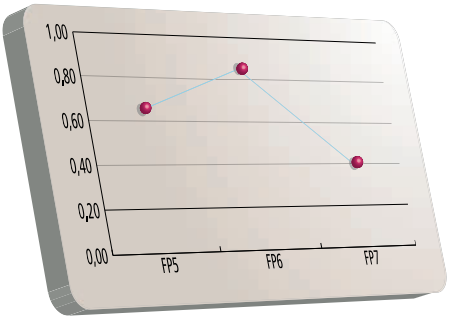


Figure 3.4 - Projects coordinated

If FP5, FP6 and FP7 are included in the count, projects coordinated by Italian organizations on the whole dimension and trend of the Italian participation in the research projects are four. As illustrated by Fig. 3.1.5., Italy ranks as second after UK in coordinated projects.

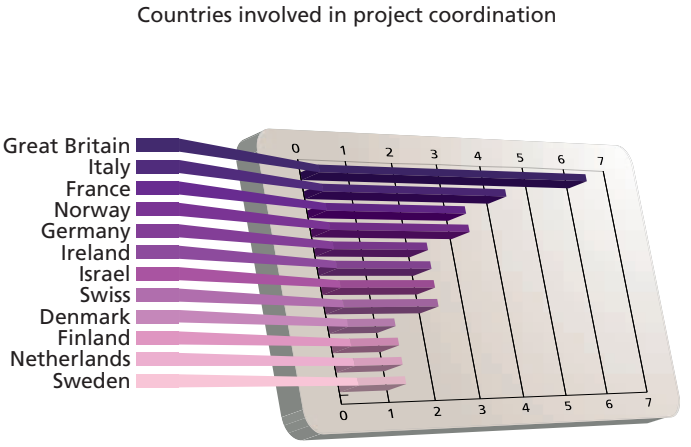


Figure 3.5 - Projects coordinated.

Under the last three FPs, more than 50% of grants were allocated to projects involving at least one Italian partner (Fig. 3.1.6).

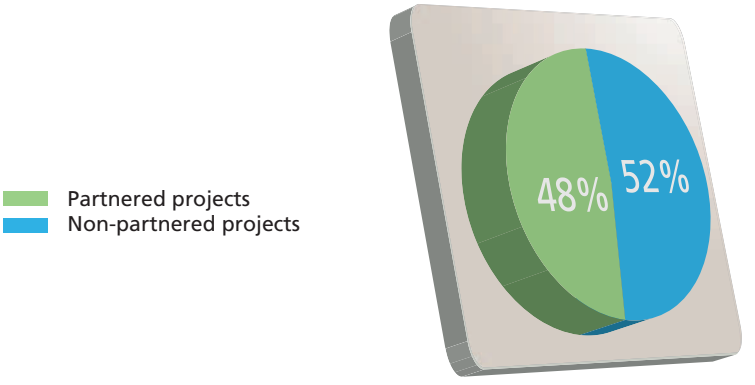


Figure 3.6 - Allocated grants.

The projects on the health and safety impacts of nanotechnologies involving Italian organizations address four main issues: i) lab research aimed at improving the knowledge of toxicological interactions among nanoparticles, environment and human beings; ii) improvement of safety procedures of nano-based productions; iii) governance of nanotechnology development and involvement of stake-holders in the health and safety risk analysis and in the setting-up of a shared regulatory framework; iv) contribution to impact analysis in technology development projects (See CANaPE).

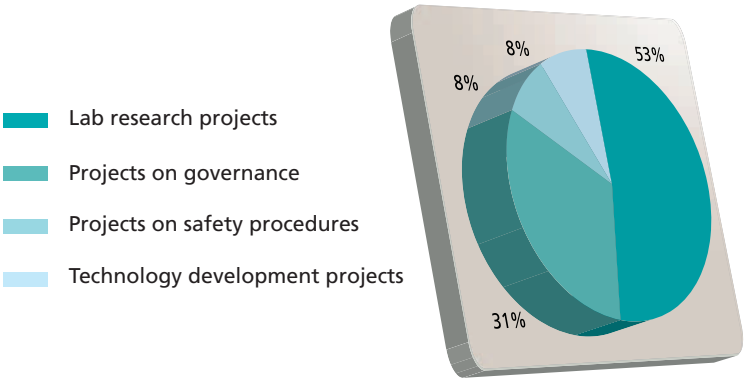


Figure 3.7 - Types of projects on the impacts of nanotechnologies funded over the running of the last three FPs.

3.2 National initiatives

3.2.1 Lombardy Region

1. **"Nanoscience for biomedical materials and applications" project**
In 2008, under the Framework Agreement with the National Research Council, the Lombardy Region received a grant of 10 million euro to launch a three-year project called "Nanoscience for biomedical materials and applications" with a regional co-fund of 5 million euro (2.5 for the Platform 1 -"Nanostructured systems for biomedical materials and applications"- and the remaining 2.5 for Platform 2 - "Development of antibiotics").
2. **The European Centre of Nanomedicine Foundation**
The European Centre of Nanomedicine Foundation was founded in Milan in July 2009 by 10 prestigious public and private research centres, under the auspices of

the President of the Lombardy Region who has provided significant financial support for the project, in collaboration with 9 public and private research centres in the territory. An allocation of 4.5 million euro is envisaged over the next three years (starting from 2009) under the Regional Operational Programme for underused areas fund - PAR-FAS (*Programma Attuativo Regionale del Fondo Aree Sottoutilizzate)*. The aim is to develop a research Centre of excellence in nanomedicine at the international level and provide innovative, non-invasive and customized solutions for the prevention, diagnosis and treatment of cancer as well as cardiovascular and neurological diseases. The Foundation was established with a joint protocol agreed on 21 March 2007 by the President of the Lombardy Region and nine "founder members":

1. IFOM Foundation (FIRC Institute of Molecular Oncology)
2. "Ca' Granda Ospedale Maggiore Policlinico" IRCCS Foundation
3. European Institute of Oncology (EIO)
4. European School of Molecular Medicine (SEMM)
5. STMicroeletronics S.r.l.
6. Genextra S.p.A.
7. Politecnico di Milano
8. University of Milan
9. University of Pavia

Moreover, 'Carlo Besta' National Neurological Institute too asked to join the Foundation and other important centres such as the National Cancer Institute of Milan and the Italian Institute of Technology of Genova showed interest in joining it.

The organs of the foundations are the Committee of Guarantors, the President and the Presidential Council, the scientific Directors and scientific Committee; their role is to carry out, promote and sustain research by reaching conventions with public and private entities or joining associations, foundations, entities and institutions pursuing the same objectives. Furthermore, the Foundation promotes and organizes seminars, training courses, demonstrations, conferences, meetings, laboratories, awards granting and scholarships. The complementarity of competence shared among founder members enables the Foundation to promote and launch interdisciplinary scientific research programmes aimed at developing innovative technologies and approaches in the field of nanomedicine. The ultimate objective is to produce a positive impact on the human health and, in general, on the capacities of the Lombardy health care system already deeply involved in the promotion of research, innovation and new technologies.

The European Centre of Nanomedicine sustains the development of advanced solutions for early diagnosis and mass screening of cardiovascular diseases and cancer based on proteomic, genomic and metabolic analysis and for the definition of customized treatments.

3.2.2 Piedmont Region

1. "Nanoparticles: from their impact on the environment and human health to safer production and usage - Nanosafe"

- Coordinator: "G. Scansetti" Centre (Interdepartmental Centre for Studies on Asbestos and other Toxic Particulates), University of Turin.
- Partners:
  - Project in collaboration with NIS;
  - Nanostructured Interfaces and Interfaces Interdepartmental Centre of Excellence (NIS);
  - Politecnico di Torino, Università del Piemonte Orientale, various SMEs.
- Funding body: Regional call for tender for the industrial research and pre-competitive development (CIPE-2006).
- Total amount of funding: 1.000.000 euro
- Start date: 2007
- End date: 2010
- Objectives: This project investigates the potential harmful impacts of some nanoparticles used in industry and produced during waste incineration. Its main aim is to improve the knowledge on safer usage of nanomaterials and management of particles released by the incinerators. Various nanomaterials with different dimensional cuts and synthesized by proponents (carbon nanotubes, composite materials, TiO_2 nanoparticles used in cosmetics, SiO_2 and Fe_2O_3) will be observed in this project. Once properly characterized, nanomaterials will be observed to define *in vitro* biological responses, such as inflammation and genotoxicity. On the basis of the results obtained from *in vitro* studies, some nanomaterials will undergo *in vivo* test in mice; TiO_2 will be tested on pig skin or reconstructed human epidermis. The knowledge gained through these tests on toxicity and biological behaviours will improve the management of production and usage of nanomaterials and serve as the driver for new screening tests to assess the toxicity of new materials.

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2. **Project for object-oriented health research financed by the Piedmont Region: Citotoxic and genotoxic damage caused by silica nanoparticles and microparticles: molecular basis and prevention and inactivation strategies (2006-2008)**

3. **Piedmont Region - Call for tenders for scientific research, Piedmont 2004. Area: Nanotechnologies and nanosciences, "Nanostructured biocompatible materials for biomedical applications".**

3.2.3 Veneto Region

1. **EuroNanoMed**
   - Title: EUROpean network of trans-national collaborative RTD projects in the field of NANOMEDicine
   - Coordinator: Atomic Energy Commission (CEA)/COMMISSARIAT ENERGIE ATOMIQUE CEA. FRANCE - Dr. Pierre-Noël LIRSAC
   - Partners:
     - Service public de Wallonie / Direction générale opérationnelle Economie, Emploi et Recherche (SPW) - *Belgium*
     - Agence nationale de la Recherche (ANR) - *Belgium*
     - Federal Ministry of Education and Research (BMBF) - *Germany*
     - VDI Technologiezentrum GmbH (VDI) - *Germany*
     - National Office for research and technology (NKTH) - *Hungary*
     - The Icelandic Centre for Research (RANNIS) - *Island*
     - Regione Veneto - Economic Development, Research and Innovation Department (VED) - *Italy*
     - Veneto Nanotech S.C.p.A. (Veneto Agency) - *Italy*
     - Ministry of Health, The Chief scientist office (CSO-MOH) - *Israel*
     - Latvian Academy of Sciences (LAS) - *Latvia*
     - Science Council of Lithuania (LSC) - *Lithuania*
     - National centre for research and development (NCBIR) - *Poland*
     - National Science Foundation (FCT) - *Portugal*
     - National Authority for Scientific research (ANCS) - *Romania*
     - National Center for Programme Management (CNMP) - *Romania*
     - Fondo de Investigación Sanitaria (FIS) Instituto de Salud Carlos IIII (ISCIII) - *Spain*
     - Industry, Trade and Tourism Department- Basque Government (ITT) - *Spain*
     - INNOBASQUE Parque Tecnológico de Bizkaia (INNOBASQUE) - *Spain*

  - Swedish Research Council (SRC) - *Sweden*
  - VINNOVA - *Sweded*
  - Swiss National Science Foundation (SNF) - *Swiss*
  - SenterNovem - *Netherlands*
  - The Scientific and Technological Research Council of Turkey (TUBITAK) - *Turkey*
- Objectives: Coordination of transnational research activities in the field of nanomedicine. Veneto Nanotech plays the role of international secretariat and is involved in the management of requests for contribution and in the state-of-the-art review of issues addressing the health impacts of nanomedicine.
- Website: http://www.euronanomed.net/

2. Nanosustain

- Complete title: Development of sustainable solutions for nanotechnology based products based on hazard characterization and LCA
- Coordinator: NordMiljö AB - *Sweded*
- Partners:
  - INSTITUTE OF NANOTECHNOLOGY - UK
  - DET NATIONALE FORSKNINGSCENTER FORARBEJDSMILJO - Denmark
  - VALTION TEKNILLINEN TUTKIMUSKESKUS - Finland
  - UNIVERSITAET BREMEN - Germany
  - VENETO NANOTECH SCPA - Italy
  - COMMISSION OF THE EUROPEAN COMMUNITIES - DIRECTORATE GENERAL JOINT
  - RESEARCH CENTRE - JRC - Belgium
  - KAUNO TECHNOLOGIJOS UNIVERSITETAS - Latvia
  - NATIONAL INSTITUTE FOR RESEARCH AND DEVELOPMENT IN MICROTECHNOLOGIES - Romania
  - NANOLOGICA AB - Sweden
  - NANOGATE AG - Germany
  - UPM-KYMMENE OYJ - Finland
  - AMROY EUROPE LTD - Finland
- Duration of project: 36 months.
- Funding body: European Commission (FP7 - NMP)
- Total amount of funding: 2.497.100 Euro
- Objectives: The main objective of the project is to develop a Life Cycle Assessment procedure. Veneto Nanotech is involved in all the project activities even though it is mostly committed in LCA studies and in the coordination of evaluation and validation activities.

3. **Impacts of innovative nanotechnological products on intracellular molecular motors and on the permeability of biological barriers.**
   - Coordinator: *VENETO NANOTECH, Italia*
   - Funding body: Veneto Region (*through ECSIN - European Center for the Sustainable Impact of Nanotechnology*).
   - Objectives: The project activities are intended to focus on the impact of nanotechnological products on molecular motors of cells and biological barriers with experiments involving different facilities such as the magnetic resonance imaging, optical imaging and electron microscopy imaging.

4. **Studies of toxicity phenomena, alterations in gene expression and mechanisms of action of nanomaterials in eukaryotic and prokaryotic cell systems.**
   - Coordinator: *VENETO NANOTECH, Italy*
   - Funding body: Veneto Region (*through ECSIN - European Center for the Sustainable Impact of Nanotechnology*).
   - Objectives:
     - To identify and develop specific techniques for chemicophysical characterization of commercial and synthesized nanoparticles;
     - to pinpoint proper technologies for the assessment of *in vitro* impact of nanoparticles on eukaryotic and prokaryotic cells;
     - to develop a series of parametric tests for the final assessment of nanoparticles on animal cells.

5. **Identification of engineered nanoparticles in toxicological tests and assessment of their harm to the environment**
   - Coordinator: *VENETO NANOTECH, Italy*
   - Funding body: Veneto Region (*through ECSIN - European Center for the Sustainable Impact of Nanotechnology*).
   - Objectives: The main objective is to improve knowledge on the environmental and eco-toxicological behaviours of nanoparticles and nanomaterials as well as to define procedures and methodologies for the assessment of the potential human and environmental exposure scenarios, taking into account the specific features these products exhibit during the different phases of their life cycle, from production to discharge/recycle.

6. **Environmental monitoring on nanotechnologicy production processes**
   - Coordinator: CIVEN
   - Start date: 01/01/2007

- End date: 30/06/2009
- Funding body: Veneto Region
- Total amount of funding: 340.000 euro
- Objectives: to census the potential sources of engineered nanoparticles in workplaces as well as quantify and characterize their emissions. Activities were divided into three different project lines: state-of-the-art analysis in the field of nanoparticles and nanotechnologies; evaluation of the presence of nanoparticles in the environment; identification of organic compounds and trace elements in nanoparticles and in fine and ultrafine particulates.

3.2.4 Projects launched under the auspices of the Ministry of Education, University and Research (MIUR)

1. Impacts of nanotubes on human health

- Coordinator:
  - University of Rome "Tor Vergata" - Dept. of neuroscience
  - University of Camerino - Dept. Molecular, Cellular and Animal Biology
  - University of Tuscia - Dept. of Agrobiology and Agrochemistry
- Funding body: MIUR Year 2005 - prot. 2005062028
- Total amount of fund: 102.000 euro
- Start date: 2005
- End date: 2006
- Objectives: to shed new light on the impacts of nanotubes on the cellular metabolism/vitality. Particular attention has been paid to the materials chosen for the tests (carbon nanotubes) which, due to the heterogeneity of preparations, may produce contradictory toxicological results. The project aimed also to investigate the wide array of potential functionalizations of nanotubes and some of their toxicological implications. In particular, the project was intended to identify chemical treatments able to reduce toxicity of nanotubes. Responses of specific cell lines have been studied in terms of apoptosis, influence on cell cycle and cell proliferation, redox modulations, potential gene alterations such as mitotic diseases and DNA damages.

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2. Study of molecular mechanisms responsible for the cell response to the crystalline and amorphous silica dusts. Identification of potential correlations between toxicity and surface state of particles.

- Coordinator: University of Turin (Prof. Dario Ghigo)
- Partners:
  - Dept. of Chemistry - IFM
  - NIS Centre of Excellence and INSTM (Materials Science and Technology National Consortium)
  - University of Turin; Dipartimento di Studio del Territorio e delle sue Risorse (DIPTERIS) - (Dept. Of Study of the Territory and its Resources), Genoa.
- Funding body: MIUR (COFIN2004, prot. 2004054901_003)
- Total amount of fund: 107.000 euro
- Start date: 2004
- End date: 2005
- Objectives: the project aims to use crystalline and amorphous silica dusts, unaltered or modified under strictly controlled experimental conditions, in such a way as they differ only for few specific surface features, in order to univocally unravel correlations between the biological effects of particles and their specific chemical and physical features. This could, on the one hand, help gain more knowledge on the pathogenic processes of silica and, on the other, identify inactivation techniques of the pathogenic potentials of dusts. Besides preparing spectroscopically and microcalorimetrically characterized silica-based materials with specific surface features, the project aimed at analyzing the interactions of materials with epithelial cells, human pulmonary fibroblasts and murine alve-

olar macrophages representing the main *in vivo* targets of the toxic inflammatory and cancerogenic action of silica. Particular attention has been paid to the effects on oxidoreductive cellular metabolism, free radical generation, oxidative damage to membrane lipids, oxidative damage to DNA, apoptosis, cellular proliferation and pro-inflammatory action.

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3. Interaction with biological systems of newly synthesized nanoparticle materials: experimental models for human health risk assessment.

- Coordinator: University of Parma, Dept. of Clinical Medicine, Nephrology and Prevention Sciences University of Parma (Prof. Enrico Bergamaschi).
- Partners:
  - University "Cattolica del Sacro Cuore" - Institute of Occupational Health (Prof. A. Bergamaschi). "G. D'annunzio" University CHIETI - PESCARA - Dept. Biomedical Science (Prof. P. Boscolo)
  - University of Rome, "Tor Vergata" - Dept. Biopathology and Imaging Diagnostics (Prof. A. Magrini)
  - Joint Research Centre - European Commission - Institute for Health and Consumer Protection ISPRA (VA)
- Funding body: MIUR
- Total amount of funding: 140.000 euro (total cost 204.000 Euro)
- Start date: 2006
- End date: 2008
- Objectives: The research aimed at unraveling all mechanisms responsible for toxicity of carbon nanotubes and metal element particles and investigating on the structure/activity relationship observed during the study and evaluation of their relevant effects on biological systems. The project also aims to develop new *in vitro* methods for the assessment of toxicity in newly synthesized nanomaterials and identify an array of tests for the risk assessment associated to the human exposure to newly synthesized materials. Five main areas have been observed: i) the transepithelial permeability of nanomaterials through the respiratory epithelial; ii) the effects caused by different nanomaterials on the following cells of biological relevance: airway epithelial cells, inflammatory monocyte-macrophagic cells, human cord-blood-derived stem cells in during T or NK lymphocytes differentiation process, human endothelial cells, peripheral blood lymphocytes; iii) basic mechanisms determining cell survival and/or apoptosis changes, cell proliferation, gene expression in the inflammatory response and oxidative stress mechanisms induced by nanomaterials: iv) DNA damages, including oxidative damage, through genotoxicity tests (Comet and micronucleus tests); v) the coherence among *in vitro* toxicity parameters applied on *in vivo* and *ex vivo* models, the latter aimed at evaluating the impact on the autonomic nervous system.

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4. Study of the mechanisms responsible for cytotoxicity and genotoxicity of silica nanoparticles and nanometrical fibrous silicates having strictly controlled size, structure and composition.

- Coordinator: University of Turin - Dept. of Genetics, Biology and Biochemistry (Prof. Dario Ghigo)

- Partners:
  - Politecnico di Torino
  - University of Bologna (Dept. of Chemistry)
  - University of Turin ("G. Scansetti" Centre)
  - University of Parma (Dept. Of Experimental Medicine)
  - University of Pisa (Dept. of Environmental Sciences)
  - Funding body: MIUR - Year 2007 - prot. 2007498XRF
  - Total amount of fund: 130.422 euro
- Start date: 2008
- End date: 2009
- Objectives: to clarify the role of the structure/activity relationship of silica-based nanostructured materials and investigate the surface reactivity and the biological effects of synthetic crystalline-amorphous silica NPs and of synthetic chrysotile nanofibers with strictly controlled and independently modifiable surface features. Nano and micrometric particles with similar chemical characteristics have been compared with a special focus on the correlation between chemico-physical features and their surface reactivity, free radical generation, induction of oxidative stress, inflammatory reaction, cytotoxicity, genotoxicity, E-M transition in cellular and *in vivo* cultures, migration across the epithelial-endothelial barrier both of *in vitro* and *in vivo* models.

References:

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5. Risks associated to the exposure to nanostructured materials: chemico-physical and toxicity studies on in vivo and in vitro models for the characterization of physiopathological mechanisms and kinetic profiles of particles.

- Coordinator: University of Pavia (Prof. Luigi Manzo)
- Partners:
  - University of Parma (Laboratory of Industrial Toxicology)
  - University of Siena
  - University of Turin
  - Funding body: MIUR
  - Total amount of fund: 176.000 euro

- Start date: 2007
- End date: 2009
- Objectives:
  - to develop a multidisciplinary research strategy for safety evaluation of nanomaterials;
  - to identify criteria and standards for the chemicophysical characterization of nanomaterials used for toxicological studies, which is key to ensure the reproducibility of toxicological tests;
  - to develop quantitative risk assessment indexes taking into account the biokinetic behaviors of nanoparticles and their effects [silica (SiO2) containing or not cadmium or cobalt and carbon nanotubes (CNTs)] on the biological system;
  - to ascertain whether the chemicophysical features and surface reactivity of nanoparticles may induce specific effects and biological mechanisms;
  - to define the "critical effects" and doses-response relationships on *in vitro* preparations (lung cells and CNS);
  - to assess the potential cellular uptake in cells and tissues (translocation) after application to cell cultures or animal administration;
  - to characterize early damage indicators in the airways (i. e. inflammatory indexes, oxidative stress markers, cytokines, chemokines, levels of nitrated proteins, proteomic profile) which can be measured in accessible biological matrixes (i.e. peripheral blood, urine, bronchoalveolar lavage).

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3.2.5 Projects under the auspices of the Ministry of Health

1. Nano-OSH Italia

- Coordinator: ISPESL - Institute of Occupational Safety and Prevention.
- Partners:
  - National Institute of Nuclear Physics (INFN), National Laboratories of Frascati
  - University of Rome "Tor Vergata"
  - "Salvatore Maugeri" Foundation
  - ARPA Puglia
- Funding body: Ministry of Health and ISPESL

- **Total amount of fund:** 465.000 euro
- **Start date:** 02/01/2008
- **End date:** 02/01/2011
- **Objectives:** to develop innovative methodologies for risk assessment in the occupational exposure to nanomaterials.
  The results of this project will help identify an integrated methodological system aimed at:
  - characterizing properly produced and functionalized carbon nanotubes (CNTs);
  - monitoring the environment in higher exposure risk workplaces;
  - monitoring exposure levels of a selected group of workers and evaluating the effects;
  - estimating the production processes where exposure risk is suspected.

These outputs are intended to be summarized in one comprehensive risk assessment model for prevention purposes.

References:

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- NNI. Strategy for Nanotechnology-related Environmental, Health and Safety Research. Executive Office of the President of the United States, National Science and Technology Council, National Nanotechnology Initiative, Feb 2008.

ANNEX 1

FACT SHEETS OF THE RESEARCH PROJECTS INVOLVING ITALIAN PARTNERS ON THE EHS IMPACTS OF NANOTECHNOLOGIES, FINANCED WITHIN THE EUROPEAN COMMISSION FRAMEWORK PROGRAMME FOR RESEARCH AND DEVELOPMENT (FP5-FP6-FP7)

FP5- EUROPEAN COMMISSION RESEARCH PROJECTS

1. NANO-PATHOLOGY

- **Title:** The role of micro and nanoparticles in biomaterial-inducing pathologies
- **Coordinator:** National Research Council (CNR), Italy (Dr. Manuela Arata)
- **Partners:**
  - UNIVERSITY OF MAINZ, Germany.
  - UNIVERSITY OF CAMBRIDGE,UK.
  - BIOMATECH SPA, FRANCE .
  - FEI ITALIA, Italy.
- **Start date:** 01/01/2002
- **End date:** 30/06/2005
- **Duration of project:** 42 months
- **Funding body:** European commission (FP5 - Quality of life)
- **Total amount of funding:** 999,937 euro. (Total cost 1.166.049 euro)
- **Objectives:**
  - To develop an innovative and technological methods of diagnosis aimed at identifying hexogen micro- and nanoparticles in unknown pathological processes.
  - To investigate patho-mechanisms.
  - To use the animal testing and in vitro models to investigate pathogenic mechanisms of disease potentially caused by micro and nanoparticles
  - To determine the pathological significance of the nanoparticles.
- **Website:** http://www.nanopathology.it/paginei/menu.htm

FP6- EUROPEAN COMMISSION RESEARCH PROJECTS

2. NANO-PATHOLOGY

- **Title:** Cellular interaction and toxicology with engineered nanoparticles
- **Coordinator:** University of Tel -Aviv, Israel (Prof. Rafi Korenstein)
- **Partners:**
  - Jrc- Institute For Health And Consumer Protection, Belgium
  - Institut National De La Sante Et De La Recherche Medicale (Inserm), France
  - Westfälische Wilhelms-Universität, Germany
  - J. Gutenberg University Of Maisz, Germany
  - Basf Aktiengesellschaft, Germany
  - Tp21 Gmbh, Germany
  - Colorobbia Italia Spa, Italy
- **Start date:** 01/11/2006
- **End date:** 30/04/2010
- **Duration of project:** 42 months
- **Funding body:** European commission (FP6 - NMP)
- **Total amount of funding:** 2.600.00 euro. (Total cost 3.651.500 euro)
- **Objectives:**
  - To unravel the physicochemical characteristics of NPs and their potential toxic effects on various organs of the human body.
  - To develop an innovative array of multidisciplinary tests and indicators for the assessment of toxicological profiles of nanoparticles.
- **Website:** http://www.fp6-cellnanotox.net/index.html

3. DIPNA

- **Title:** Development of an Integrated Platform for Nanoparticle Analysis to verify their possible toxicity and the eco-toxicity
- **Coordinator:** Consorzio Nazionale Interuniversitario Sviluppo Materiali - University of Modena and Reggio Emilia (Cnism) - Laboratory of Biomaterials, Dipt. Of Neurosciences - University of Modena and Reggio Emilia, Italy (Dr. Antonietta M. Gatti).
- **Partners:**
  - "Paris-Lodron" Universitat Salzburg, Austria
  - JRS, Institute for Health and Consumer Protection (IHCP) / European Centre for the Validation of Alternative Methods (ECVAM) UNIT, Belgium
  - Fraunhofer Institute of Biomedical Engineering, Germany
  - Grimm Aerosol Technik, Germany
  - National Research Council (CNR), Italy
  - Università della Magna Graecia di Catanzaro, Italy
  - Vlaamse Instelling voor Technologisch Onderzoek NV, Netherlands
  - Fundacio Privada Institut Catala de Nanotecnologia, Spain
  - Centre Suisse d'Electronique et de Microtechnique SA Recherche et Développement (CSEM SA), Swiss
- **Start date:** 01/11/2006
- **End date:** 31/10/2009
- **Duration of project:** 36 months
- **Funding body:** CE (FP 6 - NMP)
- **Total amount of fund:** 2.793.235 euro (Total cost 4.535.199 euro)
- **Objectives:**
  - To highlight interactions between nanoparticles and cells.
  - To improve the understanding of potential risks associated to nanoparticles and criteria to assess such risks on a case-by-case basis.
  - To develop a health risk assessment model for workers involved in nanotechnologies, citizens, final users and identify safety procedures.
  - To develop novel parameters to investigate nanoparticle pollution.
  - To setup prevention criteria and define reference standards for public authorities.
  - To create a platform to validate biodetection instruments for NPs-related risks.
- **Website:** http://www.dipna.eu

4. PARTICLE-RISK

- **Title:** Risk Assessment of Exposure to Particles
- **Coordinator:** Institute of Occupational Medicine, UK (Dr. Tran Lang)
- **Partners:**
  - National Institute of Occupational Health, Denmark
  - University of Edinburgh, UK
  - Napier University, UK
  - Forschungsz Fuer Umwelt und Gesundheit GMBH, Germany
  - Consorzio Venezia Ricerche, Italy
  - Università Cà Foscari di Venezia, Italy
- **Start date:** 01/06/2005
- **End date:** 31/08/2008
- **Duration of project:** 36 months
- **Funding body:** CE (FP 6 - NEST)
- Total amount of fund: 799.576 euro (Total cost 1.120.000 euro)
- **Objectives:**
  - To gain data on five particles potentially generated by novel and emerging technologies.
  - To assess the risk of exposure to such materials through in vitro experiments and rodent tests.
- **Website:** http://www.iom-world.com/particlerisk/

5. NANOCAP

- **Title:** Nanotechnology capacity building NGOS
- **Coordinator:** IVAM UVA BV, Netherlands (Drs. Jacques Cornelis e Pieter Van Broekhuizen)
- **Partners:**
  - PPM FORSCHUNG UND BERATUNG ARBEIT GESUNDHEIT UMWELT, Austria
  - KATHOLIEKE UNIVERSITEIT LEUVEN, Belgium.
  - AARHUS UNIVERSITET, Denmark.
  - UNIVERSITY OF ESSEX, UK.
  - TECHNISCHE UNIVERSITÄT DARMSTADT, Germany
  - FREIE UND HANSESTADT HAMBURG, BEHÖRDE FÜR WISSENSCHAFT UND FORSCHUNG, KOOPERATIONSSTELLE HAMBURG, Germany.
  - MEDITERRANEAN INFORMATION OFFICE FOR ENVIRONMENT, CULTURE AND SUSTAINABLE
  - DEVELOPMENT, Greece.
  - AMICUS, Irland
  - LEGAMBIENTE LOMBARDIA ONLUS, Italy
  - STICHTING NATUUR EN MILIEU, Netherlands
  - PUBLIC INSTITUTION BALTIC ENVIRONMENTAL FORUM (LITH. VIESOJI ISTAIGA
  - BALTIJOS APLINKOS FORUMAS), Lithuania.
  - FEDERATIE NEDERLANDSE VAKBEWEGING, Netherlands
  - UNIVERSITEIT VAN AMSTERDAM, Netherlands
  - EUROPEAN ENVIRONMENTAL BUREAU / BUREAU EUROPÉEN DE L'ENVIRONNEMENT
  - EUROPEAN TRADE UNION INSTITUTE FOR RESEARCH, EDUCATION, HEALTH AND SAFETY
- **Start date:** 01/09/2006
- **End date:** 31/08/2009
- **Duration of project:** 36 months
- **Funding body:** CE (FP 6 - SOCIETY)
- **Total amount of fund:** 1.310.000 euro
- **Objectives:**
  - to improve the understanding of environmental, occupational health and safety risks and ethical aspects of nanotechnology through a debate on nanotechnologies at European level involving environmental NGOs and trade unions.

- to develop recommendations to enable public authorities to address the health, safety and environmental risk issues related to the rapid introduction of nanotechnology into society.
- to give industry the tools to introduce a "responsible nanotechnology", i.e. to stimulate industrial and academic performers to focus on source reduction regarding nanoparticles and to make risk assessment an important dimension in their work.

• **Website:** http://www.nanocap.eu/Flex/Site/Page.aspx?PageID=&Lang=

6. SAPHIR

- **Title:** Safe, integrated & controlled production of high-tech multifunctional materials and their recycling.
- **Coordinator:** Compagnie Industrielle Des Lasers, CILAS, France (Mr. Christophe Goepfert)
- **Partners:**
  - EADS FRANCE (INNOVATION WORKS DEPARTMENT), France
  - FRAUNHOFER - GESELLSCHAFT ZUR FOERDERUNG
  - DER ANGEWANDETEN FORSCHUNG E.V., Germany
  - EADS CCR, France
  - UNIVERSITA DE SHERBROOKE, Canada
  - CENTRO RICERCHE PLAST-OPTICA SPA, Italy
  - ARKEMA France, France
  - BRITISH CERAMIC RESEARCH LIMITED, UK
  - STOCKHOLMS UNIVERSITET (STOCKHOLM UNIVERSITY), Sweden
  - CENTER FOR RESEARCH AND TECHNOLOGY HELLAS, Greece
  - QINETIQ NANOMATERIALS LTD, UK
  - INSTITUT JOZEF STEFAN, Slovenia
  - FUNDACION LABEIN, Spain
  - TEKNA PLASMA SYSTEMS INC, Canada
  - NIRO A/S, Denmark
  - INSTITUT NATIONAL DE L'ENVIRONNEMENT INDUSTRIEL ET DES RISQUES, France
  - ECOLE POLYTECHNIQUE FEDERALE DE LAUSANNE, Swiss
  - ARCELOR RESEARCH LIEGE SCRL, Belgium
  - MECACHROME SAS, France
  - ZENTRUM FUER BRENNSTOFFZELLEN-TECHNIK GMBH, Germany
  - IRD FUEL CELLS A/S, Denmark
  - C-TECH INNOVATION LTD, UK
  - COMMISSARIAT A L'ENERGIE ATOMIQUE (CEA), France
  - ALMA CONSULTING GROUP SAS, France
- **Start date:** 01/10/2006
- **End date:** 30/09/2010
- **Duration of project:** 48 months
- **Funding body:** CE (FP 6 - NMP)
- **Total amount of fund:** 8.100.000 euro (Total cost 15.800.000 euro)
- **Objectives:** The general objective of the project is the safe, integrated and

controlled production of high-tech multifunctional nanostructured products including their recycling and ensuring competitiveness.
In particular, the project aims at developing:
- Production processes during which no particle releases take place;
- Production processes consisting in linking together elementary existing or emerging processes in a safe way;
- Production processes controlled by innovative systems ensuring efficiency, reliability and traceability of products and, at the same time, a safe production.

- **Website:** http://www.saphir-project.eu/

7. NANOTOX

- **Title:** Nano-particle characterization and toxicity
- **Coordinator:** Chalex Research Ltd, UK (Pr. Mark Pullinger)
- **Partners:**
  - NANOCYL S. A., Belgium
  - BULGARIAN ACADEMY OF SCIENCES, Bulgaria
  - HELSINKI UNIVERSITY OF TECHNOLOGY, Finland
  - THE UNIVERSITY OF MANCHESTER, UK
  - CONSORZIO PER LO SVILUPPO DEI SISTEMI A GRANDE INTERFASE, Italy
  - MBN NANOMATERIALIA SPA, Italy
  - NOFER INSTITUTE OF OCCUPATIONAL MEDICINE, Poland
  - CMP CIENTIFICA SL, Spain
- **Start date:** 01/31/2005
- **End date:** 31/01/2007
- **Duration of project:** 24 months
- **Funding body:** CE (FP 6 - NMP)
- **Total amount of fund:** 408.544 euro (Total cost 399.894 euro)
- **Objectives:**
  - To examine and standardize knowledge of the physicochemical characteristics of nanoparticles and nanocrystals, their transformation and usage, their effects on the human health and the environment, their mutagenicity and genotoxicity, safety standards, etc.
  - To map the on-going research activities and their progress in an online database at European level.
  - To define guidelines and recommendations to sustain the European institutions in setting standards, regulations, policies and practical codes for a safe production and usage of nanoparticles.
- **Website:** http://www.dipna.eu

8. CANaPE

- **Title:** Carbon Nanotubes for Applications in Electronics, Catalysis, Composites and Nano-Biology
- **Coordinator:** The Chancellor, Masters and Scholars of the University of Cambridge, UK (Prof. John Robertson)
- **Partners:**
  - CRIF-WALLONIE, Belgium
  - UNIVERSITE DE LIEGE - CENTRE SPATIAL DE LIEGE, Belgium
  - UNIVERSITE MONTPELLIER II, France
  - THALES, France
  - IMPERIAL COLLEGE OF SCIENCE TECHNOLOGY AND MEDICINE, UK
  - THOMAS SWAN & CO LTD, UK
  - HITACHI EUROPE LTD, UK
  - MAX-PLANCK-GESELLSCHAFT ZUR FORDERUNG DER WISSENSCHAFTEN E.V, Germany
  - DARMSTADT UNIVERSITY OF TECHNOLOGY, Germany
  - NANOSCAPE, Germany
  - STMICROELECTRONICS SRL, Italy
  - NATIONA RESEARCH COUNCIL, Italy
  - SWISS FEDERAL LABORATORIES FOR MATERIALS TESTING AND RESEARCH, Swiss
- **Start date:** 01/06/2004
- **End date:** 31/05/2008
- **Duration of project:** 48 months
- **Funding body:** CE (FP 6 - NMP)
- **Total amount of fund:** 6.400.000 euro (Total cost 8.650.000 euro)
- **Objectives:** To enable a large scale production of carbon nanotubes through chemical vapour deposition method.
  The projects specifically aims:
  - To obtain a large scale production
  - To carry out toxicological studies on carbon nanotubes to assess potential risks to human health.
  - To test biocompatibility of nanotubes and polymers composites
- **Website:** http://www.canapeweb.com/

9. NANO DIALOGUE

- **Title:** Enhancing dialogue on nanotechnologies and nanosciences in society at the European level
- **Coordinator:** Fondazione IDIS-Città della Scienza, Atelier e Progetti di Comunicazione Department, Science Centre Department, Italy (Dr. Luigi Amodio)
- **Partners:**
  - FLANDERS TECHNOLOGY INTERNATIONAL FOUNDATION, Belgium
  - ASSOCIATION EUROPENNE DES EXPOSITIONS SCIENTIFIQUES, TECHNIQUES ET INDUSTRIELLES, Belgium
  - SCIENCE CENTRE AHHAA FOUNDATION, Estonia
  - CENTRE DE CULTURE SCIENTIFIQUE, TECHNIQUE ET INDUSTRIELLE DE GRENOBL, France
  - UNIVERSITY OF WESTMINSTER, UK
  - DEUTSCHES MUSEUM, Germany
  - ASSOCIAZIONE MACROSCOPIC QUANTUM COHERENCE AND COMPUTING, Italy
  - CIÊNCIA VIVA - AGÊNCIA NACIONAL PARA A CULTURA CIENTIFICA E TECNOLÓGICA, Portugal
  - FUNDACIÓ PARC CIENTÍFIC DE BARCELONA, Spain
  - UNIVERSEUM AB, Sweden
- **Start date:** 01/03/2005
- **End date:** 28/02/2007
- **Duration of project:** 24 months
- **Funding body:** CE (FP 6 - NMP)
- **Total amount of fund:** 850.000 euro (Total cost 935.078 euro)
- **Objectives:** To implement an integrated communication process at European level and a social debate on nanotechnologies and nanosciences. The project specifically aims to:
  - provide information and increase public awareness of the technological progresses recorded in this field;
  - to promote the social dialogue among researchers, citizens and social actors and identify their needs;
  - to provide the Commission and experts with recommendations relating to the most relevant societal concerns associated with nanotechnologies.
- **Website:** http://www.canapeweb.com/

FP 7 - EUROPEAN COMMISSION RESEARCH PROJECTS

10. NANORETOX

- **Title:** The reactivity and toxicity of engineered nanoparticles: risks to the environment and human health
- **Coordinator:** Natural History Museum Cromwell Road, UK (Ms. Vanessa Pike)
- **Partners:**
  - COMMISSION OF THE EUROPEAN COMMUNITIES - DIRECTORATE GENERAL JOINT RESEARCH CENTRE - JRC, Belgium
  - ROSKILDE UNIVERSITETSCENTER, Denmark
  - UNIVERSITE CATHOLIQUE DE L OUEST ASSOCIATION SAINT YVES, France
  - UNIVERSITE DE NICE - SOPHIA ANTIPOLIS, France
  - INTRINSIQ MATERIALES LIMITED, UK
  - IMPERIAL COLLEGE OF SCIENCE, TECHNOLOGY AND MEDICINE, UK
  - KING'S COLLEGE LONDON, UK
  - AHAVA DEAD SEA LABORATORIES LTD, Israel
  - UNIVERSITA' DI PISA, Italy
  - UNIVERSIDAD DEL PAIS VASCO/EUSKAL HERRIKO UNIBERTSITATEA, Spain
  - DEPARTMENT OF THE INTERIOR USA, United States
- **Start date:** 01/12/2008
- **End date:** 30/11/2012
- **Duration of project:** 48 months
- **Funding body:** CE (FP 7 - NMP)
- **Total amount of fund:** 3.190.000 euro (Total cost 5.190.000 euro)
- **Objectives:** To investigate health and environmental risks of nanomaterials. In particular the projects aims to observe:
  - the way the environment where nanoparticles are released impacts their chemicophysical properties and bioreactivity;
  - the way the environmental impacts the ability of particles to penetrate cells and produce toxic effects;
  - the interactions with nanoparticles identified as posing a big risk to human health.

  The research is intended to develop a risk assessment model.
- **Website:** http://www.nanoretox.eu/

11. FRAMINGNANO

- **Title:** International multi-stakeholder dialogue platform framing the responsible development of nanosciences and nanotechnologies
- **Coordinator:** Associazione Italiana per la Ricerca Industriale, Italy (Mr. Guido Frigessi Di Rattalma)
- **Partners:**
  - TECHNOLOGICKE CENTRUM AV CR, Czech Republic
  - FONDATION EURACTIV, Belgium
  - NATIONAL INSTITUTE FOR PUBLIC HEALTH AND THE ENVIRONMENT, Netherlands
  - INSTITUTE OF NANOTECHNOLOGY, UK
  - THE INNOVATION SOCIETY LTD, Swiss
- **Start date:** 01/05/2008
- **End date:** 31/03/2010
- **Duration of project:** 23 months
- **Funding body:** CE (FP 7 - SIS)
- **Total amount of fund:** 675.044 euro (Total cost 742.934 euro)
- **Objectives:** To support a dialogue on nanotechnologies involving stakeholders such as scientists, institutions, industrial community and citizens in order to define solutions for a constructive and feasible regulatory framework aimed at the promotion of a responsible development in the field of nanotechnologies. This project is intended to setup a Governance Plan outlining a deliberative process aimed at creating the conditions for a responsible development of NS&T at European level as well as including recommendations for the scientific research and political action.
- **Website:** http://www.framingnano.eu/

12. OBSERVATORYNANO

- **Title:** European observatory for science-based and economic expert analysis of nanotechnologies, cognisant of barriers and risks, to engage with relevant stakeholders regarding benefits and opportunities.
- **Coordinator:** Institute of Nanotechnology - Stirling University Innovation Park, UK (Ms. Robina Fisher)
- **Partners:**
  - Aarhus Universitet, Denmark
  - SPINVERSE OY, Finland
  - Commissariat a l'Energie Atomique (CEA), France
  - INSTITUTE OF OCCUPATIONAL MEDICINE LIMITED, UK
  - TRIPLE INNOVA GMBH, Germany
  - NMTC (NANO & MICRO TECHNOLOGYCONSULTING), Germany
  - VDI TECHNOLOGIEZENTRUM GMBH, Germany
  - TECHNISCHE UNIVERSITAET DARMSTADT, Germany
  - ASSOCIAZIONE ITALIANA PER LA RICERCA INDUSTRIALE - AIRI, Italy
  - RIJKSINSTITUUT VOOR VOLKSGEZONDHEID EN MILIEU, Netherlands
  - UNIVERSITEIT MAASTRICHT, Netherlands
  - MALSCH TECHNOVALUATION, Netherlands
  - TECHNOLOGICKE CENTRUM AKADEMIE VED CESKE REPUBLIKY VEREJNA VYZKUMNA INSTITUCE, Czech Republic
  - BAX & WILLEMS SL, Spain
  - EIDGENOESSISCHE MATERIALPRUEFUNGS- UND FORSCHUNGSANSTALT, Swiss
- **Start date:** 01/04/2008
- **End date:** 31/03/2012
- **Duration of project:** 48 months
- **Funding body:** CE (FP 7 - NMP)
- **Total amount of fund:** 4.000.000 euro (Total cost 5.140.000 euro)
- **Objectives:** To support the correlation of technological and scientific development of nanotechnologies and their social and economical impact in order to reduce it and set the development of nanotechnologies in a realistic temporal scale. In this view, the project is intended to carry out an analysis of the scientific literature and national investment and marketing strategies and to conduct surveys with questionnaires, interviews and workshops for academicians and key representatives of the industrial and financial world.
- **Website:** http://www.observatory-nano.eu/project/

13. NANOTEST

- **Title:** Development of methodology for alternative testing strategies for the assessment of the toxicological profile of nanoparticles used in medical diagnostics
- **Coordinator:** Norsk Institutt for Luftforskning*, Norway (Dr. Maria Dusinka)
- **Partners:**
  - COMMISSION OF THE EUROPEAN COMMUNITIES - DIRECTORATE GENERAL JOINT RESEARCH CENTRE JRC, Belgium
  - KOBENHAVNS UNIVERSITET, Denmark
  - UNIVERSITE DE PARIS VII DENIS DIDEROT, France
  - UNIVERSITY HOSPITALS BRISTOL NHS FOUNDATION TRUST, UK
  - INSTITUTE OF OCCUPATIONAL MEDICINE, UK
  - NATIONAL CENTER FOR SCIENTIFIC RESEARCH "DEMOKRITOS", Greece
  - NMTC (NANO & MICRO TECHNOLOGYCONSULTING), Germany
  - VDI TECHNOLOGIEZENTRUM GMBH, Germany
  - TECHNISCHE UNIVERSITAET DARMSTADT, Germany
  - UNIVERSITA CA' FOSCARI DI VENEZIA, Italy
  - SLOVENSKA ZDRAVOTNICKA UNIVERZITA V BRATISLAVE, Slovakia
  - ADVANCED IN VITRO CELL TECHNOLOGIES S.L., Spain
  - HOSPICES CANTONAUX CHUV, Swiss
- **Start date:** 01/04/2008
- **End date:** 31/09/2011
- **Duration of project:** 42 months
- **Funding body:** CE (FP 7 - HEALTH)
- **Total amount of fund:** 2.990.000 euro (Total cost 3.940.000 euro)
- **Objectives:** To develop alternative testing strategies based on in vitro and in silico models for the assessment of the toxicological profile of nanoparticles used in medical diagnostics.

The project specifically aims:

  - to define nanoparticles properties and characterize those intended to be used;
  - to observe interactions of nanoparticles with molecules, cells and organisms and to develop in vitro models to investigate their potential toxicological effects;
  - to validate short-term in vitro results obtained with in vivo models and observe the effects of nanoparticles in animals and men in order to highlight the individual sensitivity;
  - to develop in silico models of interactions with nanoparticles.
- **Website:** http://www.nanotest-fp7.eu/

chapter 4

Protocols for information gathering and exposure characterization of nanomaterials

Danilo Cottica (coordinator), *Salvatore Maugeri Foundation, Pavia*
Stefano Bellucci, *Italian National Institute of Nuclear Physics, Frascati National Laboratories*
Stefano Casciardi, *INAIL, Department of Occupational Hygiene, formerly ISPESL*
Paola Castellano, *INAIL, Department of Occupational Hygiene, formerly ISPESL*
Matteo Goldoni, *INAIL, Parma Research Centre, formerly ISPESL*
Elena Grignani, *Salvatore Maugeri Foundation, Pavia*
Ivo Iavicoli, *University "Cattolica del Sacro Cuore" of Rome*
Achille Marconi, *Italian National Health Institute*

4.1 Identification of aerodispersion sources

The "voluntary" production of nanomaterials (NMs) for nanotechnologies can be realized through two different chemicophysical approaches (INRS, 2007): the "bottom up" and "top-down" methods, whose definitions have already been provided herein. The former is the result of nanotechnology research and consists in obtaining materials in the desired configuration by assembling atoms following pre-defined schemes; the latter is widely used in the electronic industry for materials and components miniaturization. As far as the size of material under investigation is concerned, both approaches converge on the field of nanoparticles (NPs). The "bottom-up" approach refers to the chemico-physical processes whereas the "top-down" approach usually involves mechanical processes.

NPs do not always represent the final product of the technological cycle. Nanoparticles in the workplace environment are often byproducts of nucleation and condensation processes of some aerosol precursors such as gases, liquids and solids. The following are processes that most develop or involve thermal energy: metals refinery and manufacturing, high-temperature spray application, welding, grinding and carving of metals or alloys; the "undesired" products are nano metal and/or metal oxide particles exhibiting a large surface area and, usually, a low solubility.

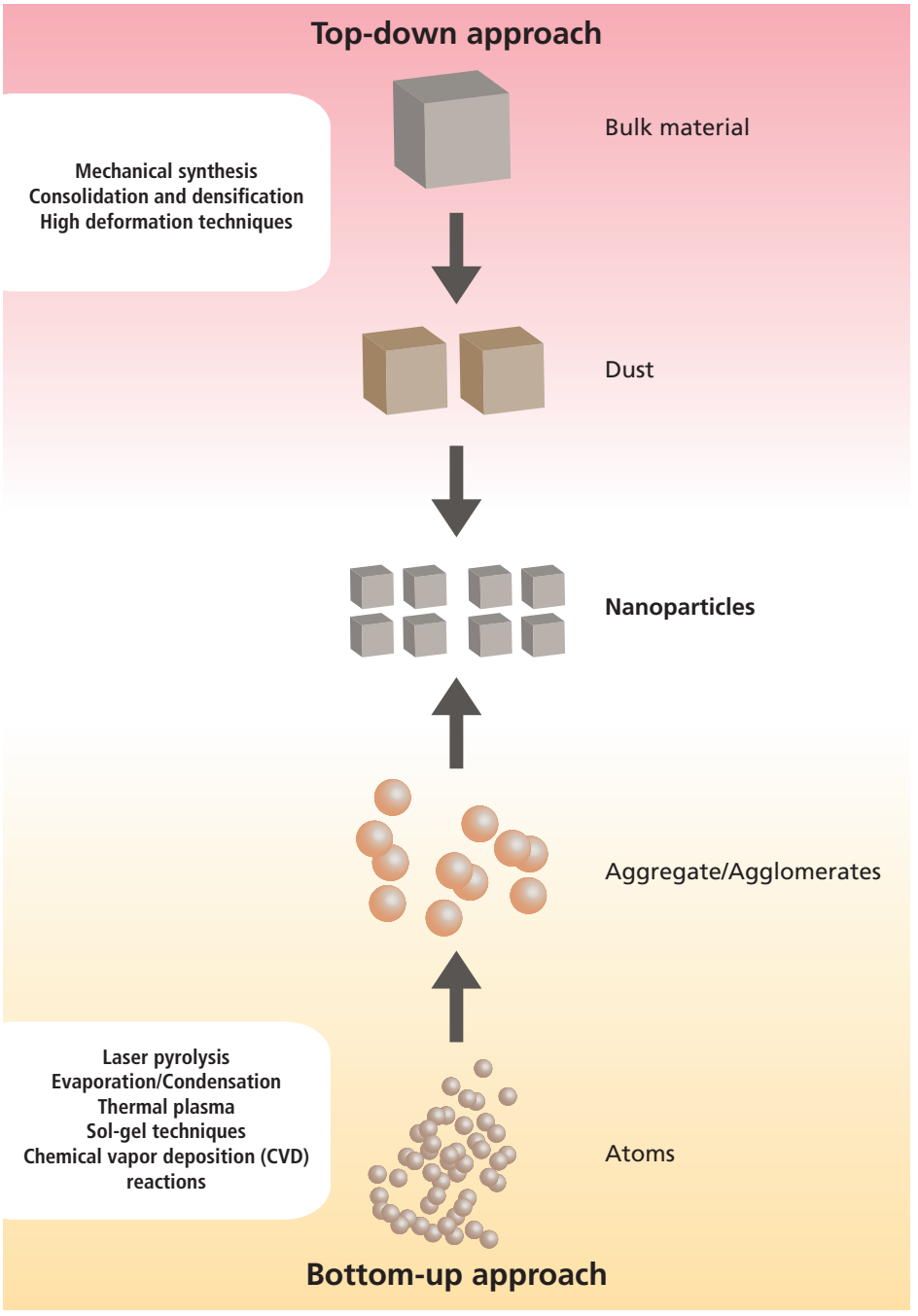

Figure 4.1 - Chemico-physical formation processes of nanomaterials (INRS, 2007).

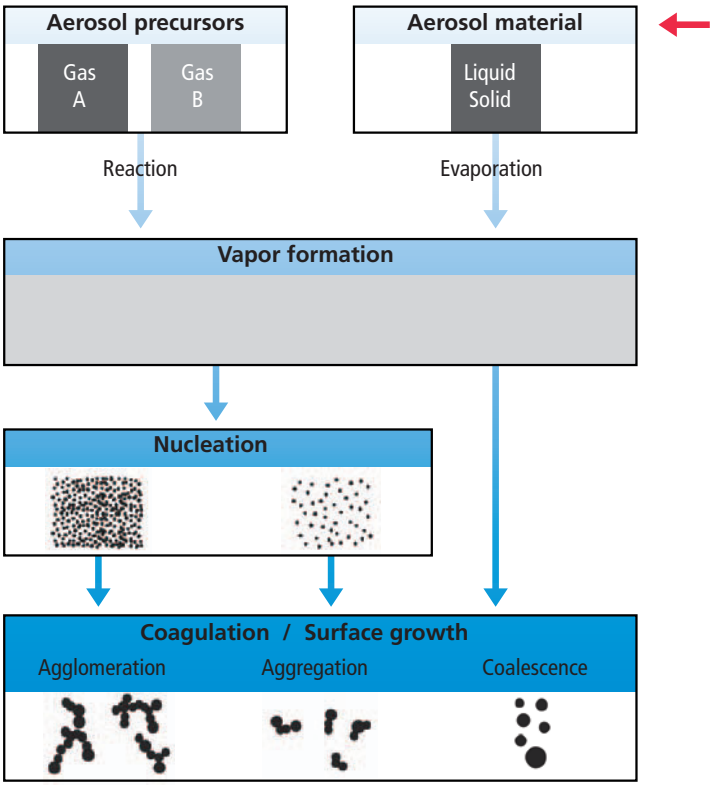

Figure 4.2 - Processes of NP formation (BIA, 2003).

Also combustion processes may produce NPs through CVD or nucleation/condensation reactions.

The characteristics of particles produced through such processes depend upon the chemical and physical conditions under which they take place. However, primary particles usually have a diameter of 10 to 15 nm and coagulate rapidly according to their concentration in the point of origin and end up becoming bigger than NPs.

Particles generated by punctual sources at high temperature and concentration (i. e. solder fumes) are destined to a rapid condensation within a short distance from the source; hence, for the assessment of the potential exposure to NPs it is fundamental to determine the distance from the source to the operator's breathing zone and, as

a consequence, the position of the sampling substrate. Conversely, in "widespread" sources the relatively lower temperatures determine a rapid slowdown of the coagulation process and, as a result, the ability of nanoparticles to maintain their size and get airborne, thus increasing the risk of exposure for the operator and/or people in the vicinity of the source.
Thermal processes which generate aerosols exhibiting a large specific surface area include the formation of carbon black, TiO_2 NPs, fumes of aluminium and silica evaporation processes. These processes usually generate agglomerated particles larger than NPs (bigger than 100 nm) with a specific surface area of more than 300 m^2/g. This group includes materials produced in wet form but used as dry dusts such as ruthenium black, palladium black and some types of TiO_2.

4.1.1 Nano-objects and NMs manufacturing processes

To date, the technological processes enabling the manufacturing of NMs and nano objects can be summarized in Tab. 4.1.

Table 4.1 - NP manufacturing processes.

Categories	Types of process
Physical processes	• Evaporation/condensation • Laser ablation • Electric discharge • Combustion flames • Laser pyrolysis • Microwaves • Ion or electrochemical irradiation • Catalytic decomposition • Vapor deposition (physical vapor deposition)
Chemical processes	• Vapor reaction (chemical vapor deposition) • Liquid reactions: chemical co-precipitation, hydrolysis, etc. • Solid reactions • Supercritical fluids with chemical reactions • Silica or metal oxide solution/gel techniques
Mechanical processes	• High energy crashing or mechanical synthesis • Welding • High energy mechanical deformation techniques: torsion, friction, lamination, etc.

These processes cause potential exposure risks, as summarized in Tab. 4.2.

Table 4.2 - Potential exposure risks associated with intentional NP manufacturing processes.

Process Synthesis	Particle Formation	Potential inhalation risk	Potential dermal and ingestion risk
Gas Phase	In air	Direct leakage from reactor Product recovery	• Aerosol particle contamination in the workplace • Product manipulation • Equipment cleaning/maintenance
Vapor Deposition	On substrate	Product recovery from reactor Processing and packaging	• Dry powder contamination in the workplace • Product manipulation • Equipment cleaning/ maintenance
Colloidal	Liquid suspension	Drying, processing and pouring of product	• Pouring/Contamination in the workplace • Product manipulation • Equipment cleaning/maintenance
Attrition	Liquid suspension	Drying, processing and pouring of product	• Pouring/Contamination in the workplace • Product manipulation Equipment cleaning/ maintenance

4.1.2 Sources of aerosol NPs

Tab. 4.3 illustrates some example provided by the *International Standardization Organisation* (ISO) of potential sources of NPs exposure with regard to technological cycles and activities involving also their unintentional formation (ISO/TR, 2007):

Table 4.3 - Sources of aerosol NPs.

Types of process	Source/activities
Hot process	• metal refining • steel casting • galvanic processes • metal cutting by thermal torch • coatings deposited via thermal spray • hot wax application • aluminium casting • iron casting • welding • laser metal cutting • firing
Combustions	• diesel engines • petrol engines • gas engines • gas heating • incinerators
Indoor aerosols	• aerosols formation from the reactions of office and cleaning equipments in gas/vapor phase
Mechanical processes	• grinding • metal grinding • polishing • high speed drilling
Dusts production	• carbon black production • TiO_2 ultrafine production • fumed silica production • fumed alumina production
Handling	• handling of nanoparticle dusts • dry-handling of colloidal deposits
Nanotechnologies	• production of carbon nanotubes • production of engineered nanoparticles in the gas-phase • spraying from suspensions and solutions of engineered nanoparticles • handling and use of engineered nanoparticles

4.2 Potential exposure routes

Potential routes of occupational nanoparticle exposure include inhalation**,** dermal contact, olfactory and ingestion.

4.2.1 Inhalation

The most common route of exposure to airborne particles in the workplace is inhalation. The deposition of discrete nanoparticles in the respiratory tract is determined by the particle's diameter (size-dependant). Agglomerates of nanoparticles will deposit according to the diameter of the agglomerate and not to that of each nanoparticle. As predicted by the IRRP deposition model for oral inhalation of 1994, inhaled particles substantially deposit primarily in the alveolar, but also in the tracheobronchial and extrathoracic regions (Fig. 4.3) (ISO, 2008; Yeh HC et al. 1996).

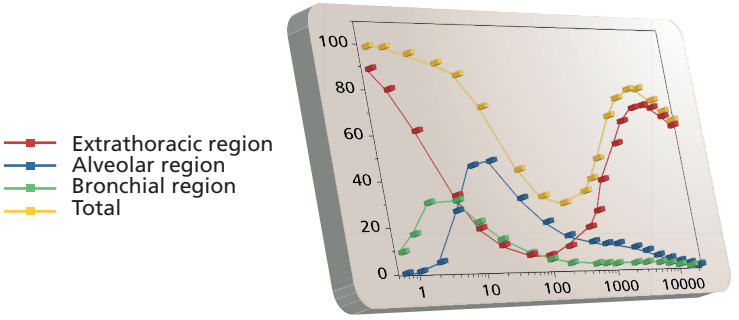

Figure 4.3 - Total and regional deposition probability of particles in the human respiratory tract based on the ICRP 66 model. The deposited fraction includes the probability of particles being inhaled (inhalability). The measurements are based on nose breathing subjects having an ordinary job.

It is worth noting that, after the deposition, the life cycle of NPs is dependant on their biopersistence (durability) and their potential for translocation to other organs and tissues. Research is still ongoing to determine the physical factors that contribute to the agglomeration and de-agglomeration of nanoparticles and the role of agglomerates in the toxicity of inhaled nanoparticles (Maynard AD and Kuempel ED, 2005).

The *International Commission on Radiological Protection* (ICRP) has recently updated and commented the deposition models of particles in airways according to their aerodynamic diameter and distinguished 5 main regions: 1) extrathoracic airway 1 (ET1) including the anterior nasal passage; 2) extrathoracic airways 2 (ET2) including posterior nasal passage, mouth, larynx and pharynx; 3) bronchial regions 2 (BB) including bronchioles and terminal bronchioles (bb); 5) alveolar-interstitial region (AI) (ICRP, 1994; ICRP, 2002; Bailey MR et al, 2003). According to the *Activity Median Thermodynamic Diameter* (AMTD), which is based on the assumption that particles with a diameter smaller than 100 nm can deposit by diffusion, the ICRP calculated the percentage of NP deposition setting the following experimental parameters:

- Log-normal distribution of particle diameter;
- 3 g/cm^3 density; density, however, scarcely affects NP deposition;
- 1.5 shape factor, i.e. compact, irregular and unspherical particles.

Tab. 4.4 illustrates the deposition of particles in workers' respiratory system compartments under normal (flux = 1.2 m^3/h) and intense (flux= 1.7 m^3/h) occupational activities and taking account of both nasal and oral breathing.

Table 4.4 - Deposition of inhaled NPs in workers' airway compartments provided by the ICRP model.

Normal flux 1.2 m^3/h - Nasal breathing - Oral breathing						
AD (nm)	**ET1 (%)**	**ET2 (%)**	**BB (%)**	**bb (%)**	**AI (%)**	**Total (%)**
5	16.0 - 7.5	18.0 - 18.0	5.6 - 6.2	26.0 - 30.0	27.0 - 30.0	92.0 - 91.0
10	8.7 - 4.2	9.8 - 9.9	3.0 - 3.2	19.0 - 20.0	47.0 - 50.0	88.0 - 87.0
20	5.3 - 2.6	5.9 - 6.0	1.8 - 1.8	12.6 - 13.0	49.0 - 50.0	74.0 - 73.0
50	3.2 - 1.5	3.4 - 3.4	1.0 - 1.0	7.2 - 7.4	31.0 - 32.0	46.0 - 45.0
100	3.2 - 1.2	3.2 - 2.4	0.8 - 0.8	4.8 - 4.8	21.0 - 21.0	33.0 - 30.0
Intense flux 1.7 m^3/h - Nasal breathing - Oral breathing						
AD (nm)	**ET1 (%)**	**ET2 (%)**	**BB (%)**	**bb (%)**	**AI (%)**	**Total (%)**
5	14.0 - 6.3	17.0 - 17.0	4.8 - 5.4	26.0 - 28.0	32.0 - 35.0	92.0 - 92.0
10	7.6 - 3.6	9.6 - 9.6	2.6 - 2.8	17.2 - 18.2	51.0 - 54.0	88.0 - 88.0
20	4.7 - 2.2	5.8 - 5.8	1.5 - 1.6	11.4 - 11.8	50.0 - 51.0	74.0 - 73.0
50	2.8 - 1.3	3.3 - 3.4	0.9 - 0.9	6.4 - 6.6	31.0 - 32.0	45.0 - 44.0
100	2.9 - 1.1	3.2 - 2.4	0.7 - 0.7	4.2 - 4.2	20.0 - 21.0	31.0 - 29.0

Starting from the assumption that the conditions are significantly different from each other (in the mouth breathing, the deposition in ET1 is evidently lower if compared to the nose breathing), let us observe the nose breathing in normal occupational activity and analyze the details.

Very small particles (5 nm) deposit in great quantity (95%) in the whole respiratory tract: 34% in the extrathoracic region (ET1 + ET2) and 27% in the alveolar interstitial

region. Total deposition decreases slightly (88%) for 10 nm particles but the alveolar deposition increases if compared to the two deposition fractions. This phenomenon becomes more and more apparent as the aerodynamic diameter increases to 100 nm and the total deposition decreases to 33%, 21% of which refers to alveolar deposition. In essence, the alveolar component gets more and more significant as the particle size increases, even if this causes a decrease in total deposition. On the basis of such data, interaction between NPs and lower airways is perhaps the most relevant from a toxicological viewpoint. Finally, it is also worth noting that, due to the size-dependant nature of the deposition models, it is assumed that structured NPs and unstructured ultrafine particles have comparable deposition rates. Conversely, interaction between NP and biological systems may vary according to the specific chemical and physical properties of NPs (see Chapter 1, par. 1.2).

Obviously, the model cannot be adapted to nanofibers, as they can be several microns in length; in this case, their deposition would be strongly dependent upon the type of fiber and almost only animal deposition models exist (Szoke R et al, 2007; Lentz TJ et al 2003; Warheit DB et al, 1994; Coin PG et al, 1992; Tanaka I et al, 1994; Yamato H et al, 1994).

Despite cellular accumulation, toxicity and toxicokinetics of inhaled structured NPs are deeply influenced by the specific chemical and physical features of particles, some common characteristics can be observed to identify the interactions between lungs and NPs (Yang W et al, 2008). At the beginning, particles are absorbed in the liquid layer lining the alveolar epithelium, the *epithelial lining fluid* (ELF), in inverse ratio to their size (Geiser M et al, 2003) without destabilizing the surfactant film (Stuart D et al, 2006). Soluble particles dissolve *in situ*. Molecules which are not soluble in mucus or in the lining fluid are not absorbed rapidly and can be physically translocated according to the region they deposit in (Oberdorster G et al Oberdorster G et al, 2006) and to the defense system of the organism. Some mechanisms may then be occurring such as mucociliary transport, phagocytosis by macrophages and endocytosis (Gumbleton M, 2001; Arredouani M et al, 2004).

Mucociliary transport is particularly efficient in the upper airways (Heyder J et al, 1986), whereas phagocytosis and endocytosis are the main transport mechanisms for particles deposited in the alveoli (Sibille Y and Reynolds HY, 1990). While microparticles are efficiently removed by macrophages, particles of diameter less than 0.26 μm may elude the macrophage system due to their small dimensions (Chono S et al, 2006; Lauweryns JM and Baert JH, 1977). This is why NPs easily interact with epithelial cells (Nel A et al, 2006). The main endocytosis phenomenon in this region is thought to be the transport via caveolae, even though this still must be demonstrated *in vivo* (Rejman J et al, 2004). Caveolae are microdomains of the cell membrane containing caveo-

line-1 and are abundantly expressed by pulmonary capillaries and by alveolar type-1 cells. Caveolae transport microparticles and particles with nanoscale diameter from lungs to blood (Oberdorster G et al, 2005; Rejman J et al, 2004). Alveolar inspiratory expansions and expiratory contractions may cause the opening (from 40 to 100 nm) and closing of caveolae thus justifying the transport of macromolecules and NPs across the alveolar membrane and the translocation of NPs through the interstice (Semmler-Behnke M et al, 2007; Brown JS et al, 2002). However, there is no univocal opinion on extrapulmonary translocation of NPs and it is still unknown to what extent inhaled NPs may reach the systemic circulation and, as a consequence, other organs (Card JW et al, 2008). Finally, particle transport through pores is also possible and it has been demonstrated that NP accumulation into cells does not take place necessarily by endocytosis but also by adhesive interactions (Geiser M et al, 2005).

4.2.1.1 Definition of Threshold Limit Values

A special focus is required on the uncertainties about differences and analogies between the biological impact of NPs deposited on the respiratory system and agglomerates/aggregates containing the same volume of material undergoing deagglomeration or disaggregation following deposition. As stated by the International Standard ISO/TR 27268 (ISO/TR, 2007), provided that the biological response is associated with the surface area of deposited aerosols, the response of a given amount of material to a fractal-like agglomerate/aggregate is assumed to be similar to that of an equivalent amount of discrete particles. Besides, if the biological interactions following deposition are dependent upon the diameter of particles, the response of discrete NPs deposited in the respiratory tract is very likely to be different from that of an equivalent amount of agglomerated/aggregated particles which do not split up under deposition.
On the basis of such premises, it is worth noting that the definition of reference regulatory standards needs to take into account some fundamental aspects; in particular, the Occupational Exposure Limit Values need to take into consideration both discrete NPs and NP agglomerates/aggregates if analogies in effects of human health from exposure are identified (with respect to a potential independence of the health impact assessment from particle size); otherwise, differentiated hygienic limits must be established.

4.2.2 Dermal exposure

In occupational settings, dermal NM exposure may occur during production, usage or contact with contaminated surfaces. It is still under debate whether and to what

extent NPs are able to penetrate the intact skin and cause harmful effects. Most of experiments have been conducted with single types of NM such as TiO_2 and ZnO on intact skin. Also, evidence indicates that nanoAg may pass through damaged skin (Larese, 2009) and nanoAu may penetrate mouse skin (Sanovane, 2009).
Potential effects on flexed and damaged human skin need further exploration (tinkle SS et al, 2003; Nohynek GJ et al, 2007; Crosera M et al, 2009). The same goes for the role of solvents in skin penetration of NPs.

4.2.3 Other exposure routes

Two more exposure routes can be found in literature: the olfactory system and the gastro enteric tract.
According to the respiratory system deposition models described in the previous chapter, it is clear that a significant amount of small particles, (with an aerodynamic diameter smaller than 50 nm) may deposit in the upper respiratory system and, in particular, in the olfactory mucosa. This deposition explains the uptake of NPs in the central nervous system via the olfactory nerves (Oberdorster G et al, 2004; Elder a et al, 2006). However, this passage is deeply influenced by the chemico-physical characteristics of particles and by the inter-species variability (Oberdorster G et al, 2005) although, to date, this has not yet been demonstrated *in vivo* on human beings.
After all, nanomaterials may be ingested through mucous which incorporates and removes NPs deposited in the respiratory tract, via contaminated food and water or oral contact with contaminated hands or surfaces (Lomer MC et al, 2002; Tiede K et al, 2008). Test results with mice show that the uptake of particles whose size is in the range of about 50 nm to about 50 µm occurs through Peyer's patches of the small intestine, although they cover a small portion of it (Jani P et al, 1990; O'Hagan DT, 1996; Gullberg E et al, 2006); the uptake of NPs through intestine enterocytes is also plausible (Carr KE et al, 1996; Hillyer JF and Albrecht RM, 2001; Des Rieux A et al, 2006). The chemical and physical factors influencing the intestine *persorption* are the charge and the size of NPs (Jani P et al, 1990; Florence AT, 1997; Hussain N et al, 2001; Gaumet M et al, 2009). To date, these findings have not been replicated with human studies. (Des Rieux A et al, 2006).

4.3 Review of characterization models for inhalatory exposure to nanomaterials

Engineered NMs in the workplace and in the environment pose an immediate challenge for an efficient health and safety management in the working and living environments. To date, little is known about what the immediate risks might be, or how

to handle them. Still less is known about how risks from new technologies can be predicted and managed in the coming years (Maynard, 2007). This can be due to the recent development of nanotechnologies as well as the shortage of information on the human exposure and working conditions. In particular, the knowledge about the occupational exposure to NMs is limited by the current technology. Nowadays, in fact, many gaps concerning identification, characterization and assessment of the potential occupational exposure have been identified due to the scarce information on size-related aspects (such as dimensions, mass, chemical composition, surface area, concentration, state of aggregation/agglomeration, water solubility and surface chemistry) which can help in determining the level of toxicity and harmfulness of interactions between NMs and the human organism but, as a consequence, do not allow the identification of hygiene-based benchmark values.
The simultaneous identification of a number of features requires the use of multiple equipments and although the more recent technology developments offer instruments capable of measuring almost all relevant parameters, they cannot be used as personal devices because of their own characteristics. Whilst awaiting the development of personal instruments able to provide the estimates of the appropriate parameters, it is necessary to develop an efficient sampling strategy that takes into appropriate consideration the interpretative limitations of the area sampling in the exposure assessments (Marconi, 2007).
Most of the information acquired is based on the documentation being prepared by the *National institute for Occupational Safety and Health* (NIOSH) (NIOSH, 2008) and by the OECD *Steering Group 8 (SG8) - Working Party on Nanomaterials* (WNPM).
As no limit values concerning the occupational exposure to NMs have been established today (except the carbon black in Japan), to address the issue of the exposure assessment an approach able to conduct a qualitative (or semi-quantitative) estimates to determine NM release during the production process is required.
Such assessment approach compares particle concentrations at the emission source to background particle concentrations. Although results from this assessment should not be interpreted as representative of worker exposure, they provide a semi-quantitative means for determining nanomaterial release in the workplace and may be useful to health and safety professionals and industrial hygienists. In addition, these data could also be useful in semi-quantitative estimate of personal exposures (Koshi, 1980) which could guide selection of appropriate exposure mitigation techniques (see dermal protection e.g.) (Wendel-de-Joode, 2003). Their acquisition is also useful to determine whether existing measures are adequate for controlling nanomaterials emissions or if additional controls might be needed.
In recent years, a number of countries have initiated survey of exposures nanotech-

nology workplaces and developed specific assessment protocols. In the United States, NIOSH has formed operating groups which have been assessing workplace processes, materials and control technologies associated with nanotechnologies since 2006[1] (Methner, 2007; Methner, 2008). As a result of such activities, evaluation of instrumentation for characterizing nanomaterials in workplace environments, as well as emission assessment guidance to semi-quantitatively evaluate workplaces where release of NMs may occur became available (NIOSH, 2007; NIOSH, 2008).

At European level, a number of different activities are currently being pursued including identification of sources of NMs, characterization of NPs, sampling and on-line detection, assessment of dermal and inhalational exposure, work on the aerosol dynamics of NMs (adherence, coagulation, aggregation and/or agglomeration), techniques addressing the background assessment versus the specific emission of NMs. More information and an information update of these activities can be found in the respective presentations of various EU research projects (NANOSAFE2; NANOSH, NANOTRANSPORT, NANODEVICE, IMPART, etc.) described in Chapter 3 of this publication.

ISO has provided important formative and orientation documents concerning the assessment and management of potential risks associated with NMs in the workplace, addressing the issues of the occupational exposure to NPs, nanostructured aerosols and NPs and engineered NPs. (ISO/TR, 2007; ISO/TR, 2008; ISO/NP TS, 2008) as well as all aspects relating to terminology (UNI CEN ISO/TS, 2010; ISO/DTS, 2010). In the United States, the American Society of Testing Materials (ASTM) published a guide for the manipulation of NMs in the workplace as early as 2007 (ASTM, 2007).

Until information on the mechanisms of biological actions associated with NMs is available, there will be no certainty about the most appropriate assessment approach to the determination of the occupational exposure. On the basis of data collected, the knowledge of a number of parameters with potential scientific relevance is required for the characterization of exposure to NMs.

Besides traditional information regarding mass and characterization, for the exposure and dose assessment, data on size-related distribution, on the surface area and/or number and, if possible, on the particle surface chimism would be needed.

Although appropriate methods exist for the assessment of such parameters, only some of them can be adopted for routinary exposures estimates. As far as NMs are concerned, a complete characterization of exposure by determining all toxicological parameters appears to be difficult to obtain today.

Next sections will address all aspects regarding the main sampling and analysis techniques established under the strategy for the occupational exposure assessment approach.

[1] www.cdc.gov/niosh/dox/2008-121/ ; www.cdc.gov/niosh/docs/2008-120/

4.3.1 Sampling and assessment systems

Currently, no sampling methods are available for the assessment of exposure to airborne nanoparticles. Every attempt to estimate exposure to NPs which characterize NMs requires the use of multiple sampling and assessment techniques (Tab. 4.5).

Table 4.5 - Summary of instruments and measurement methods used in the evaluation of nanomaterial exposures.

Metric	Method of funding (Euro)	Remarks of project* (Euro)
Mass	Size selective personal sampler	no current device with a size fraction cutoff in the nm size range is available. Gravimetric or off-line chemical analysis are therefore required. Mass could also be derived by estimates of size distribution.
	Size selective static samplers	These are the only devices offering a cut point around 100 nm (up to 10 nm) are cascade impactors.
	Tapered Element Oscillating Microbalance (TEOM)	Sensitive real-time monitors such as the TEOM may be useable to measure nanoaerosol mass concentration on-line with a suitable size selective inlet.
	Scanning Mobility Particle Sizer (SMPS)	Real time size-selective detection, based on electric mobility of particles; size range from 3 nm to 800 nm.
	Electrical Low Pressure Impactor (ELPI)	Real time size-selective detection based on inertial separation and charge of particles. Data may be interpreted in terms of number concentration. Sample collection.
Number	Optical Particle Counter (OPC)	Particles smaller than 300 nm not detected.
	Condensation Particle Counter (CPC)	Real time number concentration, up to 100 nm.
	Scanning Mobility Particle Sizer (SMPS)	Real-time size selective detection of number concentration, based on mobility diameter 3 - 800 nm.
	Electrical Low Pressure Impactor (ELPI)	Real time size-selective detection based on inertial separation and charge of particles. Data may be interpreted in terms of number concentration. Sample collection.
Surface area	Epiphaniometer	Radioactive tagging based on surface areas.
	Diffusion chargers	Sensitive to particles smaller than 100 nm according with adherence to surfaces of positive ions. Preparation is required.
	SMPS	Real time size-selective detection of number concentration based on mobility diameter 3 - 800 nm.
	ELPI	Real time size-selective detection based on inertial separation and charge of particles. Data may be interpreted in terms of number concentration. Sample collection.
	BET (Brunauer, Emmett and Teller method)	Estimates based on gas (N_2) adsorption on particle surface.
Image analysis	Scanning Electron-Microscopy (SEM); Transmission Electron-Microscopy (TEM)	Analysis of projected areas of NPs. Samples may be collected by personal samplers or size selective static samplers.

Monitoring and characterization methods, covered by ISO/TR 27628 (ISO/TR, 2007) and ISO/TR 12885 (ISO/TR, 2008) allow exposure assessments for NPs and nanoaerosols in terms of mass, concentration and surface area and are the basis for the development of new standards for the exposure characterization; most of instrumentations available today, however, are expected to be adapted in terms of compactness, portability and costs for routinary applications in the workplace.
Estimates and characterization of occupational exposure to NMs (as well as NPs and aerosols) are deeply limited by the lack of efficient instrumentation for personal sampling and, therefore, the combined use of devices for *in-situ* assessments and *off-line* sampling analysis represents, today, the best tool for the assessment of personal exposure in the workplace.
Aerosol samples can be collected using inhalable, thoracic, or respirable samplers, depending on the region of the respiratory system most susceptible to the inhaled particles. Since prevailing information suggests that a large fraction of inhaled nanoparticles will deposit in the gas-exchange region of the lungs (ICRP, 1994; Yeh, 1996), respirable samplers would be appropriate. Though, mass determination (and chemical characterization) does not provide information on particle concentration, dimension and surface but it can act as a surrogate measure if data on size distribution or specific surface area are available (Möhlmann, 2004).
The use of conventional impact techniques for determining NMs exposure is limited as the limit impact size range is from 200 and 300 nm. With low pressure impactors, such as the Electrical Low Pressure Impactor (ELPI)[2], particles are first electrically charged and then sampled. The particles are collected in the different impactor stages and the electric charge is measured by multi-channel electrometers. A similar system (nano-MOUDI) has recently entered the market[3]. These devices may measure particles of up to 10 nm as static samplers; though their dimensions and complexity do not allow a personal use. However, a personal cascade impactor is available with a lower aerosol cut point of 250 nm (Misra, 2002), allowing an approximation of nanometer particle mass concentration in the worker's breathing zone. These instruments enable the chemico-physical characterization of particles deposited on substrates but fail to differ agglomerates of NPs from single particle equal in size.
The measurement of airborne particle concentrations larger than 10 nm performed by Condensation Particle Counter (CPC) is relatively ease and can be extended without great difficulty for particles of up to 3 nm. These systems convoy in-going particles into a over-saturated vapor chamber (butylalcohol, isopropylalcohol) so that on

[2] www.dekati.com
[3] http://appliedphysicsusa.com/moudi.asp

the smallest particles, droplets from 100 to 1.000 times larger than initial particle size are formed (McMurry, 2000). Droplets, then, pass through an optical sensor measuring the attenuation of light and results are converted into concentration.
These instruments are widely used to measure ultrafine particles in the urban atmosphere (Kim, 2002; Zhu, 2002; Aalto, 2005; Marconi, 2007a). As these devices are not size selective (except initial selection), it is difficult to distinguish the different sources of NMs generated by processes from those present in the background. Such limitations have been recently addressed in a study on a carbon black production plant (Kuhlbusch, 2004). Nevertheless, the adoption of this measurement process carried out in the vicinity of potential sources has been put forward for the raw identification of NMs emitted by sources in the workplace (Brouwer, 2004).
Such devices can be used in a static way only; however, it is now available in a portable form with a size range from 10 to 1.000 nm at concentrations of less than 105 p/cm3. Instruments providing information on particle total number and size are commercially available today. Albeit more complex and expensive, the *Scanning Mobility Particle Sizers* (SMPS) can measure the size distribution of particles with a range from 3 to 800 nm (Flagan, 2001). These devices provide particles with an electrostatic charge and separate them, according to their electrical mobility, through their passage between two electrodes or a cloud of ions generated by a radioactive source. Particles, once separated, are counted with a CPC. The most recent version of this type of instrument, the *Fast Mobility Particle Sizer* (FMPS)[4], provides the size distribution even more rapidly (few seconds compared to some minutes required by SMPS) and, thanks to a series of electrometers acting as sensors of particle charges, prevents the use of a radioactive source.
These techniques allow the determination of nano-range particles but they are not able to distinguish single NPs from those formed by agglomerates of smaller particles. Some toxicological studies support the need to measure the surface area of NPs as it is shown to be more correlated to the potential biological effects (HSE, 2004; EPA, 2005; NIOSH, 2008). Traditionally, the surface area is measured through the analytic *Brunauer, Emmett and Teller* method (BET) which is adapted to bulk particulate materials using the absorption characteristics of some gases such as nitrogen, krypton and argon. The samples provided by the collection of particles (onto filters or substrates) do not permit to obtain the mass required for this analysis, which, instead, could play a role in an overall monitoring strategy if applied to the bulk material involved in the process under investigation.

[4] www.tsi.com

At present, the instrument which allows the measurements of aerosol surface- area is the epiphaniometer (Baltensperger, 1988). This device measures the quantity of radioactivity, generated by ions adhered to the particle's surface, which is proportional to the active surface area, at least for particles smaller than 100 nm. The epiphaniometer is not well suited to widespread use in the workplace because of the inclusion of a radioactive source.

More recent devices (diffusion chargers),might find a wider use in the workplace, as they use the same principles of the previous instrumentation, although they operate by generation and adhesion of positive unipolar ions to the aerosol particles surface (Keller, 2001).

These instruments are subject to potential errors in case of previously or multiply charged aerosol particles; laboratory evaluations, however, have shown a good correlation with aerosol surface area measurements obtained by *Transmission Electron-Microscopy* (TEM) for particles smaller than 100 nm (Ku, 2005); whereas a recent version of this instrument provided data which were well correlated to the surface area of particles deposited in the human respiratory tract (Wilson, 2004). The derivation of surface-area from measured aerosol size distribution can be performed as the association between particle mobility diameter and surface area in the free molecular regime has been demonstrated (Rogak, 1993; Wilson, 2004; Ku, 2005); this allows the surface area to be estimated reasonably well from size distributions with modal diameters below approximately 100 nm (Maynard, 2002). This approach, however, requires instruments and calculations which are difficult to adapt to the systematic exposure monitoring.

As already observed, some samplers enable the sampling of materials deposited onto membranes and already divided into particle size fractions.

They enable off-line investigations on NMs through chemical analysis and electron and scanning probe microcopies. The gravimetric measure, although deriving from the traditional monitoring approach, is very little sensitive to NMs made of NPs.

As for the analytical methods adopted for the chemical and physical characterization of substances contained in samples collected on filters (through the use of static cascade impactors) the use of off-line hyphenated techniques chosen in accordance with the chemical composition of NMs under investigation is frequent.

In particular, the *Inductively Coupled Plasma-Mass Spectrometry* (ICP-MS) techniques (Montaser, 1998), characterized by the combination of instruments for plasma emission spectroscopy with a mass spectrometry detection system allows qualitative and quantitative determinations inorganic components constituted by metal, natural or engineered metal oxides. Furthermore, the combination of liquid chromatography techniques (*High Performance Liquid Chromatography*, HPLC) with mass spectrometry

(HPLC-MS, HPLC-MS-MS) or photodiodes (*Diode Array Detector*, DAD) detection systems or the combined use of gaschromatography and mass spectrometry (GC-MS) allows the characterization of impurities derived from low-volatility organic compounds, possibly contained in the engineered NMs because of production processes, that strongly contribute to exposure-related occupational risk. The morphological analysis of NMs can be conducted through the scanning electron microscopy (SEM) and scanning probe microscopy. The *Scanning Electron Microscopy* (SEM) (Goldstein, 1981) provides information on the morphology (forms and sizes) of NMs smaller than 50 nm.
The *Transmission Electron Microscopy* (TEM) (Williams, 1996) provides structural information at less than 1 nm resolution. Also, TEM-based electron diffraction patterns (*Selected Area Electron Diffraction*, SAED; *Convergent Beam Electron Diffraction*, CBED; nanodiffraction) together with high resolution TEM images (*High Resolution TEM*, HRTEM) enable the study of the crystalline structure of NMs and highlight the presence of the amorphous material and reticular defects.
Information on the element composition of NMs can be acquired through the combination of the *Energy Dispersive X-ray Spectroscopy* (EDXS) and scanning and electron transmission microscopy and through the *Electron Energy Loss Spectroscopy* (EELS) together with electron transmission microscopy. Furthermore, the EELS fine spectrum edge shows chemical bonding states of elements and the oscillations observed after the picks (*Extended Energy-Loss Fine Structure*, EXELFS) allow to obtain structural information on NMs.
Scanning probe microscopy too can be used to characterize NMs (Albonetti, 2006). Such microscopes provide morphological information and the mapping of specific physical properties of NM surface at less than 1 nm resolution.
Finally, studies on the surface chemical composition of NMs and their electronic structure are carried out through conventional and advanced spectral nanoscopic techniques such as the *PhotoElectron Emission Microscopy* (PEEM), the X-ray-photoemission-spectroscopy (XPS), and the *Ultraviolet Photoelectron Spectroscopy* (UPS).
To ensure valid measurements, the following quality assurance and control steps should be taken:

- use factory calibrated direct-reading particle analyzers;
- perform daily zero-checks on all particle counters before each use;
- calibrate pumps before and after each sampling day;
- submit for analysis any process, background, and bulk material samples along with field and media blanks to a laboratory;

Today, only some types of reference NMs to be included in the quality control program are available, such as gold NPs (RM 8011, RM 8012, RM 8013) developed by the *U.S. National Institute of Standards and Technology* (NIST) and silica NPs (CRM-

No IRMM-204) provided by the *European Commission Joint Research Centre - Institute for Reference Materials and Measurements* (IRMM), Geel, Belgium[5].
These types of nanoparticles have a spherical shape and are size-certified and can be used for the calibration and quality control of particle size measurement instruments.
The main hindrance to the manufacturing of reference NMs is posed by the lack of consensus on metrological parameters and methods.

4.3.1.1 Critical analysis of instruments used for exposure estimation and measurement

The measurement strategy based on the determination of emissions for exposure estimates is susceptible to different limitations concerning: 1) the variability on number concentration of NPs in the workplace; 2) the upper limit intervals of CPSs; 3) the state of aggregation of aerosol NPs; 4) the scarce selectivity of particle counters.
As for the point 1, orders of magnitude difference can exist in aerosol number concentrations, depending on the number and types of sources of particle emissions. Monitoring over several days and during different seasons can provide a better understanding of the variability that might exist in airborne particle number concentrations found in background measurements and in measurements made at sources where nanomaterials are handled.
As for point 2, since the upper dynamic range of the CPC is 100.000 p/cm3, a dilutor, consisting of a modified *High Efficiency Particulate Air* (HEPA) filter cartridge placed upstream of the inlet, can extend the range of the CPC when particle number concentrations are greater than 100.000 p/cm3 (Peters, 2006).
The issue illustrated in point 3 arises from the fact that during sampling, the size distribution of NMs and their state of aggregation (or agglomeration) may be unknown and, consequently, direct-reading particle counting and sizing instruments may allow only a semi-quantitative evaluation of the potential emissions. Since the size of airborne manufactured nanomaterials and the degree of agglomeration may be unknown at the time of sample collection, the use of direct-reading, particle sizing/counting instruments may provide a semi-quantitative indication of the magnitude of potential emissions, provided background particle number subtraction can be successfully accomplished. The particle number concentration measurements taken with CPC and OPC will provide a measurement of particles larger than the ISO definition of nanoparticles (approximately 1 to 100 nm) (UNI CEN ISO/TS, 2010).

[5] For further information, visit http://ec.europa.eu/dgs/jrc/index.cfm?id=2820&obj_id=220&dt_code=HLN&lang=en

However, the two particle counters can be used simultaneously to obtain a semi-quantitative size-differential evaluation of the aerosol being sampled.
The CPC provides a measure of total particles per cubic centimeter of air in the size range of 10-1.000 nm. The OPC can provide the total number of particles per liter of air within a minimum of four specific size ranges: 300 - 500 nm; 500 - 1.000 nm; 1.000 - 10.000 nm, and > 10.000 nm. The data from the CPC and OPC should be used together to determine the number concentration of nanoscale particles.
For example, a high particle number concentration obtained by the CPC, in combination with a high particle number concentration in the small size range (300-500 nm) provided by the OPC, may indicate the possible presence of nanometric particles. Conversely, a low CPC particle number concentration, in combination with a high OPC particle number concentration in the larger size range (> 1.000 nm) may indicate the presence of larger particles and/or nanoparticle agglomerates.
These assumptions of nanoscale particles versus larger particles and/or nanoscale particle agglomerates may be verified by TEM or SEM analysis.
Selectivity, illustrated in point 4, is a critical issue in exposure characterization by using airborne particle number concentration.
Airborne nanoparticles are present in many workplaces and often originate from multiple sources such as
combustion, vehicle emissions and infiltration of outside air. Particle counters are generally not selective to particle source or composition, making it difficult to differentiate between incidental and process-related nanoparticles using number concentration alone. The CPC and OPC are used to identify sources of nanoparticles and the filter-based samples are used to verify the size, shape, and chemical composition of the nanoparticles with the goal of differentiating between incidental and engineered nanoparticles.

4.4 Approach to the evaluation of the occupational exposure to nanomaterials: the strategy

The strategy for occupational exposure assessment is worthy of consideration. Currently, estimates of personal exposures derive from static instruments and, as a consequence, for a reliable interpretations of the results it is essential to identify every potential source of nanomaterials, both internal and external to the workplace, to record the air flow regimes and the distance of the operator from the source and from the instrument location.
On the basis of the occupational hygiene aspects described in this review, the conclusion that can be agreed is that, to date, no sampling method for the characteri-

zation of NMs is available. It is therefore necessary to adopt a multiple instrument approach and develop an appropriate sampling strategy.
In order to implement a correct strategy for occupational exposure assessment of nanomaterials to the human health, the *Scientific Committee on Emerging and Newly Identified Health Risks* (SCENIHR, 2006), within the European Commission, has assumed that:

1. to date, no common opinion exists on parameters aimed to represent the most appropriate measure for exposure assessment (mass, number, surface area, surface chemistry, etc.);
2. no personal samplers are available today for the assessment of NMs;
3. innovative sampling methods and new occupational and environmental exposure assessment strategies are required;
4. occupational exposure limit values (TLV) for airborne chemicals need to be established taking into account the different biological effects associated to discrete NPs or agglomerates/aggregates of NPs.

Whilst awaiting the development of a more appropriate method to assess the exposure to biologically active NPs, the scientific community agrees to recommend a multiple instrument approach to get the best characterization of aerosol particles in the workplaces involved in the production, handling and usage of NPs.
This approach requires the use of static samplers and, therefore, makes it difficult for individual exposure assessment through personal sampling or epidemiological elaborations to comply with the exposure limit values (as, to date, there is no reference regulatory standards for nano chemicals). On the basis of such premises, the development of an appropriate sampling (and/or estimate) strategy, before and during the monitoring, is required (Tab. 4.6).

Table 4.6 - Considerations to take into account before and during sampling (from Technical Report ISO/TR 27628/2007)

Considerations	Objective	Instrument
Source	To identify and localize single/multiple nanoaerosol sources in the workplace; to identify the aerosols in the workplace environment.	Condensation particle counter (CPC); record remarks on emission generating activities.
Ventilation	To monitor the airflow and the aerosol transmission in workplace environment.	Anemometer; Smoke tube; gas tracers; observations and recording of door openings, etc.
Workplace activities	To interpret data directly recorded by instruments in view of the variations in exposure parameters.	Observation technique: monitoring the emission generating activities; CPCs.
Worker's behavior	To interpret spatial differences in the residence time in the different sites.	Observation technique: monitoring the worker's distance from the source. Sample reading.

Identifying an appropriate sampling place is key for a reliable interpretation of data and for an accurate estimate of personal exposure.
The first step to take consists in identifying NPs source in the workplace and verifying the presence of potential external sources such as additional industrial activities or vehicular traffic, etc. which may influence the indoor concentration of the nanoscale aerosol count.
The use of static NPs samplers implies that the sampling event takes place in the vicinity of the potential source of emission to allow for more accurate determination of NM release and to identify locations where the occupational exposure is more likely to occur. As a consequence, the results from this type of sampling should be interpreted as an indicator of NMs release and the possible need for more efficient controls.
In order to obtain data on the background measurements, exposure estimates should also be conducted before the manufacturing or handling of NMs. Then, these data can be compared to the background values to evaluate a potential increase in the parameters that have been chosen. Simultaneous particle concentration measurements inside and outside the workplace can also be conducted using two identical instruments: the external measurement is then computed and subtracted from the internal NP concentration. However, this expensive approach is acceptable only if the external particles do not undergo any changes during their transportation inside the workplace.
Furthermore, as already noticed, since aerosols consist of complex mixtures of different origins, sampling and analysis techniques should be optimized in order to identify the potential involuntary emitting sources (i.e. through source profiles provided by the Principal Component Analysis).
It is important, therefore, to develop assessment protocols for the occupational exposure to NPs, optimized in all their above-mentioned phases, aimed at determining the *chemical composition* and the *size distribution* of NPs and microparticles in the workplace through a multi-parametric approach based on different sampling and analysis techniques.
The air flow is considered another important parameter to be monitored for determining the aerosol transmission in the workplace. The most appropriate instrument for this estimate is a smoke generator, although it cannot be used simultaneously with measurements as it generates aerosols.
During the exposure verification, all work activities should be observed in order to provide further information useful in the interpretation of results from static sampling. Attention must be paid to the additional sources of aerosol emission, such as the use of specific equipments, presence of smokers or secondary sources like resuspen-

sion of aerosol deposits due to air movement induced by movement of people or vehicular activities. Although the dust resuspension is assumed to be characterized by large particles, the process may play a role in the release of NPs in the air. Finally, variations in the distance of the operator from the source should be monitored during sampling time. The results of multiple area sampling can be used for the estimate of personal exposure.

In the initial assessment, samples of multiple surfaces can be useful if potential NMs contamination may occur due to sedimentation, leak-induced emissions or migration of materials from one workstation to another.

To improve the comparability of exposure data, the accepted practice of giving personal exposure as an eight-hour-shift value should also be observed in the case of nanoaerosols. As a consequence, wherever possible exposure measurement results concerning shorter measurement intervals should be converted into shift data by time weighted recalculation. In all cases, where short-term exposure itself is the target of investigations, the time base of measurements needs to be documented. A time base of 15 minutes for short-term exposure measurements is recommended as it is generally used in occupational hygiene.

Fig. 4.4 shows the strategy based on NIOSH and OECD indications.

The strategic approach centered upon the acquisition of specific parameters to determine the presence and the identification of NPs allows a semi-quantitative estimate of personal exposure.

However, as this type of analysis focused on static or area samplings, special attention must be paid to the estimate of the occupational exposure (ISO/TR, 2008).

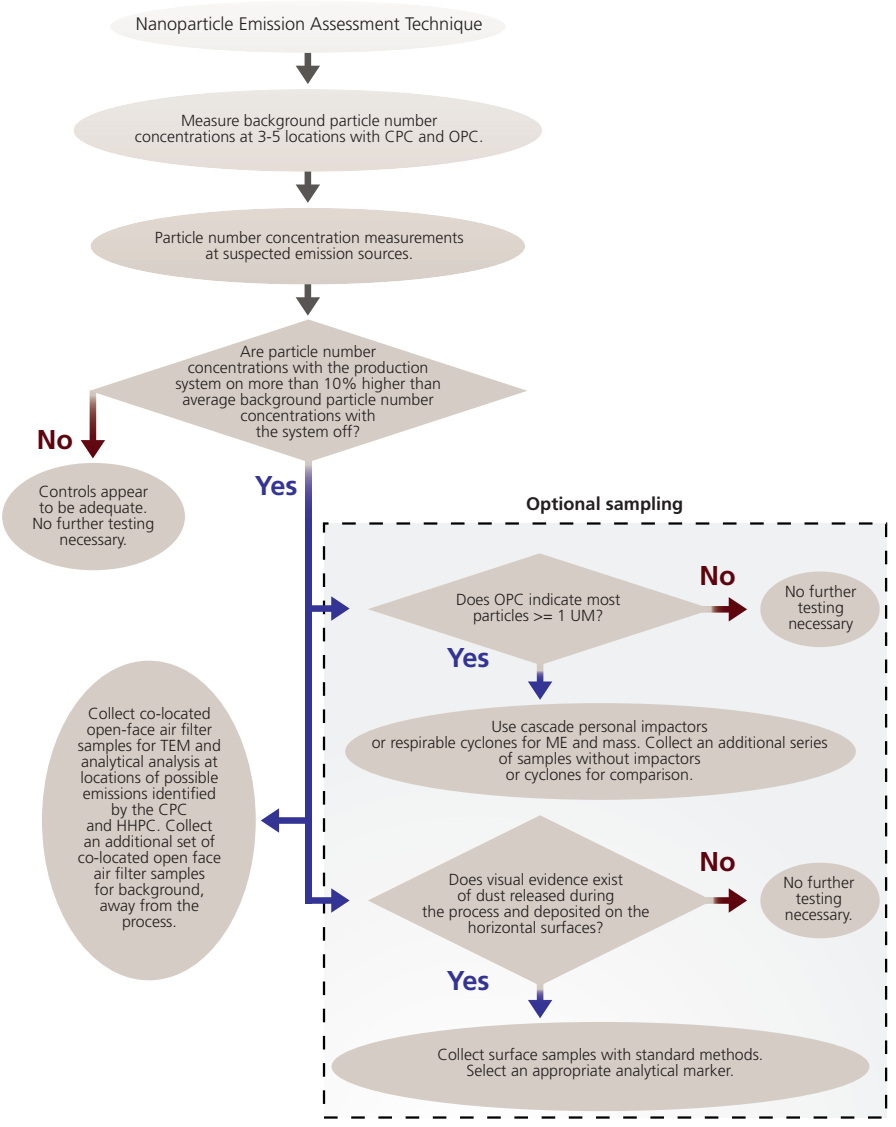

Figure 4.4 - The proposed NIOSH and OECD sampling strategy.

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chapter 5

Effects of engineered nanomaterials on health

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Preface

The growing production and use of engineered nanomaterials (NMs) in workplaces, the potential exposure risk for an increasing number of workers and the paucity of data available on health risks associated with such compounds make it necessary to implement the knowledge regarding the potential biological effects (either at the molecular-cellular or organ-system level). Due to the recent production, dissemination and use of engineered nanomaterials and the complexity of exposure assessment, no epidemiological studies and information on the toxic effect of NMs on exposed populations are available today. So far, studies have been mainly conducted *in vitro* or on animals (primarily mice) and the effects of NMs on organs and systems are sometimes extrapolated from results obtained at the cellular level.

Recent studies have highlighted the potential cytotoxic and genotoxic-oxidative effects at cellular level as well as the respiratory, dermal, immunologic, neurotoxic and cardiovascular effects caused by NMs.

Most of the investigations on the effects of NMs conducted at cellular level use high concentration of such compounds and mainly show cytotoxic effects. The few studies available today on the exposure to low concentrations of engineered NMs have pointed out genotoxic, oxidative and inflammatory effects that may be implied in a cancerogenesis process. Most of these studies use carbon nanotubes and metal oxide particles that may cause direct or indirect DNA damage by oxidative stress induction.

The cellular effects of NMs are dependent on size, surface area and chemicophysical

properties (such as metal contaminants and surface charges) which determine their reactivity and aggregation state. These properties make difficult the study on the effects and mechanisms of action of NMs.

Some experimental evidences show that engineered nanoparticles (NPs) are able to penetrate the systemic circulation and reach organs and systems. The main routes for NPs uptake are assumed to be lungs, nasal mucosa, skin and gastroenteric apparatus with subsequent accumulation in many tissues such as kidneys, muscles, spleen and thigh bone (Singh et al, 2006). At the organ and system level, *in vivo* studies have investigated the effects on the respiratory, nervous and cardiovascular system of rodents; to date, scarce data on the immune and dermal systems are available.

Several studies have demonstrated that engineered NMs, in particular carbon nanotubes and metal NPs, may induce oxidative stress and pulmonary inflammatory processes. Most studies concern carbon nanotubes (CNTs) and the adverse effects on the respiratory system seem to be correlated with the toxicity on different cell populations, capacity of fibrosis induction, asbestos-like activity, bio-accumulation and the potentially low levels of bio-degradation of NMs. In particular, some similarities have been observed between pathogenic properties of multi-walled carbon nanotubes and the properties of asbestos fibers in terms of inflammatory response and oxidative stress.

In vivo studies on the effects of NPs on the Central Nervous System (CNS) mostly involve metal NPs and demonstrate neurotoxic effects that are mainly induced by oxidative stress. There is scientific evidence that inhaled NPs are able to displace from the uptake sites to the CNS via trans-synaptic transport or to be captured through the nerve endings of the nasal (olfactory and trigeminal nerves) or tracheo-bronchial mucosa (vagus nerve afferences). Furthermore, inhaled NPs penetrate the respiratory barriers and, through the circulation, can reach the CNS by crossing the blood-brain barrier (BBB) in the case of its malfunctioning due to specific pathological factors.

Studies investigating the potential effects of engineered NPs on the cardiovascular system have been mainly conducted *in vivo* on rodents exposed to CNTs and have provided evidence that they can have effects on atheroma development, arterial thrombosis and blood platelet aggregation; the critical aspects of some of these studies, though, are represented by doses, routes of administration and small number of animals involved. Other studies have evaluated the potential effects of CNTs on the systemic inflammation which is thought to be one of the main predisposing factors for atherosclerosis and have shown that both multi-walled carbon nanotubes (MWCNs) and single-walled carbon nanotubes (SWCNs) - MWCNs, in particular - are able to activate systemic inflammation parameters such as granulocytes, IL-6, CXCL 1, IL-5, CCL11, CCL22 and neutrophil activation biomarkers.

The few data available on the immunological effects of NPs suggest that, once entered the systemic circulation, NPs interact with proteins circulating or deposited on the cell surface determining an autoimmune response. NPs may also interfere with the opsonization process and, as a consequence, with the clearance of extraneous material (i.e. microorganisms) normally eliminated by this process or, finally activate the complement, which can be either harmful or beneficial according to circumstances.

Dermal exposure to NPs may cause local effects on the skin or be used as a route of uptake into the systemic circulation. Further investigations on the different types of NMs are needed as their diffusion and dermal effects may vary according to their size and chemical composition. To date, most of the knowledge in this filed comes from the pharmaceutical industry which has studied the effects of titanium dioxide (TiO_2) and zinc oxide (ZnO) nanoparticles used in sunscreen formulations, whereas very little information relating to other type of NPs is available.

Fig. 5.1 illustrates the biokinetics of nano-sized particles. While many uptake and translocation routes have been demonstrated, others still are hypothetical and need to be investigated (Oberdoster et al, 2005).

In this chapter are reported the main available studies on the biological effects induced by the engineered NMs both at cellular and molecular level (the genotoxic and cytotoxic effects are mostly addressed as they provide more information related to the mechanisms of action of such materials) (Fig. 5.2) and at organ and systems level (reporting immunological, dermal, respiratory, CNS-related and cardiovascular effects).

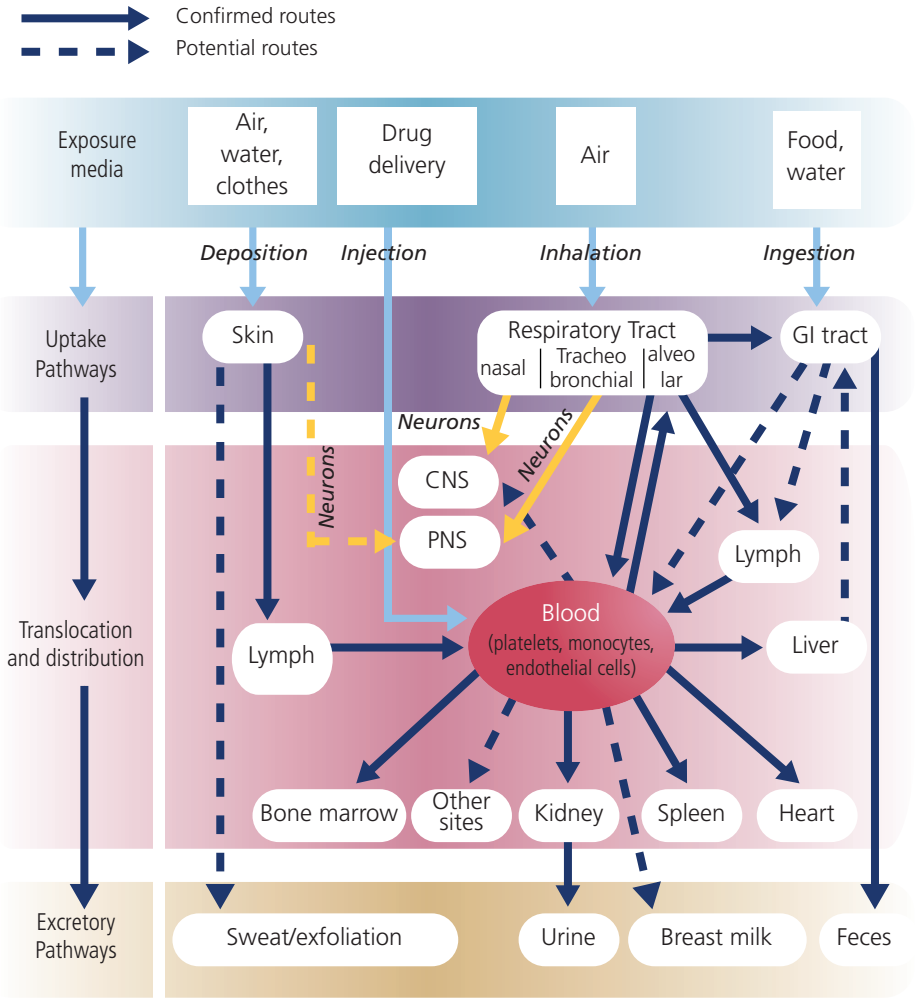

Figure 5.1 - Biokinetics of NPs. CNS- Central Nervous system. PNS - Peripheral Nervous System. (from Oberdoster et al, 2005).

Will be considered toxicity studies on NMs such as multi-walled carbon nanotubes (MWCNs) and single-walled carbon nanotubes (SWCNs), fullerenes, metal and metal oxides NPs, quantum dots, representative of NMs already on the market or about to enter it, that are included in the list provided by the OECD (Organisation for Economic Co-operation and Development) published in Chapter 1.

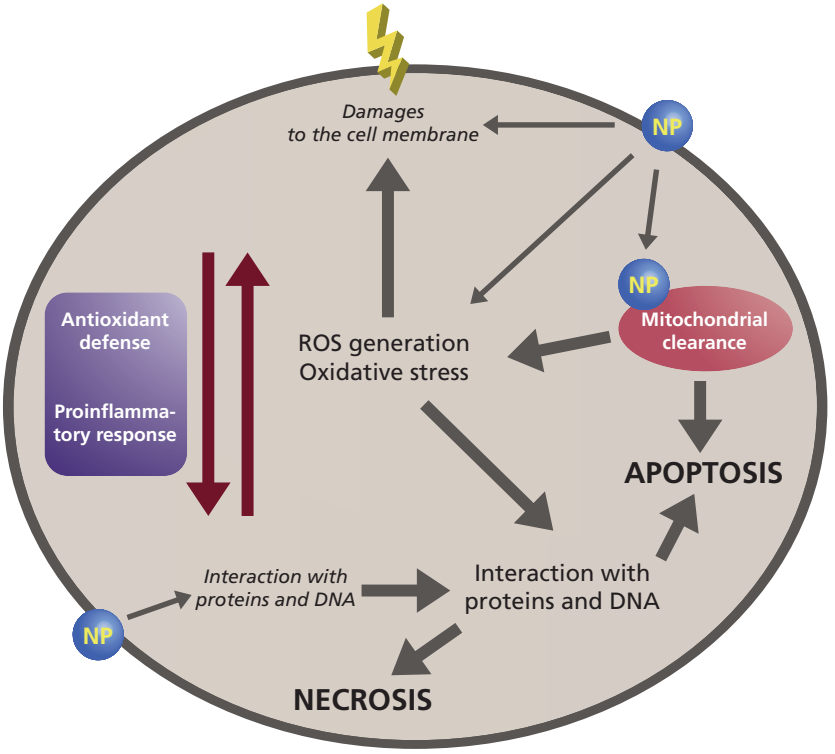

Figure 5.2 - Cytotoxic and genotoxic effects of NPs.

5.1 Genotoxic and oxidative effects

Most of the studies on the effects of NMs have focused on high-dose exposures. Recent research data, on the exposure to low concentration of engineered NMs, however, demonstrated that they may cause DNA damages and induce oxidative and inflammatory effects that could be involved in the carcinogenic process (Singh et al, 2009); great uncertainty, however, still exists and results remain contrasting. Most of these studies use carbon nanotubes and metal oxide particles which may cause, directly or indirectly, DNA damage by induction of oxidative stress. The genotoxic effects of NMs are dependent on size, high surface area and chemicophysical properties (such as metal contaminants and surface charges) which determine their reactivity and aggregation state. These properties give NMs unexpected genotoxic properties

which make complex the study of their effects and mechanisms of action (Yang et al, 2008). According to their size and state of aggregation, NMs are able to penetrate the cell by passive diffusion or receptors-mediated or proteins-mediated endocytosis, then enter the nucleus through the nuclear membrane (if sufficiently small) and through nuclear pore complexes or after the dissolution of the nuclear membrane during the cell division (if larger or aggregated). Once entered the nucleus, they can damage the genetic material directly through the interaction with the DNA and histone proteins or indirectly through the inhibition of nuclear proteins involved in the processes of DNA replication and transcription. The genotoxic damage can be indirectely induced also through the interaction with other cell proteins like those involved in the cell division process, through the induction of oxygen free radicals, inflammatory processes or through the alteration in functionality of proteins involved in the DNA damage recovery. Tab. 5.1 illustrates the main mechanisms of action hypothesized in current literature on the genotoxic and cytotoxic effects of NMs.

5.1.1 Carbon-based nanomaterials

Carbon nanotubes (CNTs)

The study of the genotoxic effects of carbon nanotubes (CNTs) is of great importance due to the similarities to the asbestos which is known to damage DNA and induce carcinogenesis mediated by high biopersistence, local generation of free radicals and subsequent prolonged inflammatory response. To date, studies on the genotoxic and oxidative effects of single-walled carbon nanotubes (SWCNs) or multi-walled carbon nanotubes (MWCNs) are quite contradictory, probably due to the variability of their characteristics (purity, size, shape, presence of metal contaminants, functionalization), dispersion medium, presence of surface charges and exposure-related conditions which are not always explained in details. Fibrous NMs may induce genotoxicity directly through the interaction with DNA (SWCNTs have been observed in the nucleus) or the mitotic fuse and indirectly through the induction of oxidative stress and inflammatory responses (Migliore et al, 2010). *In vivo* studies on rodents have indicated that SWCNTs may induce oxidative stress and inflammatory response (Folkman et al, 2009; Jacobsen et al, 2009). Induction of inflammation, fibrosis and pulmonary granuloma in mice exposed to MWCNTs has been reported in a pilot study conducted by Poland et al (2008); long-term studies have demonstrated that MWCNTs could promote the mesothelioma development (Sakamo 2009, Takagi 2008). Conversely, in other studies there is no evidence of oxidative or inflammatory effects on rodents exposed to MWCNTs (Mitchell et al, 2007; Elgabli et al, 2008). Most of the *in vitro* studies conducted so far on carbon nanotubes have involved the SWCNTs and high-

lighted the induction of oxidative stress and DNA damages in different cell types. In particular, generation of free radicals, accumulation of peroxidation products and decrease in the antioxidant activity in human keratinocytes (Shvedova et al, 2003a), induction of reactive oxygen species (ROS) in rat pulmonary cells (Sharma CS et al., 2007), ROS generation and DNA damages in human mesothelial cells (Pacurari et al, 2008) and DNA damage in human bronchial cells (BEAS-2B) (Lindberg et al, 2009) have been observed. In particular, Lindberg evaluated the effects of exposure to commercial carbon nanotubes (SWCNTs> 50%, other CNTs about 40%) in BEAS-2B cells for 24, 48 and 72 hours through *comet assay* and micronucleus (MN) test. While dose-dependent increases of the DNA damage, with more evident effects for prolonged exposure, have been observed with the *comet assay*, the micronucleus test demonstrated an evident effect only after a 48 hour exposure. The genotoxicity observed in the study can be correlated to the fibrous nature of materials used and the presence of metals like Co and Mo. Also in the study carried out by Pacurari et al (2008) on the human mesothelial cells exposed to SWCNTs containing metal contaminants (Ni, Y and Fe) and showing DNA damage and ROS generation in *comet assays,* it has been hypothesized that the induction of DNA damage, partly related to the oxidative stress, could be caused not only by the presence of metal impurities but also by SWCNT-induced ROS.

Some studies (Jacobsen et al, 2008; Zeni et al, 2008) have shown that highly pure SWCNTs are thought not to cause DNA breakages or increase DNA mutations frequency; whereas, in other studies the induction of DNA damage has been observed. In particular, Jacobsen et al (2008), in a study that evaluates by *Fpg comet assay* the direct-oxidative damage in murine lung epithelial cells exposed to highly pure SWCNTs, found oxidative stress induction but no DNA breakages. While DNA damage in Chinese hamster lung fibroblasts (V79) exposed to pure SWCNTs has been observed using *comet assay* by Kisin et al (2007).

In a study on commercial SWCNTs and MWCNTs, ROS formation and a decreased membrane potential in rat macrophages and human lung cells have been observed; conversely, SWCNTs purified by acid treatment had no effects. This leads us to the conclusion that metal traces associated with the commercial nanotubes are responsible for the biological effects. (Pulskamp et al, 2007). The genotoxic potential of purified MWCNTs has been reported by Muller et al (2008a), through the use of two complementary approaches based on the MN test both *in vivo* (after intratracheal administration) and *in vitro* (on rat lung cells). This study demonstrated that micronuclei (MNs) may be induced by both clastogenic and aneugenic events. In addition, MWCNTs may induce point mutations that may be responsible for their carcinogenicity (Zhu et al, 2007). A recent study by Wirnitzer et al (2009), however, has demon-

strated that agglomerates of MWCNTs (baytubes) do not show genotoxic activities (induction of chromosome aberrations) in V79 cells.

Fullerenes

Fullerenes are thought to be less toxic than carbon nanotubes. Several studies demonstrated that fullerenes have antioxidant properties without significant cito-genotoxic effects; in other studies, though, induction of oxidative stress, DNA breakages, increased MNs, mutagenicity and chromosome aberrations have been observed (Singh et al, 2009). In particular, the oral exposure to low doses of C_{60} induces the formation of high levels of 8-oxo-7,8-dihydro-2'-deoxyguanosine (8-oxo-dG) in rat liver and lungs (Folkmann et al, 2009). Colloidal dispersions of C60 fullerenes in water have been shown to have genotoxic effects on human lymphocytes estimated by the *comet assay* (Dhawan et al, 2006). In addition, a recent study has demonstrated that exposure to C_{60} fullerenes induces the formation of MNs in A549 lung cells and DNA damage in lungs of mouse (Totsuka et al, 2009).

Discrepancy in the available findings on the genotoxic effects of fullerenes is probably due to factors such as exposure lenght, preparation and cell types; today, as no chemicophysical characterization is available, it is difficult to compare existing data.

5.1.2 Metal and metal oxide nanoparticles

Transition metal ions (cadmium, chromium, cobalt, copper, iron, nickel, titanium and zinc), released by specific NPs, may induce the production of hydroxyl radical (**.**OH), which is one of the main species causing DNA damage. Furthermore, Fe(II) may cause the H_2O_2 production from the molecular O_2. Metal nanoparticles like silver and cobalt appear to have genotoxic effects such as the induction of increased expression and phosphorylation of p53, DNA breakages and chromosome aberrations. Gold NPs also appear to indirectly induce DNA damage, by an oxidative response dependant on cell type and particle size. Metal oxide NPs (TiO_2, ZnO, SiO_2, Fe_xO_x) cross the cell membrane and concentrate in the perinuclear region indirectly inducing genotoxic damage by oxidative stress promotion (Sharma et al, 2009; Park et al, 2008a) and inflammatory response, or they enter the nucleus (TiO_2 and SiO_2) where aggregate with the nuclear proteins involved in DNA replication and transcription, inhibit them and, as a consequence, induce DNA damage. An *in vitro* study by Karlson et al (2008) compared the genotoxic effects of metal oxide particles (CuO, TiO_2, ZnO, $CuZnFe_2O_4$, Fe_3O_4, Fe_2O_3) with those of carbon NPs and MWCNTs on A549 cells using *comet assay* and the 2',7'- dichlorofluorescein diacetate (DCFH DA) for the detection of ROS concentrations. All particles, except iron oxides,

proved to cause DNA damage after 4 hour exposure; CuO particles proved to be more powerful followed by TiO_2 particles. Furthermore, CuO particles determined highest oxidative damage and showed to be the only ones able to induce an increase in intracellular ROS levels.
TiO_2 particles induce sister chromatid exchanges (SCE), increase in MN frequency, DNA damage, increase of hypoxanthine-guanine phosphoribosyltransferase (HPRT) gene mutations, kilobase deletions in mouse embryonic fibroblasts (MEF) (Singh et al, 2009).
Though, there is not an unanimous opinion on the genotoxicity of these materials. In addition, the cellular response induced by NPs of TiO_2 is dependant on their size and shape and unfortunately no sufficient information is provided by current studies for data comparing. IARC classified TiO_2 NPs as possibly carcinogenic to humans (Group 2B) based on sufficient evidence in experimental animals. Induction of lung carcinoma has been observed in rodents after inhalation or tracheal instillation of TiO_2 particles (Pott and Roller, 2005 and Baan et al, 2006) and genotoxic (induction of NM and DNA damage) and oxidative (induction of 8-hydroxy-2 deoxyguanosine) effects on mice exposed to TiO_2 NPs contained in drinking water have been found (Trouiller et al, 2009). Iron oxide nanoparticles are usually covered with polyethylene glycol (PEG), dextran or dendrimers which increase their solubility and biocompatibility or with complex molecules such as antibodies, peptides, hormones or drugs which improve their clinical applications; nevertheless, the stability of these coating layers remains unknown.

5.1.3 Quantum dots (QDs)

At this time, information on the genotoxic effects of QDs is at a very preliminary stage; QDs consist of a nucleus containing metal elements some of which are highly toxic (Cd, Te, Se and Pb), a protective coating layer (cap/shell) mainly made of ZnS and functional coating groups (carboxylic group, amine group and polyethylene glycol) which make them sufficiently hydrophilic, enhance their biocompatibility and bioactivity and make them more stable by reducing their potential toxicity (Singh et al, 2009). QDs sliding through the nuclear membrane pores may interact with the histone proteins in DNA inducing breaks of the DNA chains, activation of p53 genes and chromatinic condensation. The addition of coating groups (eg. ZnS) is thought to have a protective effect as it reduces cyto-genotoxicity, as highlighted by recent studies, even though long-term stability of the protection has not been yet adequately tested. If QDs are hold in the organism for a very long time, the protection coating layer may be degraded under photolytic and oxidative conditions and a sub-

sequent penetration through the nuclear membrane pores and induction of cyto-genotoxic effects may occur (Landsiedel et al, 2009). Several studies have demonstrated the protective effects that zinc sulfide gives to the QDs, reducing their toxicity. The preparation and purification process of materials play an important role in determining the genotoxicity of QDs. While some evidence is provided of the interaction between QDs and the cellular nucleus, very few studies have focused specifically on their genotoxicity.

5.1.4 Conclusions

In conclusion, to date, relatively limited information is available on the genotoxicity of the engineered NMs. Most of the *in vitro* studies have been conducted by the *comet assay* -evaluating direct or oxidative DNA damage- which provided positive results for fullerenes, SWCNTs, MWCNTs, TiO_2 nanoparticles, CdSe/ZnS QDs, gold NPs and by the MN test -evaluating the clastogenic and aneugenic effects - that produced positive results for TiO_2, SiO2, CoCr, zinc oxide particles and TiO_2 + UV-visible irradiation (Landsiedel et al, 2009; Lindberg et al, 2009). *In vivo* studies mainly conducted on rodents involve, in most cases, carbon nanotubes, which may induce oxidative stress, inflammation, fibrosis and mouse lung granuloma (Poland et al, 2008), fullerenes - responsible for oxidative stress and DNA damage in rats (Folkmann et al, 2009; Totsuka et al, 2009) - and, more recently, TiO_2 NPs which proved to induce genotoxic and oxidative effects in mice (Trouiller et al, 2009).
The contradictory findings of the various studies are due to the lack of detailed information regarding the chemicophysical characteristics and production process of materials under investigation but also the dispersion media and treatments which may influence cell uptake, interactions with biological macromolecules and, as a result, toxicity. In addition, further genotoxicity studies using simultaneously multiple tests is needed taking into account the ability of NMs to interact with biological fluids, dispersion media, colouring agents and other reagents that may influence the results. Furthermore, since most of the studies on NMs genotoxicity, performed so far *in vitro,* use short-term exposure, more studies on the effects of prolonged exposure are auspicable.

Table 5.1 - Mechanisms of action of NMs

Nanomaterials	Mechanisms of action	
Carbon nanotubes	*SWCNTs*	Decrease in the cellular adhesion and cell proliferation, induction of apoptosis. Oxidative stress and DNA damage.
	MWCNTs	Cell penetration and reduction in cell viability and IL-8 release. ROS generation. Inflammation, lung fibrosis and granuloma and development of mesothelioma.
Fullerenes	Considered less toxic than CNTs. ROS production. Induction of DNA damage, mutagenicity and induction of chromosome aberrations and micronuclei.	
Metal and metal oxide NPs	*TiO_2, ZnO, SiO_2 and Fe_xO_x*	Induction of DNA damage through ROS production and inflammatory response.
	Al_2O_3	ROS production. Pro-inflammatory response.
	TiO_2	ROS and SCE induction, increased micronucleus frequency and lung carcinoma. Glutathione depletion and oxidative stress as a result of photoactivity and redox properties. Membrane disruption.
	ZnO	ROS production. Dissolution and release of toxic cations. Lysosomal damage. Inflammation.
	Ag	Dissolution and Ag+ release, inhibition of respiratory enzymes and ATP production. ROS production. Disruption of membrane integrity and transport processes.
	Ag and Co	Enhanced expression and phosphorylation of p53, DNA breakages and chromosome aberrations.
	CdSe	Dissolution and release of toxic Cd and Se ions.
	Fe_3O_4	Liberation of toxic Fe^{2+}. Interference on the electronic and/or ion transport activity in the cell membrane.
	CuO	induction of DNA damage and oxidative stress.
Quantum Dots	Penetration into the cell nucleus through membrane and induction of breakages in DNA chain. Activation of p53 and chromatinic condensation. Decreased cyto-genotoxicity due to ZnS.	

5.2 Cytotoxic effects

The available studies on the cytotoxic effects of nanomaterials are numerous and demonstrate that NPs may produce a wide range of cytotoxic effects (Tab. 5.1). The ability of NPs to induce cytotoxic effects has been attributed to size, large surface area, and chemical-physical properties that influence their state of aggregation, interaction with the cells with respect their uptake and reactivity. This variety of factors complicate the study of effects and of their mechanism of action.

5.2.1 Carbon-based nanomaterials

Single-walled carbon nanotubes (SWCNTs)

Cui et al. (2005) investigated SWCNT cytotoxicity in human embryonic kidney (HEK 203) cells and showed decreases in cellular adhesion ability, cell proliferation and induction of apoptosis, all these effects were dose- and time-dependent. These researchers also found that SWCNTs could cause cell cycle arrest in G1 phase. A study (Manna et al. 2005) performed on four different cell lines showed oxidative stress and dose-dependent cell viability. Authors found for human keratinocytes (HaCaT), uterine cervix carcinoma cells (HeLa), human alveolar (A549) and lung cancer cells (H1299) that inhibition of cell growth induced by SWCNTs may be a common cytotoxic response because all four cell lines showed similar loss of cell viability.

Many studies have been performed to verify the different theories that could explain CNT cytotoxicity. Some authors studied the effect of residual metal catalyst particles in the SWCNTs on cytotoxicity. Exposure to human keratinocytes (HaCaT) to SWCNT material containing 30% by weight of iron (Shvedova et al. 2003a) produced oxidative stress and cellular toxicity accumulation of peroxidative products, antioxidant depletion, and loss of cell viability after 18h of SWCNT exposure. Incubation of HaCaT cells with a metal chelator (deferoxamine) reduced cytotoxicity of SWCNTs, indicating a protective role of iron chelator. In addition, results showed ultrastructural and morphological changes in cultured human cells. A 26 wt%, iron-rich SWCNT resulted in a significant decrease of the GSH content and accumulation of lipid hydroperoxides in murine macrophages (RAW 264.7) (Kagan et al. 2006).

Nanoparticle aggregation is considered one of the factors which affect the toxicity of NMs, but as evidenced by Lewinski et al. (2008) in their review, studies produced conflicting results and therefore the effect of the carbon nanotube aggregation is still in doubt. A group of researchers (Wick et al. 2007) conducted a study to assess the influence of the degree and kind of agglomeration of SWCNTS on cytotoxicity. They treated mesothelioma cell line (MSTO-211H) with four samples of SWCNTs. The four samples of SWCNTs were: the starting material called CNTs-rm; the CNTs-rm purified termed CNT-agglomerates; CNT-bundles and CNT-pellet. To prepare the last two samples the CNTs-rm was suspended in non-ionic and biocompatible surfactants (polyoxyethylene sorbitan monooleate), centrifuged to separate the suspended carbon nanotubes (CNT-bundles) from mainly non-tubes carbon fraction (CNT-pellet). The asbestos (crocidolite) was employed in the present study as control material. Authors also measured the two metal ratio (Ni/Y) in all materials. All CNT fractions, except the well dispersed CNT-bundles, were aggregated after the incubation time to micron-sized structures. The toxicity of CNT-bundles was less than that of CNT-ag-

glomerates. Of these two samples the Y, Ni and the carboneous material content were similar indicating that the difference in toxicity is not based on that, but the difference was the degree of dispersion of CNT-agglomerates. Moreover authors postulated that cytotoxic response induced by CNT-agglomerates comparable to asbestos was due to the stiffness and larger size, making nanotubes similar to asbestos. Tian et al. 2006 assessed the cytotoxicity of refined and unrefined SWCNTs on human fibroblast cells. Authors found that refined SWCNTs were more toxic that unrefined SWCNTs and they attributed the greater toxicity of refined SWCNTs to much larger surface area. In addition, authors suggested for the lower cytotoxicity of unrefined SWCNTs that their ability to group together in bundles could create larger and thus less harmful materials.

Some authors evaluated the influence of SWCNT functionalization on cytotoxicity concluding that functionalized SWCNTs are less cytotoxic than pristine SWCNTs. Kam et al. 2004 treated human promyelocytic leukemia (HL60) and human T cells (Jurkat) with functionalized SWCNTs (carboxyl-, fluorescein-, biotin- coated) and they found no toxicity to the cells. Sayes et al. (2006a) performed *in vitro* cytotoxicity screens of three SWCNT samples on cultured human fibroblasts. The SWCNT samples used in this exposure include SWCNT-phenyl-SO3H and SWCNT-phenyl-SO3Na, SWCNT-phenyl-(COOH)2 and one SWCNT stabilized in Pluronic F108. Authors observed that as the degree of sidewall functionalization increases, the SWCNT sample becomes less cytotoxic. Further, sidewall functionalized SWCNT samples are substantially less cytotoxic than surfactant stabilized SWCNTs.

Multi-Walled Carbon Nanotubes (MWCNTs)

Monteiro-Riviere et al. 2005 exposed human epidermal keratinocytes (HEK) to chemically unmodified MWCNTs. High-resolution transmission electron microscopy (HRTEM) images depict multi-walled structures that resemble a 'bamboo' shoot. Transmission electron microscopy (TEM) analysis revealed the presence of MWCNTs within cytoplasmic vacuoles of the HEK and the nanotubes were more numerous within cells as treatment concentration and exposure time increased. The number of keratinocytes containing MWCNTs increased from 59.1% at 24 h to 84.0% at 48 h at the 0.4 mg/ml dose. The MWCNTs induced the release of the proinflammatory cytokine interleukin 8 from HEKs in a time dependent manner. Researchers suggested that this response might be the cumulative effect of both MWCNTs attaching to the plasma membrane as well as being internalized by the cell. Authors, also, evidenced this response was not due to the presence of the iron catalyst, since iron was not detected in the MWCNTs before or after exposure using two independent techniques.

Sato et al. 2005 conducted a study on the effect of length on CNT (testing two different lengths 220 nm and 825 nm) cytotoxicity using the human acute monocytic leukemia cell line (THP-1) in vitro and did not see any significant effect. However, the degree of inflammatory response in subcutaneous tissue in rats showed length-dependent inflammation. Granulation tissue containing macrophages, fibroblasts, and foreign body giant cells was observed around aggregates of both 220 and 825 nm MWCNTs. More 220 nm MWCNTs were observed phagocytosed by macrophages than 825 nm MWCNTs. Of the 825 nm CNTs visible within phagocytic cells the majority were not surrounded by membranes indicating cytoplasmic localization. Bottini et al. (2006) found dose- and time-dependent cytotoxicity in human T lymphocytes and Jurkat T leukemia cells. Both pristine and oxidized MWCNTs also induced apoptosis in freshly isolated primary human T lymphocytes in a dose-dependent and time-dependent manner. Furthermore, oxidized MWCNTs appeared to be more toxic than the pristine CNTs. Jia et al. (2005) evidenced a dose-dependent reduction in cell viability in alveolar macrophage (AM) after exposure to MWCNTs with purity greater than 95%. In a study (Flahaut et al. 2006) conducted to investigate of the cytotoxicity of MWCNts towards human umbilical vein endothelial cells (HUVEC), authors concluded that no citotoxic effects were found for any sample. Although they observed that for MWCNTs having large surface area a decrease of HUVEC viability seemed to appear with increasing the dilution of their suspension. Authors attributed this result to the aggregation of MWCNTs or to improvement their interaction with the cells due to higher dispersion at lower concentrations.
Muller et al. (2005) demonstrated that the short MWCNTs are more toxic that the long ones. Researchers tested purified MWCNTs and purified ground MWCNTs on peritoneal macrophages. MWCNTs were ground in an oscillatory ball mill, this treatment reduced MWCNT lengths, but it did not affect the other characteristics of the material. The cytotoxicity and TNF-α expression of purified MWCNTs were significantly lower than the ground MWCNTs. In addition, purified ground MWCNTs induced a TNF-α response and dose-dependent cytotoxicity similar to asbestos and carbon black.

Fullerenes

The available studies seem to indicate that the cytotoxic response induced by fullerene depend on type cells used. No cytotoxic effects were found in macrophage cell lines exposed to fullerenes, instead a dose-dependent cytotoxic response was detected by several authors in other cell lines. Fiorito et al. (2006) found that fullerenes did not stimulate the release of NO by murine macrophage cells in culture, their uptake by human macrophage cells was very low, and that they did not induce apoptosis and cell death compared to graphite particles, suggested that fullerenes

were not cytotoxic. Jia et al. (2005) after exposing alveolar macrophages to C_{60} for 6h did not observe cytotoxicity in a dose range from 1.41 to 226.00 μg/cm^2. Porter et al. (2006) found that no cytotoxicity to human monocyte macrophages was elicited by C_{60}, despite the fact that they were internalized and contained within the cytoplasm, nucleus, and lysosomes. C_{60} aggregates were also apparent along the plasma membrane, which was suggested by the authors to promote the development of lipid peroxidation observed by other investigators. Sayes et al. (2004) evaluated the cytotoxicity of four different water-soluble fullerene species (nano-C_{60} aggregates; C_3; $Na^{+}_{2\text{-}3}[C_{60}O_{7\text{-}9}(OH)_{12\text{-}15}]^{(2\text{-}3)\text{-}}$; $C_{60}(OH)_{24}$) on human dermal fibroblasts (HDF) and human liver carcinoma cells (HepG2). Authors showed that nano-C60 was cytotoxic to HDF and HepG2 cells at the 20 ppb level. The C_3 and $Na^{+}_{2\text{-}3}[C_{60}O7\text{-}9(OH)12\text{-}15]^{(2\text{-}3)\text{-}}$- water-soluble fullerene species were less cytotoxic to HDF or HepG2 cells, while $C_{60}(OH)_{24}$ showed no cytotoxicity up to its limits of solubility. This provided striking evidence that water-soluble functional groups on the surface of a fullerene molecule dramatically decrease the toxicity of pristine C_{60}. In a further study Sayes et al. (2005) demonstrated cytotoxicity mediated through enhanced ROS production, lipid peroxidation and membrane damage for nano-C_{60} (0.24-2400 ppb) in a variety of cell lines (dermal fibroblasts, hepatocytes and astrocytes). Moreover, the oxidative damage and toxicity of nano-C_{60} were prevented by addition of L-ascorbic acid to the culture medium as an antioxidant. Rouse et al. (2006) investigated the biological response of amino acid-derivatized fullerenes in human epidermal keratinocytes (HEK). HEK viability significantly decreased in a dose-dependent manner after 24 h and 48 h. Uptake of amino acid-derivatized fullerenes began after 24h exposure to concentrations above 0.004 mg/ml.

5.2.2 Metal and metal oxide nanoparticles

Lanone et al. (2009) evaluated the toxic effect of 24 nanoparticles of similar equivalent spherical diameter and various elemental compositions on human alveolar epithelial (A549) and monocyte/macrophage (THP-1) cell lines. Copper- and Zinc based nanomaterials appeared to be the most toxic of all compounds tested. Copper-Zinc mixed oxide was as toxic as Copper or Zinc by itself. Titania, Alumina, Ceria, Silver, Nickel and Zirconia-based nanomaterials showed moderate toxicity, and no toxicity was observed for Tungsten Carbide. Exposure of THP-1 cells to Cobalt nanomaterial induced toxicity only when incorporated as a Nickel-Cobalt-Manganese mixed variants, but not as Cobalt alone.

Braydich-Solle et al. (2005) tested silver (Ag of 15 nm), molybdenum (MoO_3 of 30 nm,) and aluminium (Al of 230 nm) NPs on a spermatogonial stem cell line (C18-4)

using as a positive control for toxicity cadmium oxide. Results demonstrated a concentration-dependent toxicity for all types of particles tested, whereas the corresponding soluble salts had no significant effect. Silver nanoparticles were the most toxic while molybdenum trioxide (MoO_3) nanoparticles were the least toxic. In addition, results showed an increased number of apoptotic cells, and that increase was dose-dependent at lower concentrations (1-5 µg/ml for cadmium oxide, and 10-50 µg/ml for the nanoparticulates tested). More cells became necrotic as the concentrations increased. In the case of molybdenum nanoparticles, a small number of apoptotic cells began to be observed starting at a concentration above 25 µg/ml, and few necrotic cells were observed at concentrations below 50 µg/ml.
Lin et al. (2006a) assessed the toxicity of cerium oxide NPs (20 nm) in human lung cancer (A549) cells. Cell viability decreased significantly as a function of nanoparticle dose and exposure time. Moreover, exposure
to CeO_2 nanoparticles of 3.5 to 23.3 µg/ml produced significant oxidative stress in A549 cells.

5.2.3 Quantum dots (QDs)

The release of free Cd^{2+} ions from CdSe-QD after surface oxidation is an important factor for their toxicity. Cadmium is a know toxic agent that induces cell death via mitochondrial damage and oxidative stress. Encapsulation of the CdSe-QD with a ZnS shell has been shown to reduce toxicity. Several authors evaluated the effect of secondary coatings on the cytotoxicty of QDs. Shiohara et al. (2004) investigated the cytotoxicity caused by three QDs covered with mercaptoundecanoic acid (MUA-QD) in three cell types (african green monkey kidney cell- Vero, uterine cervix carcinoma cell - HeLa, and primary human hepatocyte). These QD-types emitted green, yellow, and red light respectively. Authors showed that cell viability decreased with increasing concentration of MUA-QDs. Hoshino et al. (2004) reported that treatment with QD-capping material mercaptoundecanoic acid (MUA) alone (without QD) for 12 hr caused severe cytotoxicity in murine T-cell lymphoma (EL-4) cells at 100 µg/ml.
Ryman-Rasmusse et al. (2007) utilized primary human neonatal epidermal keratinocytes (HEKs) to determine the cytotoxic and inflammatory potential of CdSe core/ZnS shell QDs of two sizes (QD 565 with a diameter of 4.6 nm; QD 655 with diameters of 6 nm by 12nm) and three different surface coatings (polyethylene glycol (PEG), PEG-amines, and carboxylic acids). Cytotoxicity was observed for QD 565 and QD 655 coated with carboxylic acids or PEG-amine by 48 hours, with little cytotoxicity observed for PEG-coated QDs. Only carboxylic acid coated QDs significantly increased release of IL-1b, IL-6, and IL-8. Voura et al. (2004) treated melanoma cells (B16F10)

with dihydroxylipoic acid (DHLA)-capped CdSe/ZnS QDs and they observed no detectable difference in growth between QD-treated and untreated cells. Hanaki et al. (2003), exposing african green monkey kidney (Vero) cells to 0.24 µg/ml CdSe/ZnS QDs capped with 11-mercaptoundecanoic acid (MUA) and coated with sheep serum albumin (SSA,) found no effect of QDs on cell viability.
Lovric et al. (2005) found that CdTe QDs coated with mercaptopropionic acid (MPA) and β-Mercaptoethylamine were cytotoxic to rat pheochromocytoma cells (PC12) in culture at concentrations of 10 *µ*g/mL. Uncoated CdTe QDs were cytotoxic at 1 *µ*g/mL. Cell death was characterized as chromatin condensation and membrane blebbing, which was symptomatic of apoptosis. In addition, smaller positively charged QDs were significantly more toxic than larger equally charged QDs at the highest concentration. QD distribution was in part dependent on nanoparticle size, smaller cationic QDs (2r=2.2±0.1 nm) were often found in the nucleus and larger cationic QDs (2r=5.2 nm) were distributed throughout the cytoplasm. The mechanisms involved in cell death were considered to be due to the presence of free cadmium or free radical formation.

5.2.4 Conclusions

In conclusion, available data show cytotoxic and apoptotic effects for carbon nanotubes (mainly unfunctionalized single-walled carbon nanotubes) but the cytotoxicty can be attributed to the state of aggregation, the presence of metal catalysts, functionalization groups, length and size distributions. Fullerenes seem to be less cytotoxic even though the response depends on the cell types: several studies showed that they are not cytotoxic for macrophages but they induced toxic effects in other cell types. Metal NPs exhibit a wide range of cytotoxic response which depend on metal types: some toxic effects have been observed for silver-, copper-, zinc-, molybdenum- and aluminium-based NMs. In addition, cytotoxic effects of QDs depend on release of metal ions, particle size and type of coatings.
The available studies evidenced a wide range of cytotoxic effects depending on the combined effects of a variety of physicochemical properties, this underlines the need for a thorough physicochemical characterization of each type of nanomaterial prior to toxicological studies.

5.3 Respiratory effects

In vitro and *in vivo* tests showed that lung is the main target organ for NP toxicity. Furthermore, inhalation is the primary route of NP uptake.

5.3.1 Uptake

While airways are a strong barrier to NPs, in the alveoli gas exchanges between lungs and blood do take place. At this level, in fact, the interstitial thickness is of only 5 μm. As most of the engineered NPs affect both occupational and environmental settings as aerosols or colloidal suspensions, the lung exposure resulting from inhalation is the most likely route of exposure for men (Maynard et al, 2004). Interactions between engineered NPs and lung parenchyma may vary according to the spherical or elongated shape. Spherical NPs deposit in lung regions according to their size and physical structure (Oberdoster et al, 2005). Once deposited in the alveoli, spherical NPs appear to translocate into the pulmonary interstitial sites probably by transcytosis and, then, into the systemic circulation. Berry et al. (1977) were the first to describe translocation of NPs across the alveolar epithelium using intratracheal instillations of 30-nm gold particles in rats. Unlike spherical NPs, fiber-like particles (i. e. carbon nanotubes) are able to elude the macrophage surveillance system (frustrated phagocytosis) and, therefore, penetrate the general blood circulation. This macrophage circumvention is more noticeable for longer fibers.

5.3.2 Carbon-based nanomaterials

Carbon nanotubes (CNTs)

Many authors have studied the cytotoxic effects of SWCNTs and MWCNTs on human or inhuman lung cell lines. Cytotoxic effects observed with the MTT essay in murine macrophage cell line RAW 264.7, in human alveolar macrophage cell line THB-1 and human lung carcinoma cell line A549 after exposure to 5 μg/ml of multi-walled carbon nanotubes (Soto et al, 2007) were similar to those produced by asbestos. Similar findings were observed in RAW 264.7 cell line and alveolar macrophages exposed to SWCNTs and MWCNTs (Murr et al, 2005; Jia et al, 2005). Less cytotoxicity was observed in A549 cells exposed to a concentration range of 1.56-800 μg/ml SWCNTs. Significant cytotoxic effects were recorded at 400 and 800 μg/ml SWCNTs and in the absence of serum with alamar blue, neutral red and MTT assays (Davoren et al, 2007). Other authors, on the contrary, demonstrated that exposure to 5, 10, 50 and 100 μg/ml of purified SWCNTs and MWCNTs did not determine toxic effects on the same cell lines and on rat macrophages NR8383 (Pulskamp et al, 2007). In particular, no toxicity was observed with WST-1 assay, although MTT revealed a dose-dependent decrease in cell viability. Some authors claimed that the reason for this discrepancy may depend on the ability of CNTs to interfere with MTT assay (Worle-Knirsch et al, 2006; Mointeiro-Riviere and Inman 2006). A number of studies, however, showed

the toxicity of these NPs through other cell viability tests. A significant decrease in the number of cells was observed in RAW 264.7 cells treated with 12.5-30 μg/ml SWCNTs (Dutta et al, 2007). In A549 and BEAS-2B human bronchial epithelial cell lines, clonogenic assay demonstrated a significant decrease in the size of cell colonies if exposed to 0-400 μg/ml SWCNTs (Herzog et al, 2007). Finally, human mesothelioma cell line MSTO211H treated with 0-30 μg/ml of unpurified SWCNTs determined a decrease in cell proliferation (Wich et al, 2006).

Existing literature on CNTs shows that their cytotoxicity could be linked to the presence of metal impurities. In fact, the exposure of BEAS-2B cells to SWCNTs having a content of 30% iron and 20% nickel produced cytotoxic effects such as the decrease in cell viability, reduced glutathione and an increased hydroxyl radicals production (Shvedova et al, 2007b). Similar effects have been reported in A549, BEAS-2B and RAW 264.7 cell lines (Kagan et al, 2006) exposed to SWCNTs with iron content; while unremarkable toxic effects have been observed in purified SWCNTs (Herzog et al, 2007; Kagan et al, 2006). Purified SWCNTs, indeed, do not induce the release of ROS and inflammatory mediators. Exposure to purified SWCNTs did not stimulate the intracellular ROS production or nitric oxide (NO) production in RAW 264.7 cells (Kagan et al, 2006; Shvedova et al, 2005). Likewise, in A549 and NR8383 cells treated with purified MWCNTs and SWCNTs (5-100 μg/ml), no nitric oxide, IL-8 or TNF-α production has been observed, while unpurified NPs induced ROS generation (Pulskamp et al, 2007). Finally, Wang L. et al (2008) found that exposure of lung fibroblasts to SWCNTs resulted in a 70% increase in collagen production and cell proliferation. These findings confirm the *in vivo* fibrogenic responses caused by CNTs.

Few *in vivo* studies have reported the cytotoxic effects of CNTs on the respiratory system. Intratracheal instillation of 1-5 mg/kg of SWCNTs in rats produced a transient lung inflammation followed by a non-dose-dependent series of multifocal granulomas (Warheit et al, 2004). In male rats treated with 0.1-0.35 mg of purified and unpurified SWCNTs, a dose-dependent formation of interstitial granulomas was observed (Lam et al, 2004). Similar results were found by Shvedova et al (2005) who observed that exposure to SWCNTs (0-40 μg/ml) of female rats via pharyngeal aspiration induced an acute but transient inflammatory response as well as a dose-dependent development of epithelioid granulomas and the onset of progressive interstitial fibrosis. Among *in vivo* tests, of particular interest are the experiments of inhalation toxicity. Shvedova et al. compared the responses resulting from exposure via pharyngeal aspiration (5-20 μg/ml) against exposure via inhalation (5 mg/m^3, 5 h/day for 4 days) in C57BL/6 mice. Both studies reported a significant and acute inflammatory response which, through the induction of oxidative stress, could lead to the onset of a multifocal granulomatous pneumonia followed by a persistent interstitial fibrosis (Shvedova et al, 2008b).

Nevertheless, inhalation exposures lead more easily to the induction of the aforementioned cytotoxic effects. The oxidative stress induced by SWCNTs is one of the most important mechanisms of lung toxicity. The treatment of NADPH oxidase-deficient C57BL/6 mice with 40 μg SWCNTs determined an accumulation of polymorphonuclear leukocytes, an increased production of apoptotic cells and pro-inflammatory cytokines as well as decreased production of anti-inflammatory cytokines, TGF-β and lower levels of collagen deposition (Shvedova et al, 2008c). On the basis of such results, authors concluded that NADPH oxidase-dependent ROS generation plays a leading role in the regulation of the pulmonary response to SWCNTs. In the same mice fed with vitamin E-deficient diet, the injection of 40 μg SWCNTs resulted in a more severe decrease of pulmonary antioxidants and induced a far more significant inflammatory response (Shvedova et al, 2007a).
Muller et al (2005), after intratracheal instillation of 0.5, 2 and 5 mg of MWCNTs in Sprague-Dawley rats, observed a significant acute inflammatory response. Two months after the treatment, agglomerates of MWCNTs deposited in the airways induced collagen-rich granulomas and alveolitis. Exposures to MWCNTs in C57BL/6 mice via pharyngeal aspiration have resulted in dose- and time-dependent pulmonary inflammation (Sriram et al, 2007). However, inhalation of MWCNTs at doses ranging from 0.3 to 5 mg/m^3 in the same rats (7 and 14 days, 6h/day) did not determine any pulmonary damage or inflammation (Michell et al, 2007). It has been recently hypothesized that the structural defects of the carbon framework may be one of the major factors governing the lung toxicity observed in Wistar rats after dosing 2 mg of MWCNTs by intratracheal instillation (Fenoglio et al, 2008; Muller et al, 2008b). Takagi et al (2008) reported that the intraperitoneal injection of 3 mg of MWCNTs in p53 +/- mice resulted in mesothelioma six months after treatment. However, the high dose of MWCNTs raised concern about their ability to cause asbestos-like lesions (Shvedova et al, 2009). Poland et al (2008), using a more reasonable dose of MWCNTs, reported that intraperitoneal instillation of "long" MWCNTs (50 μg) but not "short" MWCNTs in rats induced a significant inflammation response in the abdominal wall with formation of the so-called foreign body giants cells.

Fullerenes

Alveolar macrophages exposed to fullerenes have produced cytotoxic effects at very high concentrations (226 μg/cm^2) (Jia et al, 2005).
Cytotoxicity of fullerenes is associated with their solubility degree and is inversely proportional to the number of hydroxyl and carboxyl groups on the surface of the nanoparticle (Sayes et al, 2004). *In vitro* test performed on lung epithelial cells

showed that the toxic effects of insoluble fullerenes are 3-4 times more acute than for soluble fullerenes (Sayes et al, 2007a).
Intratracheal instillation in mice of 0.2-3 mg/kg of soluble and insoluble fullerenes demonstrated that both induce a transient inflammatory response one day after treatment. Moreover, three months after treatment, in mice exposed to 1.5-3 mg/kg of fullerenes an increased lipid peroxidation has been observed (Sayes et al, 2007a). There was not significant histopathological lesion in the respiratory tract of Fischer 344 male rats during the inhalation exposure to 2.22 mg/m^3 of fullerenes, 3h/day for 10 consecutive days (Baker et al, 2008).

5.3.3 Metal nanoparticles

Many *in vitro* and *in vivo* studies investigated the potential cytotoxic effects and biocompatibility of metal NPs.
The exposure of BEAS-2B cell to higher and higher doses (5-40 µg/ml) of titanium dioxide NPs (TiO_2) enable NPs to penetrate into the cytoplasm and localize in the perinuclear region. Moreover, as doses increased, the cell viability decreased by 40% when compared with the control; on the contrary, according to authors, the increased dose-dependent oxidative stress observed would be responsible for the induction of cytotoxicity (Park et al, 2008b).
Similar effects were found in A549 cells exposed to TiO_2 NPs concentrations ranging from 3 to 400 µg/cm^2 (Singh et al, 2007). Sayes et al (2006b) observed cytotoxic effects in the same cell line but only at relatively high concentrations (100 µg/ml), and demonstrated that induction of the inflammatory response and cytotoxicity were time- and dose-dependent. Significantly lower levels of cytotoxicity have been observed with concentrations ranging from 0.25 to 100 µg/ml under analogous experimental conditions (Simon-Deckers et al, 2008). Whereas, no cytotoxic effects or mitochondrial damage were observed by Karlsson et al (2009) with concentrations of 40 to 80 µg/ml. These discordant findings could be caused by the different characteristics of NPs such as dimensions, crystalline structure, chemical composition and surface area.
Also in rats, intratracheal instillation of 1 to 5 mg/kg of different types of TiO_2 NPs induced pulmonary effects due to the chemical composition and crystalline structure of NPs (Warheit et al, 2007). BAL analysis showed that Ti NPs induce a significant inflammatory response and acute cytotoxic effects. In a similar study, ICR male rats treated with 0.1 and 0.5 mg of TiO_2 have showed significant morphological and histological alterations in the pulmonary tissue such as the breakage of the alveolar septums, proliferation and hyperplasia of type II pneumocyte apoptosis, macrophages

accumulation, apoptosis of epithelial cells and the induction of emphysematous lesions (Chen et al, 2006). In the same animal models, exposure to 5, 20 and 50 mg/kg of TiO_2 NPs determined the induction of proinflammatory Th1 and Th2 cytokines, proliferation of B cells and formation of granulomatous lesions and deposition of proinflammatory proteins (Park et al, 2009). Groups of male Crl:CD (SD) rats were intratracheally instilled with 1.5 and 5 mg/kg of TiO_2 particles of different size and aggregation state; after one week of treatment, NPs elicited a higher inflammatory response than larger particles equally administered (Kobayashi et al, 2009). Whereas, after one month, a similar inflammatory response in the different groups of animals was observed. Therefore, authors hypothesized that the inflammatory response could be associated with the particle different surface area (1.58 m^2/kg for NPs and 0.05 m^2/kg for fine particles).

The cytotoxic effects of silver (Ag) NPs have been observed in murine germ cells, in rat hepatic cells, in human gliobastoma cells and in human lung fibroblasts (IMR-90). In this cell line, the treatment of silver NPs resulted in a significant decrease in the ATP intracellular concentrations, higher levels of mitochondrial damage and a sensible increase in ROS generation (Asharani et al, 2009). According to the authors, Ag NPs would induce dysfunctions in the mitochondrial respiratory chain and ROS accumulation and block the ATP synthesis.

Yet, a study of the A549 cells comparing the different effects produced by metal NPs revealed that Ag NPs would induce significantly less cytotoxic effects than Ti NPs and would exhibit the same toxicity as Zn NPs (Park et al, 2007).

In Sprague-Dawley rats exposed through inhalation (6 h/day for 90 days) to Ag NPs at concentrations of 0.7×10^6, 1.4×10^6 and 2.9×10^6 particles/cm^3 small granulomatous lesions and the onset of a chronic alveolar dose-dependent inflammation were observed (Sung et al, 2008). In a similar study conducted on rats exposed to similar concentrations of Ag NPs (0.6×10^6, 1.4×10^6 and 3×10^6 particles/cm^3), the same authors noted, through histopathological examinations of lung tissues, the presence of an inflammatory cell infiltrate, chronic alveolar inflammation and small granulomatous lesions (Sung et al, 2009).

The literature suggests that iron (Fe) NPs have scarce cytotoxic power; indeed, exposure of A549 cells to concentrations of 0.24, 2.4, 24, 240, 2400 ppb of Fe NPs induced insignificant effects on their mitochondrial activity, DNA content and the cell glutathione production (Cha et al, 2007). No significant cytotoxic effects were found in other studies of the same cell line (A549) (Karlsson et al, 2209; Karlsson et al, 2008).

On A549 cells, copper (Cu) NPs are able to induce more genotoxic and cytotoxic effects, a higher mitochondrial membrane depolarization and a significant increase in oxidative stress than micrometric particles of the same composition(Karlsson et al,

2209; Karlsson et al, 2008). Similar results were obtained with the exposure of HEp-2 human larynx epithelial cell line to 4-400 μg/cm^2 of Cu NPs (Fahmy et al, 2009). Indeed, authors claimed that the dose-dependent cytotoxic effects observed in the exposed cells could be linked to the significant cell oxidative stress.
In the human bronchial epithelial cell line BEAS-2B, exposed to Ce NPs at increasing concentration (5, 10, 20, 40 μg/ml), higher levels of ROS production, increased cell death and induction of genes involved in oxidative stress responses have been reported (Park et al, 2008b). The increased intracellular reactive oxygen species would be responsible for caspase-3 activation and the consequent induction of apoptosis which enables Ce NPs would realize to produce their cytotoxic effects. The involvement of ROS in the cytotoxicity of Ce NPs has been confirmed by a study conducted by the same group of authors on BEAS-2B cells exposed to 1 μg/ml (Eom et al, 2009).
In A549 cells exposed to different concentrations of Zn NPs for 24 hours, a high apoptosis and a significant increase in the intracellular ROS have been observed (Park et al, 2007). In a study exposing different cell lines to concentrations of Zn NPs varying from 0.0001 μg/ml to 30 mg/ml, a higher cellular damage was observed in the rat lung epithelial cell line (L2) (Sayes et al, 2007b). In addition, Zn NPs induced different cytotoxic effects. During the same study, an *in vivo* test was performed on Crl:CD (SD) IGS BR rats intratracheally instilled with 1 and 5 mg/kg of Zn NPs which produced a potent but reversible inflammatory response which was resolved by 1 month postinstillation exposure (Sayes et al, 2007b).
In A549 cells, SiO_2 NPs at 10, 50 and 100 μg/ml induced a time- and dose-dependent decrease of the cell viability and an increase in ROS, malondialdehyde and lactate dehydrogenase (LDH) production. (Lin et al, 2006b). *In vivo* intravenous administration (2 times a week for 4 weeks) of 10 and 30 mg/kg of silica NPs to BALB/c rats caused no respiratory damage (Nishimori et al, 2009).

5.3.4 Conclusions

Generally speaking, *in vitro* tests suggest that engineered NPs can elicit significant cytotoxic effects on the biological systems under investigation. Indeed, murine cells of rat and human respiratory systems treated with SWCNTs, MWCNTs, metal or silica NPs have been shown to cause a decrease in cell viability and proliferation, size and number of cell colonies and an increase in ROS generation and in the production of extracellular and intracellular oxidative stress.
In vivo tests, on the other hand, demonstrate that the exposure to NPs induce a significant and acute lung inflammatory response, granulomatous and emphysematous lesions, collagen deposition and consequent interstitial fibrosis and the production

of significant levels of oxidative stress. In addition, as for MWCNTs, the ability of NPs to cause lesions similar to those of asbestos still needs to be ascertained.
The aforementioned effects on the respiratory system suggested that the industrial use and practical applications of NPs should be reduced (Shvedova et al, 2009). However, NPs exhibit very different toxicity profiles and, as a result, generalizations are impossible. Further studies are therefore required to define the real toxicity of such NMs; to this purpose, an accurate chemicophysical characterization of NPs under investigation will be fundamental.

5.4 Dermal effects

Dermal exposure to NPs may induce irritative and allergic local effect on the skin and be used as entryway into systemic circulation. To date, few data are available on dermal risks associated to NPs but initial experimental results suggest their potential ability to trigger dermal effects and penetrate skin layers; however, today, further research on the wide range of NPs is recommended as their diffusion and dermal effects on the skin may differ significantly. The exposure to carbon nanotubes is known to induce different dermal effects from those of metal NPs whose behaviours vary according to the size and type of the metal involved. Additionally, to date, most of the knowledge in this field comes from the pharmaceutical industry which has observed the effects of titanium dioxide (TiO_2) and zinc oxide (ZnO) nanoparticles used in skin care formulations, whereas very little information relating to other type of NPs is available.

5.4.1 Uptake

Human skin (1.5 m^2 in area in an adult human) normally functions as strict barrier for NPs (Argyle et al, 2009). This is due to the substantial impermeability of the outer layer of the skin (epidermis), whereas the inner layer (dermis) is richly supplied with blood vessels, tissue macrophages, lymphatic vessels, dendritic cells and five different types of sensory nerve endings. As a consequence, if engineered NPs could penetrate the dermis, they would enter the bloodstream and convey through lymph nodes in the lymphatic circulation and elicit an immune response. Kim et al (2004) demonstrated that NPs intradermally injected migrate towards regional alveoli, possibly through microphages and Langherhans cells. Studies on the dermal uptake of NPs (Alvarez-Roman et al, 2004; Baroli et al, 2007; Bennat et al, 2000; Ryman-Rasmussen et al, 2006; Larese et al, 2009) produced discordant results, probably due to the use of different technologies and methodologies and, above all, to the lack of standardized protocols. Moreover, mechanical flexions, irritant detergents, and chemicals may

increase the skin NPs uptake. Although today no convincing experimental evidence suggests that NPs may permeate the epidermis and reach the dermis, indirect clues exist supporting this possibility. Carbon nanotubes have been shown to induce damage to human keratinocytes which are the predominant cell types in the epidermis (Shvedova et al, 2003a; Monteiro-Riviere et al, 2005): as the trans-epidermal passage is very likely to take place in the case of anatomical damage, repeated exposures to engineered NPs could cause epidermal damage first and then allow the passage of NPs into the dermis. This possibility has occupational and social implications since NPs are widely used in many cosmetic products such as lotions or shampoos, cleansing creams or sunscreen formulations.

Irritative effects

Some NMs have been shown to produce irritative and inflammatory effects (Crosera et al, 2009). In particular, carbon nanotubes may have an irritative action on the skin and conjunctiva due to their mechanical effects: Kishore et al (2009) reported reversible irritative effects of some nanotubes on the conjunctiva, while no effect in the animal skin was observed. Ryman-Rasmussenn et al (2007), on the contrary, found cytotoxic and irritative effects in quantum dots through *in vitro* tests on keratinocytes where cytokine liberation was observed. Silver NPs too may induce cytotoxic effects on *in vitro* keratinocyte cell cultures; however, to date no data on workers exposed to or users of these particles are available (Zanette et al, 2009).

Allergic effects

To date, no data on the potential allergenicity of NPs are available; however, current data suggest that an increased exposure risk is associated with metal NPs. Since NPs exhibit a larger bioavailable surface area, it has been hypothesized that they are able to interact significantly with dermal immune system and induce an allergic response caused by contact with particles containing allergenic metals such as cobalt, nickel and chromium. Yet, no data confirming such hypothesis are available and further studies in this field would be needed. Presumably, no allergic response should be caused by NPs containing non-allergenic metals (gold, silver). Some preliminary results, on the other hand, suggest that fullerenes may have a leading role in the inhibition of the *in vitro* and *in vivo* IgE-mediated allergic response, thus blocking the histamine release (Ryan et al, 2007).

5.4.2 Carbon-based nanotubes

Carbon nanotubes and fullerenes

Little is known on the dermal effects caused by carbon nanotubes (CNTs). Rouse et

al (2007) demonstrated the ability of fullerenes to penetrate intact skin and, primarily, in the flexion areas; in 2007, Ryan et al hypothesized that fullerenes might have a major role in the inhibition of the IgE-mediated allergic response.
Data available today on potential dermal effects of CNTs are mainly based on *in vitro* studies conducted on keratinocytes and fibroblasts. Shvedova et al (2003a) demonstrated that single-walled carbon nanotubes (SWCNTs) induce oxidative stress on immortalized human keratinocytes (HaCaT), loss of viability and microscopic alterations. In a study on keratinocytes exposed to increasing doses of SWCNTs, the increase in interleukin-8 (IL-8) release observed after 24 and 48 hours from the highest dose (0.05 mg/ml) induced an irritative dose-dependent reaction (Zhang et al, 2007); whereas, Sayes et al. (2006a) found that the cytotoxic response of human dermal fibroblasts exposed to SWCNTs was dependent on the degree of functionalization of nanotubes. In addition, the surface exposed has shown to be proportional to their potential toxicity. Irritative and inflammatory effects of multi-walled carbon nanotubes (MWCNTs) on keratinocytes (Monteiro-Riviere et al, 2005; Witzmann and Monteiro-Riviere et al, 2006) and cytotoxic effects on dermal keratinocytes (Ding et al 2005) were also observed.

5.4.3 Metal and metal-oxide nanoparticles

Metal nanoparticles
Ag NP-based products have multiple applications, especially due to the recognized and non-toxic antimicrobial activity of silver. Keratinocytes and fibroblasts cultures have been studied to investigate the potential toxic effects of silver NPs released from nanosilver containing dressings (Leaper et al, 2006). Findings showed that bandage extracts covered with silver NPs inhibit the keratinocyte proliferation and alter their morphology (Paddle-Ledinek et al, 2006). Furthermore, Poon and Burd (2004) demonstrated that nanosilver crystals prove toxic to keratinocytes and fibroblasts. Some Authors suggested an increased dermal penetration of nanosilver associated with damaged skin compared to intact skin (Larese et al, 2009). Little is known about NP elimination mechanisms after transdermal uptake; yet, there is evidence that intradermal nanoparticles can gain access to systemic distribution through subcutaneous lymphatics vessels. (Gopee et al, 2009).
Gold nanoparticles are used in several medical applications as they proved to be safe (Connor et al, 2005); however, cytotoxic effects on human keratinocytes were observed *in vitro* by Wang S. et al (2008).
Papageorgiou et al (2007) compared the cytotoxic and genotoxic effects of NPs and micron-sized particles of cobalt/chromium alloy on human fibroblasts cultures and

reported that NPs induce more DNA damage and exhibit more cytotoxicity than micron-sized particles.

Metal-oxide nanoparticles: titanium dioxide (TiO_2) and zinc oxide (ZnO)
These are the most used NPs in sunscreen formulations as they protect against UV rays efficiently and do not exhibit the typical white colour of creams containing micronic particles. Tan et al. (1996) excised skin from human volunteers following 2-6 week applications of a sunscreen formulation containing TiO_2. Later, other authors demonstrated, however, that TiO_2 localizes only in the outer layer - *stratum corneum* - and does not penetrate skin to an appreciable depth (Shulz et al, 2002; Mavon et al, 2007) even though a small amount of TiO_2 can be found in hair follicles (Lademann et al, 1999). The 2007 European project NANODERM, the broadest investigation of dermal penetration of TiO_2 NPs thus far, led to the conclusions that TiO_2 is safe as it does not penetrate the deeper layers of epidermis. The same goes for the zinc oxide particles that remain on the surface of the skin and in the *stratum corneum*. (Cross et al, 2007).
Although these results deny that these particles may penetrate the deeper layers of the skin, more recent investigations highlight the ability of some NPs to penetrate flexed (Rouse et al, 2007) or damaged skin (Larese et al, 2009); so, after repeated applications, such formulations are likely to allow skin penetration of NPs. Newman, in a recent interview (2009), suggested that further studies are required to understand whether under real conditions an increased skin uptake of these metal is observed.
The penetration of these NPs in the deeper layers of the epidermis is an important aspect concerning the safety of nanoparticles as small sizes and large surface could induce different effects on immune system or cells if compared to materials exhibiting higher granulometric parameters. Small size may enhance their ability to perform their immune action and act as haptens and provoke allergic or autoimmune reactions (Newmann et al, 2009); however, current studies are not enough to express a definitive opinion on this issue.
Nevertheless, relatively high concentrations of TiO_2 NPs are known to induce cytotoxic and inflammatory effects *in vitro* (Cai et al, 1992; Wamer et al, 1997; Dunford et al, 1997; Sayes et al, 2006b). *In vitro* cytotoxic effects have also been demonstrated for ZnO NPs (Huang et al, 2009; Yuan et al, 2009).
Of great importance are the photocatalytic properties of TiO_2 and ZnO NPs that have resulted in their use in photovoltaic cells for electron production. At cellular level, these properties induce ROS generation and damage the DNA. Dunfort et al (1997) demonstrated that TiO_2 particles determined DNA chain breakages in fibroblasts cul-

ture following UVA and UVB rays exposure. In 2002, Uchinno confirmed the ability of TiO_2 to generate hydroxyl radicals and, in 2006, Hidaka et al studied the DNA damages induced by TiO_2 and ZnO following UV exposure and found an increased alteration in cellular DNA. Opposite findings were reported by Dufour et al (2006) who compared the chromosomal alterations induced on hamster ovary cells by UV treatment with and without ZnO NPs but no significant differences were observed. Authors concluded that chromosomal aberrations may be linked to UV irradiation rather than ZnO exposure.
In a recent review published by L'Oreal, Nohynek et al (2009) focused on the risk associated with the use of NPs in UV protective products and concluded that there is scientific evidence that NMs used in cosmetic formulations and sunscreens do not produce risks to human skin or health and protect against the adverse effects of UV irradiation, such as the skin cancer.
However, it was noted that nano-sized products may induce new biological effects when compared to traditional formulations and this requires more studies to be conducted in order to reflect the normal conditions of use of these products and determine the safety of NPs they contain (Newmann et al, 2009).
Today, all sunscreens are thought to contain NPs and the European Union is calling for new and specific labeling of these products under the framework of the new cosmetics directives; however, the scientific community tends to collect more data before launching new products under the "no data, no market" approach (http://www.framingnano.eu/newsletters/FramingNanoNewsletter5_morenews.htm#nanocosmetics).
In 1999, the U.S. Food and Drug Administration approved the marketing of cosmetics containing NPs without the new labeling but is now reassessing its views; in 2007, a teamwork proposed a series of marketing guidelines requiring new safety testing and additional information for the marketing of products containing NPs.

5.4.4 Quantum dots

Some studies underlined cytotoxic and irritative responses such as the cytokine release in keratinocytes cell cultures exposed to quantum dots (Ryman-Rasmussen et al, 2007; Zhang et al, 2008).

5.4.5 Conclusions

Finally, there is literature evidence on the ability of NPs to perform an irritative actions on keratinocytes and partially penetrate the *stratum corneum* of the skin and the epidermis; yet, data are not enough to reach definitive conclusions. No significant

evidence on human exposure exists even though, starting from 1997, NPs are widely used in a number of cosmetic formulations and sunscreens. Further research is therefore needed to assess the dermal risk associated with NP exposure.

5.5 Effects on the Central Nervous System

Different types of engineered NPs are currently used for selective drug delivery or the development of pharmacological, therapeutic and diagnostic agents associated with Central Nervous System (CNS)-related pathologies (Uwatoku et al, 2003; Bianco et al, 2005; Olivier, 2005; Silva, 2006). However, many *in vitro* and *in vivo* studies observing the ability of nanoparticles (NPs) to provoke cytotoxic effects has been reported in literature. The current state of knowledge on the effects on CNS resulting from exposure to NMs is reported below in order to ascertain whether or not the use of nanotechnologies may pose risks to this organ system.

5.5.1 Uptake

NPs, most often absorbed via the inhalation route, are able to translocate from the penetration site and reach the CNS in different ways. The transnasal uptake route assumes a peculiar relevance as it represents the unique direct pathway from the external environment to the central nervous system. The possibility for the engineered NPs to rapidly reach the CNS via this route is suggested by an *in vivo* study involving the inhalation exposure of rodents to 35 nm carbon NPs (Oberdoster et al, 2005). The authors were able to detect the material at the level of the olfactory bulb shortly after the exposure. This finding suggests undoubtedly the use of the transnasal uptake route. Analogously to the poliovirus, following the inhalation exposure, NPs travel through a mechanism of trans-synaptic transport towards the CNS and, thus, are directly picked up in the CNS by nerve endings in the nasal (olfactory nerve and trigeminal nerve) and tracheobronchial mucosae (afferent vagus nerve) (Oberdoster et al, 2004; Muller et al, 2004; Kreuter et al, 2004).
Furthermore, inhaled NPs penetrate the respiratory barriers and, through the circulation, reach the CNS by crossing the blood-brain barrier (BBB) in the case of damages due to hypertension or encefalomielitis.
The BBB is the most selective epithelial barrier and is aimed at reducing the paracellular passages. Intact BBBs protects CNS against exposures to substances carried by the blood; however, intrinsic characteristics linked to surface charges and/or morbid conditions like hypertension or encefalomielitis may alter the integrity of the BBB and enable NPs to cross and distribute and, as a result, cause CNS toxicity (Muldoon et al, 1999).

NPs conveyed by the blood flow are thought to exhibit the intrinsic ability to alter epithelial cell membranes properties and/or disrupt the BBB tight junctions.
NPs are known to induce oxidative stress and generate reactive oxygen species (ROS) that affect the epithelial cell membranes and cause BBB damages and dysfunctions. Apart from the physical properties of the endothelial plasma membranes and NP sized, the NP surface electrostatic charges play a major role in the non-endocytic transport across the BBB (Hagenbuch and Meier, 2003).
Specific studies (Lockman et al, 2004) demonstrated that neutral NPs and low concentration anionic NPs do not alter the integrity of the BBB, while high anionic and cationic concentrations of NP surface charges induce BBB dysfunction.

5.5.2 Carbon-based nanomaterials

Although most of the *in vitro* and *in vivo* studies on the assessment of NM neurotoxicity used metal NPs, some works on the effects on the CNS resulting from exposure to non-metal NMs are reported.
Chen et al (2008) investigated the response of human endothelial cells of the brain microvasculature following the exposure to increasing doses of carbon NPs. Treatment with carbon NPs altered mitochondrial membrane potential, induced oxidative stress and decreased the expression of tight junction proteins; however, the cytotoxic effects were significantly less if compared to those provoked by the exposure to aluminium NPs. In addition, unlike metal NPs, the decrease in the cell viability was observed at the highest exposure dose only (10 mM).
Recently, neurotoxic effects of carbon nanotubes (CNTs) on primary mixed neuroglial cultures derived from spinal cord and dorsal root ganglia of chicken embryos exposed to single-walled carbon nanotubes (SWCNTs) have been investigated (Belyanskaya et al, 2009). The main cytotoxic effect on neuro-glial cultures of CNS and peripheral nervous system (PNS) was a significant decrease in DNA content. This effect was higher in cells treated with aggregated SWCNTs compared to those exposed to dispersed SWCNTs. In addition, the decreased vimentin expression, a specific glial cells protein, suggests that the decrease in DNA content is mainly due to the action of NPs on this type of proteins. Finally, the analysis of the ion conductance and resting membrane potential indicates that SWCNTs are able to influence these parameters in PNS neurons, while the elecrophysiological properties of CNS neurons are not modulated by the SWCNTs exposure. That being said, results demonstrate that the exposure of PNS and CNS neuro-glial cultures to SWCNTs induces significant cytotoxic effects on the nervous tissues and, in particular, on glial cells.
Largemouth bass (*Micropterus salmoides*) after exposure to 0.5 ppm of fullerenes

(nC60) showed that a trans-synaptic transport mechanism enables NMs to reach the CNS through the olfactory nerve (Oberdorster E, 2004). The analysis of the cerebral tissue in animals treated with fullerenes showed a sensible increase in the lipid peroxidation products resulting from the oxidative stress induced by NPs.
The same modalities of penetration in the CNS have been observed in rodents exposed to 36 nm carbon black NPs (Oberdorster G, 2004). This study showed a significant accumulation of nanomaterial at the level of the olfactory bulb.
Successive investigations demonstrated that these NPs are able not only to reach the CNS through the above mentioned transport mechanism but also to significantly alter the normal functions of the cerebral tissue. Tin-Tin-Win-Shwe et al (2006) showed that the exposure of male BALB/c rats to 14 nm and 95 nm carbon black NPs induced a deep alteration in the gene expression of proinflammatory cytokines (IL-1β and TNF-α) and chemokines (CCL2 and CCL3) at the level of the olfactory bulb. In particular, treatments with 14 nm NPs induced the activation of microglia cells which, as a consequence, caused a significant increase in proinflammatory cytokines and chemokines. This mechanism explains the inflammatory response observed in the olfactory bulb.
Using the same animal model, the same research group investigated the effects caused by the exposure to carbon black NPs on extracellular levels of neurotransmitters in the olfactory bulb (Tin-Tin-Win-Shwe et al, 2008). Analogously to the previous investigation, the intranasal instillation of 14 nm carbon black NPs induced increased levels of IL-1β and TNF-α. Compared with the control, higher extracellular levels of glutamate and glycine have been observed in the olfactory bulb. Based on these findings, authors hypothesized that NPs, once in the CNS, may cause the release of neurotransmitters within the extracellular fluid and trigger the secretion of proinflammatory cytokines that are responsible for the neurotoxic damage.

5.5.3 Metal nanoparticles

Titanium dioxide (TiO_2) NPs have been tested *in vitro* and their potential effects on CNS cells have been investigated. Recently, it has been noted that the exposure of murine microglial N9 cells to TiO_2 NPs may induce relevant cytotoxic effects (Li et al, 2009). In fact, a significant decrease in N9 cell viability and apoptotic morphologic alterations even at low exposure doses (16 µg/ml) have been observed. This type of cellular response is thought to be responsible for the potential adverse effects of nanoparticles on the CNS.
Whereas, Long et al (2006) showed that the exposure of the immortalized murine microglia cell line (BV2) to Degussa P25, a TiO_2 NPs mixture of anatase and rutile

(70:30), determined a rapid and prolonged release of reactive oxygen species (ROS). In order to assess the effects on CNS cells, the same research group (Long et al, 2007) exposed the BV2 cells, rat mesencephalic neurons (N27) and a primary culture of embryonic Sprague-Dawley rat striatal cells to the same concentration of Degusse P25. TiO_2 NPs induced a significant increase in the hydrogen peroxide (H_2O_2) production in microglial cells and influenced the molecular mechanisms associated with the cell cycle control, inflammation and apoptosis.

Conversely, the exposure to Degussa P25 determined no cytotoxic effect in isolated rat dopaminergic neurons (N27) even after 72 h of exposure. Yet, the neurotoxicity assessment of the material detected in the embryonic rat striatal cell cultures containing microglial cells evidenced a significant decrease in the cell viability even at low exposure doses (5 ppm). Based on these findings, authors concluded that the strong neurotoxic effects of TiO_2 NPs observed in the complex cell cultures of the nervous tissue are mediated by the microglial ROS generation.

Apart from the cytotoxicity mediated by microglial ROS, it has been hypothesized that NPs are able to induce "excite-toxicity" and, as a consequence, the nerve cell death. To this regard, a study carried out by Aleseenko et al (2008) examined the uptake and release of glutamate (one of the most important excitator neurotransmitters) and ROS production in synaptosomes isolated from Wistar rat brains. The exposure of synaptosomes to ferritin (containing ferric oxide phosphate particle with a diameter of 7 nm) inhibited the glutamate uptake. Furthermore, these results showed increased ROS levels in cell cultures. Authors suggested that the increased oxidative stress induced by NP exposure may inhibit the synaptosomal glutamate uptake. Hence, the excessive extracellular glutamate concentration cause a prolonged exposure of neurons to the excitator neurotransmitter that is likely to be responsible for the cell damage and death induced by "excite-toxicity".

A further molecular mechanism able to alter the normal functions of the CNS is associated with the ability of NPs to interfere with voltage-dependent potassium currents. In fact, hippocampal CA1 neurons isolated from Wistar rats exposed to copper NPs showed an efficient inhibition of the potassium delayed rectifier current (I_k) (Xu et al, 2009).

One more interesting aspect related to the cytotoxic effects of NMs on CNS is the relationship between NPs and the blood-brain barrier (BBB). Chen et al (2008) exposed human brain microvasculature endothelial cells (HBMECs) to increasing doses of aluminum oxide NPs. Results showed that the treatment induced a significant decrease in the cell viability, alterations in the mitochondrial membrane potential, augmentation of oxidative stress and decrease in tight junction proteins expression. So, according to the authors, the integrity of the BBB represents one the main objectives of aluminum NPs whose toxic action would alter the mitochondrial functions.

Astrocytes have been reported to attenuate the neurotoxic effects and play a significant role in modulating the blood-brain barrier permeability. For this reason, the influence of NMs on the BBB may be assessed also through the exposure of such glial cells to NPs, in order to observe alterations in their normal morphology and functionality, adhesion capability and cell growth. Experiments conducted on Sprague-Dawley rats indicated that the exposure of immature astrocytes isolated from their cerebral cortex to iron oxide NPs (Fe_3O_4 or y-Fe_2O_3) inhibits the adhesion capability and the subsequent cell growth; on the contrary, iron oxide NPs do not alter morphology or cell growth in mature astrocytes, while a significant augmentation of mitochondrial activity has been observed (Au et al, 2007).

Even more significant cytotoxic effects have been detected in human astrocytoma U87 cells exposed to increasing concentrations of TiO_2 NPs (< 25 nm), manganese oxide (< 50 nm), zinc oxide (< 100 nm) (Lai et al, 2008). The results showed that TiO_2 NPs are able to induce a significant decrease in the cell survival, that the increase in the cell death is dose-dependent and that the most acute cytotoxic effects are caused by zinc oxide NPs, while manganese oxide NPs was the least toxic. As for the cellular response, findings of this study show that U87 cells treated with TiO_2 NPs exhibit three different models of cell death: apoptosis, necrosis and apoptosis-necrosis.

After 60 days exposure, tissue distribution and toxicity of TiO_2 NPs (10 nm, 25 nm, Degussa P25 (21 nm) and 60 nm) topically administered in BALB/c rats demonstrated that, among NMs, only Degussa P25 was able to reach the brain tissue (Wu et al, 2009). Histopathology of the CNS did not reveal pathological alterations. This partly confirms data in the literature according to which NPs are able to cross the BBB and reach, albeit in quite small amount, the CNS (Kreyling et al, 2002; Lockman et al, 2004; Oberdorster et al, 2004).

Similar results were obtained after a single oral administration in CD1 (ICR) rats of 5 g/kg of different sized TiO_2 particles (25 nm, 80 nm and 155 nm). In groups exposed NPs accumulated in the CNS and slight brain lesions of the hippocampus region consisting of vacuoles and fatty degeneration of the brain tissue were also observed (Wang et al, 2007).

In the same animal models, acute neurotoxic effects after intraperitoneal injection of anatase TiO_2 NPs were observed (Ma et al, 2010). In particular, in rats exposed to higher doses of nanomaterials the production of superoxide anions and hydrogen peroxides showed to be significantly increased. The resulting oxidative stress induced the increase in lipid peroxidation levels, the release of nitric oxide, the reduction of glutamic acid and the decrease in levels of acetylcholinesterase and antioxidant enzymes activities. In addition, authors observed significant morphologic alterations in nerve cells assuming filamentous shapes or turning into inflammatory cells.

A study conducted by Shimizu et al (2009) showed that the maternal exposure of female ICR rats in pregnancy to TiO_2 NPs induced a significant alterations in the expression of genes related to apoptosis, oxidative stress and CNS development in the progeny.
Finally, in CD 1 (ICR) rats treated with intranasally instilled TiO_2 NPs of 80 nm (i.e. rutile) and 155 nm (i.e. anatase) acute adverse effects on the CNS were observed (Wang JX et al, 2008). NPs are able to reach the CNS via transsynaptic transport after the uptake into the nerve endings embedded in the mucosa of the nasal cavity and then accumulate mainly in the olfactory bulb and in the hippocampus (Bodian and Howe, 1941a; Bodian and Howe, 1941b; Tjalve et al, 1995; Oberdorster et al, 2004). These findings highlighted that the exposure to TiO_2 NPs determine an increase in the oxidative stress levels, an augmentation of the inflammatory response and a significant increase in the levels of Tumor Necrosis Factor-α, (TNF-α) and interleukin-1beta. Toxic effects proved to be slightly higher in anatase-instilled animals, which suggested that the crystal structure of nanomaterials could play a prominent role in response induction.
Similarly to the previous investigation, the intranasal instillation in CD 1 (ICR) rats of Fe_2O_3 NPs (21 nm) induced significant neurotoxic effects such as the increased oxidative stress and morphological signals of cell damage in some CNS regions of treated animals (Wang et al, 2009). Authors also noted neuronal-dendritic degenerations, alterations in cellular membrane, increase in lysosomes of the olfactory bulb, rough endoplasmic reticulum dilatation and increase in hippocampal lysosomes.
It has been demonstrated that including manganese oxide NPs (30 nm) are able to reach the CNS of Fisher 344 rats via transsynaptic transport after inhalation exposure (Elder et al, 2006). Nanomaterials have been identified in various brain regions; however, analogously to previous studies, the preferential NP accumulation in the olfactory bulb was observed. Moreover, in regions where materials accumulate, an increase in the levels of TNF-α and glial fibrillary acid protein - specific indicator for reactivity or astrocyte damage - was found. The increased gene expression of such protein is thought to indicate the presence of an inflammatory response in tissues involved.
ROS generation is reported to induce, among other things, neurotoxicity in male C57BL/6N rats exposed to 23 nm silver NPs (Rahman et al, 2009). In fact, the various brain regions (caudate nucleus, frontal cortex and hippocampus) of treated animals showed a significant induction of oxidative stress and DNA damage. In addition, results demonstrated that silver NPs are able to emulate the expression of a number of genes involved in the induction of oxidative stress and in the production of antioxidant agents. ROS generation associated with gene modulation could negatively influence the immune system and cause cell apoptosis as well as significant neurotoxic effects.

A study of Fisher 344 rats, investigated the effects on the BBB and the brain's vascular system after the exposure to aluminium NPs (Chen et al, 2008). The intravenous instillation of 8 to 12 nm aluminium NPs resulted in a decreased tight junctions protein expression and in a marked fragmentation of occludin and claudin-5 which play a paramount role in the regulation of the BBB integrity.
Similar results were obtained after the intraperitoneal injection of the same material in male Sprague-Dawley rats (Song et al, 2008). The ultrastructural analysis of the BBB showed that aluminium NPs cause damage to cell membranes, cytoplasmic organelles and tight junctions in the brain capillary endothelial cells. In addition, analogously to the previous investigation, a significant reduction of occludin and F-actin expression was observed. Authors suggested that neurotoxicity of aluminium NPs is associated with its ability to influence permeability and alter the BBB integrity.
NP uptake within body's fluid compartments is likely to alter the CNS functions under normal conditions and/or its responses to additional stress such as hypothermia. Nanoparticles containing metals (50-60 nm Cu, Ag and Al) are thought to cause CNS dysfunctions in normal animals and aggravate pathologies following hypothermia (Sharma HS and Sharma A, 2007). NP exposure determined more significant motor function disturbances in Ag-treated rats than Cu and Al-treated animals (Sharma HS and Sharma A, 2007). The influence of metal NPs on the BBB has been investigated also in Sprague-Dawley rats treated with 50-60 nm Cu, Al and Ag NPs (Sharma et al, 2010). NPs were instilled by intravenous and intraperitoneal injection and cortical perfusion. Findings showed that the BBB integrity was deeply altered by NM exposure, the most acute adverse effects were caused by intravenous instillation and cortical perfusion; copper and silver NPs proved to be the most toxic. The ability of NPs to alter the BBB integrity is associated with the induction of the oxidative stress which may, in turn, induce the release of neurochemical substances, cytokines and ROS and damage the endothelial cells (Sharma HS and Sharma A, 2007; Sharma et al, 2009). As a consequence of NP-induced BBB rupture, macromolecules reach the CNS and, generally, induce angiogenic oedemas (Sharma 2006a-b; Sharma et al, 2006c). Chronic instillation of metal NPs results more often in brain oedemas than spinal cord oedemas and the oedemigenic activity proved to be more pronounced in Ag NPs than Cu and Al NPs.
Oedemas in NP-treated animals are likely to determine neuropathological effects and neurodegenerative alterations.
Finally, the BBB damage induced by NP activity enables the passage of a number of toxic substances which would not normally come into contact with the CNS microenvironment and result in morphological and functional cell alterations (Sharma 2004, b; Sharma, 2006a-b).

Neurons of NP-treated animals show intracellular alterations such as cytoplasmic condensation and chromatolysis, cell membrane damage and karyoplasmic densification exhibiting nucleolar eccentricity.

5.5.4 Conclusions

Generally speaking, NPs may reach the CNS through two different mechanisms: transsynaptic transport through the olfactory nerve after inhalation or intranasal exposure and the uptake across the BBB following the intravenous, intraperitoneal, oral or percutaneous NM administration (Lai et al, 2000; Borm et al, 2006). The easy passage of NPs cross the BBB is the reason why, in recent years, they have been widely used in drug delivery to the CNS in the biomedical field or for the development of therapeutic and diagnostic agents of some neurodegenerative diseases (Uwatoku et al, 2003; Bianco et al, 2005; Olivier, 2005; Silva, 2006). However, the current state of knowledge on the potential toxic effects of engineered NMs on the nerve tissues is still extremely limited and fragmentary; yet, there are some *in vitro* and *in vivo* studies found in the literature on the ability of NPs to induce significant neurotoxic effects on animal and human neurons and glial cells as well as on several animal models. NMs exhibit toxicity mainly through significant oxidative stress induction at the CNS level. Furthermore, NPs are thought to alter the normal BBB integrity and modulate the expression of several genes related to the inflammatory response and apoptosis.

So, it seems clear that, to ensure a safe use of nanotechnologies, better information and deeper knowledge of molecular mechanisms underlying the revealed neurotoxic effects are required.

Finally, apart from metal NMs investigated so far, more studies are needed to test the potential neurotoxic effects of engineered NMs, i.e. carbon NPs and quantum dots.

5.6 Cardiovascular effects

Scientific interest in potential cardiovascular effects of engineered NPs is due to the known strong correlation between high levels of nano-sized atmosphere particulate (ultrafine particles) and cardiovascular events such as stroke, myocardial infarction, arrhythmias and sudden death (Mossman et al, 2007). In addition to the analogous chemical components observed in both ultrafine particles and engineered NPs, nanometer sizes represent an additional element in terms of potential toxicity: to date, there is clear evidence that the cytotoxicity of ultrafine or nanoparticles is greater than that of the same mass of larger particles of similar chemical composition

(Donaldson et al, 2001). This specific toxic characteristic of NPs is likely to derive from the strong correlation between total area and mass resulting in very large surface in contact with biological materials.
Engineered NPs could possibly induce adverse effects on the cardiovascular system through two mechanisms:

A. A secondary indirect biopersistence in lung regions: this could lead to a persistent and chronic local inflammatory process and to the release into the circulation of mediators (i.e. cytokines) which, in turn, are able to induce a persistent, albeit slight, systemic inflammation. A number of studies have shown that chronic inflammatory response may be one the major factors leading to atherosclerosis and subsequent acute effects (myocardial infarction and strokes) which can be associate with such degenerative process (Ross, 1999).
B. A direct effect resulting from the ability of engineered NPs to cross the lung parenchyma and come into contact with cells (i.e. endothelial cells and platelets) and soluble products (i.e. coagulation proteins) playing a role in the induction of cardiovascular damage.

5.6.1 Carbon nanotubes

The most investigated NPs in terms of potential adverse effects on the cardiovascular system are the carbon nanotubes. Particular attention has been paid to the potential effects of these NMs on the physiopathological pathways of cardiovascular damage such as hypercoagulability, atheroma formation, systemic inflammatory state, endothelial dysfunction/damage (including the oxidative damage) and alterations of the cardiovascular activity regulated by the autonomic nervous system. In this review, the effects on modes of action will be treated separately; therefore, investigations analyzing more than one potential mode of action will be cited more than once.
As for coagulation alterations, Radomski et al (2005) demonstrated that both single-walled carbon nanotubes (SWCNTs) and multi-walled carbon nanotubes (MWCNTs) may induce an enhanced platelet aggregation *in vitro*: this could result in ischemic damage in humans, as confirmed by the efficient anti-atherosclerotic treatments used to inhibit platelet activity (Ben-Dor et al, 2009). Of particular interest was the fact that this work was performed on human platelets and, as a consequence, proved to be more clinically applicable compared to other investigations performed on animal cells. The weak point (however typical of almost every currently existing toxicological experiment on engineered nanomaterials) is that doses used are several orders of magnitude higher than that of potential occupational or environmental human exposures. Radomski's findings were partly confirmed by an *in vivo* investi-

gation on the lungs of Swiss rats after a single exposure to MWCNTs (intratracheal instillation) (Nemmer et al, 2007): platelet alterations were slight over a 24-hours period and tended to disappear 6 hours after exposure. It is worth noting, however, that this investigation had involved MWCNTs only (which had lower effects than SWCNTs in the Radomski investigation) and that the potential long-term effects of chronic exposure had not been evaluated. Based on available data, therefore, we can conclude that the effects of platelet aggregation are not significantly marked; however, additional studies are needed to identify the presence and the entity of such potential pathways of cardiovascular damage. It is interesting to notice that both authors (Ben-Dor et al, 2009; Nemmar et al, 2007) conducted a parallel *in vivo* investigation to evaluate whether *in vitro* or *ex vivo* findings could be applied to the *in vivo* arterial thrombosis formation. In particular, they evaluated the possibility that carbon nanotubes might accelerate or enhance thrombus formations in the rat physically and chemically damaged carotid artery: in relation to the relatively mild results concerning the platelet aggregation, both experimental models showed an unquestionable effect of carbon nanotubes on the acute carotid artery thrombus formation. This finding indicates that besides platelets, other components taking part in the complex coagulation process might be involved after exposure to carbon nanotubes. In fact, this hypothesis might be proved right by recent results obtained by Erdely et al (2009) who observed a significant augmentation of the total and active PAI-1 (protein playing a leading role in the inhibition of fibrinolitic cascade) in rats exposed to carbon nanotubes. Also in this case, the effects were evaluated by using an acute experimental method (4 h after exposure) and, therefore, need to be confirmed with a more plausible models of chronic human exposure. The formation of atherosclerotic plaques induced by repeated pulmonary exposures to SWCNTs in rats genetically predisposed to atherosclerotic damage was observed by Li et al (2007). This study is of great interest as it shows the possible induction of damage following repeated, relatively low-dose exposures which represent more plausible conditions for men. Yet, it is worth noting that hyperlipid diet fed to genetically predisposed rats resulted in the development of atherosclerosis; among rats fed on normal diet, atherosclerotic effects in rats given SWCNTs did not differ from rats receiving saline solution. Further studies on this issue are therefore necessary; however, these findings suggest that analogous risks may be hypothesized in workers having high cardiovascular risks or chronically exposed to SWCNTs. As an example, obesity among workers with family history of cardiovascular diseases may be associated with genetic predisposition (strong family history) or environmental cofactor (experimental models of rat hyperlipid diet or human obesity). With respect to the induction of systemic inflammations, Salvador-Morales et al (2006) demonstrated in an *in vitro* study that carbon nan-

otubes, primarily SWCNTs, are able to activate the to activate the complement system via the classical pathway (involved in most inflammatory processes in humans) while two already mentioned *in vitro* studies reported contradictory results (Erdely et al, 2009; Li et al, 2007). In fact, Erdely et al (2009) observed an increased neutrophil count, augmentation of soluble inflammatory mediators (IL-6, CXCL1, IL-5, CCL11, CCL22, CXCL2, S110a8, IL8rβ and Mac-1) and higher expression of pro-inflammation associated genes in the aortic arch. In contrast, case-control study conducted by Li et al (2007) revealed no alterations in MCP1, IL-12, IL-6, TNF-α, and IFN- levels. It is worth mentioning, however, that above mentioned studies had two different objects: Erdely et al evaluated the acute model, whereas Li et al focused on the chronic model. It may be hypothesized, therefore, that exposures to carbon nanotubes may determine acute, but short-lasting, inflammatory response.

Absence of cytotoxicity in endothelial cell cultures was reported by Flahaut et al (2006) by two standard assays (Neutral Red and MTT assays): after 24 h exposure to mixtures of carbon nanotubes, no sign of cardiovascular damage was found. Conversely, the cited *in vivo* study by Erdely et al (2009) showed the activation of the aortic endothelial cells that resulted in the increased expression of E-selectin (adhesion molecule which is expressed only on activated endothelial cells) which plays a major role in the initial process of atherosclerosis. Both studies could be interpreted as indicating that although carbon nanotubes do not induce evident endothelial cell damage, they have been shown to cause endothelial dysfunctions which is paramount in the onset of the atherosclerotic disease. From Flahaut's work it resulted that the MTT assay may prove to be technically inadequate for assessing cytotoxicity of carbon nanotubes (Wörle-Knirsch et al, 2006).

The induction of oxidative damage to cardiovascular cells is suggested by Simeonova's et al (2007) investigation demonstrating that SWCNTs were able to determine *in vitro* LDL oxidation in human aortic endothelial cells. Since phagocytosis of oxidized particles by tissue macrophages induces the formation of the so-called foam cells which can be detectable in the initial stages of atherosclerotic disease, the result of Simeonova's work is of paramount interest in the relationship between nanotube exposure and the onset of atherosclerosis. This physiopathological mechanism was also detected by Li et al (2006) who observed oxidative damage to mitochondrial DNA in the aorta and depletion of natural antioxidants (i.e. reduced glutathione after chronic exposure to SWCNTs).

Finally, a very recent *in vivo* study conducted by Legramante et al (2009) demonstrated that repeated intratracheal instillations of SWCNTs may determine alterations in the autonomic regulation of the cardiac activity. In particular, alterations in the baroreflex control have been observed. This physiologic system permits a continuous

adaptation of the cardiac activity to the variations of the venous pressure in order to maintain a constant organ perfusion: in healthy subjects, as the cardiac pressure lowers, the cardiac frequency increases; the opposite occurs in the case of raised cardiac pressure. Alterations of this homeostatic mechanism lead to a higher risk of cardiovascular diseases. Many studies, in fact, show that subjects with malfunctioning baroreflecx control have a higher risk of sudden cardiac death, infarction relapse and arrhythmias (La Rovere et al, 1998). Interestingly, Legramante et al obtained their outcomes from sub-chronic administration (data assessed 2 weeks after the first SWCNT administration) and suggested that the evaluation of the baroreflex activity should be taken into account when assessing chronic exposures to SWCNTs.
Finally, existing experimental evidences suggest that exposure to carbon nanotubes may activate multiple physiopathological pathways of cardiovascular damage. Furthermore, data need to be interpreted with extreme caution as relating to acute exposure (expected human exposure is generally chronic) and high doses of materials (human populations are expected to be exposed to much lower doses of material). Of particular interest is the ability of such material to induce not only the formation of atherosclerotic plaques following chronic exposure but also the acute thrombus formation in predisposed subjects. It is known, indeed, that major ischemic events (as myocardium infarction and ictus) occur in patients with chronic conditions, such as atherosclerotic disease, which can be associated, under specific circumstances (instable atherosclerotic plaques) with acute thrombus formation causing complete arterial occlusion. Therefore, the question of whether carbon nanotube exposure may play a role in both processes need to be addressed. Based on the current state of knowledge, it may be hypothesized that the physiopathological pathways of atherosclerotic disease may be associated with endothelial dysfunction and oxidative damage to key elements of arterial cells (mitochondrial DNA); acute thrombus formation, on the contrary, is more likely to derive from the activation of proteins involved in the coagulation cascade rather than from direct effects on platelets. Other mechanisms (induction of a persistent and chronic systemic inflammatory state) are less likely to induce atherosclerotic damage since it has been demonstrated that they are able to determine transitory and relatively slight adverse effects. Fine nerve processes which can be responsible for irreversible alterations of neurogenic control of the cardiovascular activity should also be considered.

5.6.2 Conclusions

Before reaching conclusions on the potential cardiovascular effects of engineered NPs, more studies aimed to provide a more accurate reflection of the expected occupational and environmental exposure conditions appear mandatory.

5.7 Immunological effects

The scarce data (mostly *in vitro*) currently available on the potential effects of NPs on the immunological system suggest that NPs, once entered the systemic circulation, might be able to interact with proteins deposited or circulating on the cell surface, thereby exposing amino acid residues normally not exposed (cryptic epitopes), and stimulating a potential autoimmune inflammatory response (Labarre et al, 2005). One more potential damage mechanism may be triggered by the interference with opsonization processes and, as a consequence, with the clearance of foreign materials (i.e. microorganisms) usually eliminated by the process itself (Moghim and Patel, 1998).

5.7.1 Carbon nanotubes

In vivo studies demonstrated a series of potential effects of CNTs on the immune system. Koyama et al (2006) evaluated the immune response of rats to subcutaneous administration of single-walled carbon nanotubes (SWCNTs) and multi-walled carbon nanotubes (MWCNTs) for 3 months. Authors observed that this material is able to induce major histocompatibility complex Class I and Class II within two weeks from administration. This response could underlie the peculiar hystopathological picture (granuloma formation) detected after lung exposure. Mitchell et al (2007) in a inhalatory study on rats observed a suppressed T-lymphocyte dependent antigen response associated with increased levels of interleukin-10 (IL-10) which indicates altered immune function. The functional meaning of this alterations is still uncertain, given the complex interrelations among various parts of the immune system. These results, however, are very likely to be associated with a reduced ability to kill infections; previous environmental and epidemiological studies, indeed, showed a reduced ability to spontaneously eradicate infections after exposure to ultrafine environmental particulate.

An effect on the innate immunity and, in particular, on the ability of lung macrophages to phagocytize *Listeria monocytogenes* was observed by Shvedova et al (2008a) after lung exposure to SWCNTs of rats subsequently infected with such germ. Even though the induction modalities are still unknown, the findings of this study are outstanding in terms of potentially increased receptivity to infections among workers chronically exposed to SWCNTs. Conversely to previous findings, Dumortier et al (2006) found no adverse effects of carbon nanotubes on macrophages, T and B lymphocytes *in vitro.* It is important to note, however, that carbon nanotubes used in this study were functionalized by hydrosoluble groups; this process induces alterations in the chemicophysical characteristics of such NMs and attenuates their cytotoxicity.

An indirect correlation between engineered NPs and the immune system is represented by the interaction between nanoparticles and the natural history of diseases with an immune component such as amyloidosis. Primary amyloidosis is induced by monoclonal alterations of plasma cells (cell normally involved in the humoral immunity response) responsible for the extracellular deposition of fibrillar substance called amyloid. Recently, Linse et al (2007) observed that the presence of MWCNTs induced a dose-dependent increase in the formation of the critical nucleus, a crucial stage in the fibril formation. Therefore, the interaction with engineered NP might reveal or accelerate the course of some autoimmune diseases.

5.7.2 Conclusions

Also due to the high doses used, existing data are insufficient to conclusively express an opinion regarding toxic effects of engineered NPs on the immune system. Notwithstanding, they will stimulate further investigations on this issue.

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chapter 6

Risk assessment and risk management

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6.1 Precautionary principle

In environmental and health decision-making, the so-called "Precautionary Principle" represents the tool for cases in which controversial opinions need to be addressed without adequate scientific justification.
The precautionary principle may be invoked where urgent measures are needed in the face of a possible danger to human, animal or plant health or to protect the environment where scientific data do not permit a complete evaluation of the risk. This principle is applied mainly to protect human health.
The EC Treaty contains only one explicit reference to the precautionary principle, namely in the title on environmental protection. However, in practice, the scope of this principle is far wider and also covers consumer policy and human, animal and plant health.
Since the precautionary principle is not defined in the Treaty or in other Community instruments, the Council in its Resolution of 13 April 1999 requested the Commission to develop clear and effective guidelines for the application of the principle. In February 2000, the Commission issued a *Communication on the Precautionary Principle* aiming to build a common understanding of how to assess, manage and communicate all risks that science is not yet able to fully evaluate (European Commission's Communication, 2000). The Commission's Communication is a response to this request: the Commission reviews respectively all factors invoking the precautionary principle and the measures resulting from it. It also puts forward guidelines for the application of the principle.
According to the Commission, the precautionary principle may be invoked when the

potentially dangerous effects of a phenomenon, product or process have been identified by a scientific and objective evaluation, and this evaluation does not allow the risk to be determined with sufficient certainty. Hence, the implementation of the principle belongs to the general framework of risk analysis (which includes risk evaluation) and, more particularly, to the context of risk management which corresponds to decision-making.
Hence, the precautionary principle may only be invoked if three conditions are met:

1. Identification of potentially adverse effects.
2. Evaluation of the scientific data available.
3. The extent of scientific uncertainty.

As regards the measures resulting from the use of the precautionary principle, they may take the form of a decision to act or not to act. The response depends on a political decision and is a function of the level of risk considered "acceptable" by the society on which the risk is imposed.
The precautionary principle should be informed by three specific principles:

1. implementation of the principle should be based on the fullest possible scientific evaluation. As far as possible this evaluation should determine the degree of scientific uncertainty at each stage;
2. any decision to act or not to act pursuant to the precautionary principle must be preceded by a risk evaluation and an evaluation of the potential consequences of inaction;
3. once the results of the scientific evaluation and/or the risk evaluation are available, all the interested parties must be given the opportunity to study the various options available, while ensuring the greatest possible transparency.

Besides these specific principles, the general principles of good risk management remain applicable when the precautionary principle is invoked. These are the following five principles:

- proportionality between the measures taken and the chosen level of protection;
- non-discrimination in application of the measures;
- consistency of the measures with similar measures already taken in similar situations or using similar approaches;
- examination of the benefits and costs of action or lack of action;
- review of the measures in the light of scientific developments;
- the burden of proof.

Nanomaterials (NMs) have peculiar characteristics and their industrial use creates new opportunities but also news risks and uncertainties. An increasing number of workers and consumers are more and more exposed to such materials due to their growing production and application. Thus, more information on potential environmental and health effects of NMs is required.

The understanding of the occupational exposure to emerging nanomaterials is very limited. In addition, exposure evaluation techniques are not completely developed. Several *in vivo* and *in vitro* methods are being debated and defined to investigate the potential health effects of NMs and to characterize their chemicophysical features (Satterstorm FK et al, 2008).
The Community Strategy 2007-2012 on health and safety at work includes the issue of nanotechnologies in the framework for new and emerging risks identification. Furthermore, the European Commission issued a publication illustrating a plan of action to implement a safe, integrated and responsible approach to nanotechnologies, then welcomed by the European Parliament (European Commission's Communication, 2005; European Parliament, 2006; European Commission's Communication, 2007). In addition, a Code of Conduct for an ethical and safe nanotechnology development was issued by the European Commission (European Commission's Communication, 2004). Currently, several initiatives are being launched with a view to guaranteeing nanotechnology and paving the way to its safe and responsible development.
In 2008, European Commission supported the definition of a responsible approach managing and structuring the development of nanotechnology (EU Recommendation, 2008). The European Economic and Social Committee stressed the need for nanotechnology development, addressing ethical issues in close parallel with environmental, health and safety issues, all along the life cycle of their scientific applications (Opinion of the European Economic and Social Committee, 2008).

6.2 Risk Assessment Approach

As already stated, the European strategy sustains a responsible approach to the development of all activities associated with NMs. Until further investigations are conducted on potential toxicological effects of NMs, extreme caution is advised.
To date, a number of tools are available to guarantee a high level of occupational safety standards and, as a consequence, a safe management of NMs.
Framework Council Directive on Health and Safety of Workers 89/391/EEC has been implemented in Italy by way of the Leg. Decree 81/08 and subsequent amendments and integrations. The chemical safety is regulated by the Council Directive 98/24/EC on the protection of the health and safety of workers from the risks related to chemical agents at work.
Finally, the REACH (Registration, Evaluation, Authorization and Restriction of Chemicals) Regulation which entered into force on 1st June 2007 should guarantee a higher protection of workers and consumers from the risks associated with chemical agents like NMs.

Hence, risk assessment processes for NMs can be most likely similar to those currently used for chemical substances. The European Commission acknowledged, in principle, that potential risks to health, safety and environment posed by NMs are covered under current regulatory framework (Communication form the Commission to the European Parliament, 2008).
In particular, the "risk assessment" is to be considered as the collection of the knowledge-based and operational procedures required for the "assessment of health and safety risks of workers associated with the exposure to NMs", according with specific work activity.
Risk assessment is a complex and iterative operation which necessarily requires, in every environment or workplace, a series of successive and consequent operations such as:

1. the identification of sources of occupational exposure to NMs detected in the operational cycle;
2. the identification of subsequent NM exposure risks with respect to the specific work activity;
3. the assessment of NM exposure risks in relation to the welfare protection policies identified.

With respect to the specific work activity, this process may result in:

- no exposure risk;
- controlled exposure risk;
- exposure risk.

In the first case, no critical issues related to the job processes have been observed; in the second one, periodical monitoring is required; in the third case, prevention and protection measures must be taken in order of priority.
Here are the logical steps for every operational phase of the exposure risk assessment.

1. **Step I.** Identification of sources of occupational exposure to NMs.
   Besides a brief, albeit accurate, description of the job process, this phase provides information concerning:
   - the objectives of the work or operation, followed by the description of the technological process, equipments, plants, machinery employed and/or produced substances, intermediates;
   - the technological cycle of job activities (including cleaning, maintenance, waste treatment and disposal and concurrent operations);
   - the working environment (work compartment, laboratory, etc.);
   - the structural characteristic of the working environment (area, volume, doors, windows, etc.);

- the number of operators for activities performed;
- the handling of NMs in the workplace.

The description of the work cycle or operational activity will provide a more detailed picture of all processes performed in a given workplace and, as a consequence, will enable the analytic identification of sources of occupational exposure to NMs. In this phase, workers' involvement and participation is essential for the identification of potential risk sources in the job process.

At the end of Step I, risk sources which may objectively pose both accidental and environmental/hygienic exposure risks must be identified (through hazardousness evaluation, functioning, etc.), not taking into account the risk sources that, due to their nature, structure or use do not pose exposure risks.

2. **Step II.** Identification of subsequent NM exposure risks.
   This is a generally complex phase designed to ascertain whether hazard and/or risk sources which have been identified in the previous phase may pose true occupational risks from NM exposure. For this aim to be met, it is necessary to take into account:
   - the operating modalities of the work activity (manual, automatic, instrumental) or operations (closed-cycle, segregated or protected);
   - the processing operations on the basis of time elapsed and material used per working day;
   - planning of activities (permanence in the workplace, concurrent activities);
   - security measures and/or preventive/protective systems for workers.

   Apart from the identification of the intrinsic potentials of risk sources (equipments, plants, chemicals, etc.), it is important to identify the residual risks, taking into account operating modalities, prevention and protection measures and exposure characteristics (shielding, segregation, intrinsic protections, extractor fan, ventilation, insulation, warning signals) as well as protection interventions.

3. **Step III.** Assessment of NM exposure risks
   In this phase, decisions concerning the evaluation process are made. Such decisions must rely on qualitative methods integrated, as much as possible, with quantitative methods. These are particularly suitable if the extent of damage is expected to be great and serious. Qualitative methods, on the contrary, are best used to evaluate alternative security measures and identify the best performance. In detail, this phase is aimed to:
   - verify that safety standards are correctly applied to equipments, plants and machinery during operations;

- verify the acceptance of working conditions, with respect to the objective review of risks, duration of activities, operating modalities and exposure determinants on the base of data obtained under similar exposure conditions in same operative sector and a consolidated experience. It is worth stressing that a "unitary" indicative guidance of risk factors will be defined for homogeneous working situations and, on the basis of such assessment, integrated interventions will be carried out according to specific measures of protection on a case-by-case basis;
- verify hygiene and security of work through documents analysis (nanomaterial safety data sheets);
- estimate risk parameters in order to obtain an objective quantitative evaluation and subsequent benchmark analysis (this phase is not yet completely developed as the exposure assessment approaches for nanomaterials still need to be defined).

At the end of this step, the most suitable protection and prevention measures will be defined (see next paragraph). Once protection and prevention measures are identified, risk assessment must be repeated in order to verify whether an acceptable residual risk has been achieved.
Finally, risk assessment procedures and results must be documented.

6.3 Potential prevention and protection measures

According to the precautionary principle, it is necessary to minimize the exposure to NMs. This can be achieved by reducing the exposure durations and/or the number of people exposed and the concentrations of NMs.
To date, the control of airborne exposure to nanoparticles can most likely be accomplished using a wide variety of engineering control techniques similar to those used in reducing exposures to general aerosols (Ratherman S, 1996; Burton J, 1997). Furthermore, a correct installation and maintenance of engineering controls (e. g. exhaust ventilation systems) should be ensured at process locations where exposure might occur.
Elements of such a program should include the education and training of workers on the proper handling of NMs and correct use of the Personal Protection Devices (PPDs).
The foremost information prevention tool is represented by the nanomaterial safety data sheets where information and risks for human health and the environment associated with handling and use of NMs are reported.
According to the BSI (British Standards Institute, 2008), potential prevention and protection measures should be prioritized as follows:

1. Elimination of the nanomaterial

2. Substitution of the nanomaterial
3. Insulation/confinement or segregation of the source
4. Environmental protection (measures aimed to detect, limit and expel NMs)
5. Work organization
6. Individual protection (complementary use of IPDs and technical measures)

To date, the potential prevention and protection measures are:

a. Substitution:
   - of dusty preparations with others containing bound nanoparticles, thus preventing their diffusion (dispersions, pastes, granules, compounds, etc.);
   - of spray applications with others containing low aerosol concentrations (brush on or immersion techniques).

b. Source enclosure:
   - use of closed cycle machines;
   - automation - robots.

c. Environmental protection measures:
   - extractor fan;
   - workplace ventilation systems;
   - localized suction units;
   - alarm systems;
   - correct use of production systems;
   - exhaust air filtration (HEPA filters for recirculation of workroom air);
   - separation of the workroom and adaptation of ventilation system (small depression).

d. Security measures and work organization:
   - minimizing the exposure duration;
   - minimizing the number of people exposed;
   - limiting accesses to the workplace;
   - no smoking;
   - operating far from flames, heat sources and sparkles;
   - information/formation on hazards and protection measures;
   - labeling;
   - safety sheets;
   - warning signals;

- security services (emergency shower and eye wash, first-aid information on substances used in the workplace).

e. Hygienic and personal protection measures:
   - gloves;
   - masks and respirators;
   - correct use of laboratory equipments and instruments;
   - pro pipet fillers;
   - automatic pipets;
   - disposable materials;
   - containers for temporary storage of waste.

6.4 Examples of good practices

6.4.1 Risk assessment

This paragraph illustrates an example of a correct risk assessment approach, based on the "control banding" method (Giacobbe F et al, 2009).
The example can be applied to lab research and industrial production.
In addition to the preliminary identification of the adverse affects of NM exposure and the relevant risk sources, this example is made up of the following 10 factors:

A. numerousness of the exposed workers,
B. frequency of exposure,
C. frequency of direct manipulation,
D. dimensions of the nanoparticles,
E. nanoparticles behaviour (e.g. dispersion or agglomeration),
F. effectiveness of Personal Protection Devices (PPDs) used,
G. work organization/procedures,
H. toxicological characteristics of the substances,
I. risk of fire and explosion
J. suitability of workspaces and installations.

The aforesaid factors are denominated "factors level risk" and one of them may assume three increasing values: 1 (low), 2 (medium) and 3 (high), referred to as "risk levels". Since the use of nanomaterials presents nowadays unknowns about the effective level of danger, the risk assessment takes into consideration these important aspects through the help of an appropriate index denominated "corrective factor". Such index assumes a value within the range 0.5 and 2.0 in accordance to the established level of scientific knowledge. Particularly, it assumes the following values:

0.5 - good scientific knowledge; 1.0 - sufficient scientific knowledge; 2.0 - insufficient scientific knowledge.
The evaluation algorithm is:

$$\text{Evaluation Risk} = \sum_{i=A}^{j} \text{(Factor level risk)i} * \text{(Corrective factor)}$$

The evaluation algorithm covers normal work conditions as well as abnormal (e.g. breakdown air filter) and emergency situations (e.g. package cracking with uncontrolled spill of the product).
According to the evaluation outcome, risk levels may be subdivided into three increasing levels ("low", "middle" and "high").

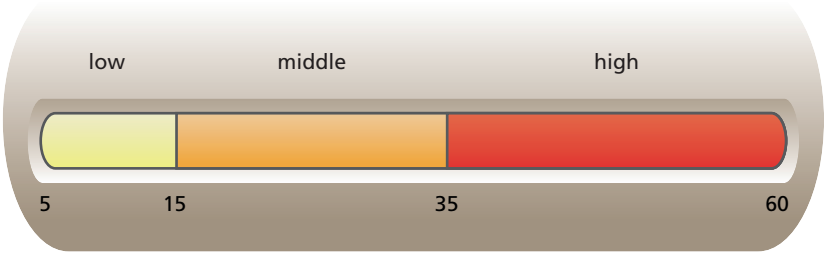

Figure 6.1 - Risk level are subdivided into: "low" (5 ÷ 15); "middle" (16 ÷ 35); "high" (36 ÷ 60).

The "high" level risk needs the activation of immediate interventions to reduce the final result of the evaluation at least to the "middle" level risk.
For the factor lever risk expressed in point G (work organization/procedures), good work practices are the training for laboratory personnel that must be informed of the risks associated with workplace hazards; the equipment cleaning/maintenance; use and maintenance of Personal Protection Devices (PPDs).
The evaluation model has been experimented within research laboratories handling nanomaterials for the realization of photovoltaic cells. Due to their electrical properties, both single-walled carbon nanotubes (SWCNTs) and metal oxide nanoparticles (TiO_2) were used.

The above nanomaterials have been purchased in single stocks of reduced dimension in weight (less than 10 g). The single products are equipped of material safety data sheet that report the information relating to the risks associated with handling and use. For the realization of photovoltaic cells, the researcher (worker) is not involved in the direct handling of the nanomaterials in solid state. These are initially scattered in water in order to reduce and control the density (SWCNTs have a density equal to about 1 g/cm^3). The nanodusts in TiO_2 are worked with solvents so that to obtain a cream paste. Every single working phase is developed avoiding the contact with skin and the breathing of aerial dispersion dusts. Researchers must wear the protection devices (gloves and masks) and carry out the manual operations using a dry box.

To date, specific procedures on how to regulate the premises entrances and potential emergency situations (e.g. uncontrolled spill of SWCNTs dust due to an accidental overturning of the package) have not been drawn up yet.

Table 6.1 - Factors level risk for factors specific (A ÷ J).

	Risk level Risk parameters	Low 1	Medium 2	High 3
A	Numerousness of the exposed workers	1÷2 units	3÷5 units	more than 6 units
B	Exposure frequency	< 2 h/day	> 2h/day and < 6h/day	> 6 h/day
C	Direct manipulation frequency	> 70 nm	> 10 nm and < 70 nm	< 10 nm
D	NM dimensions	high agglomeration tendency	medium agglomeration tendency	high dispersion tendency
E	NM behaviours	< 2 h/day	> 2 h/day and < 4 h/day	> 4 h/day
F	Effectiveness of the PPDs used	PPD used: - rubber gloves (hands) - safety glasses or goggles (eyes) - laboratory coats (skin) - mask with filter HEPA (respiratory)	Partial use of PPDs	No use of PPDs
G	Work organization/procedures	good work practices	simple and limited procedures	any procedure or free access to the workspaces
H	Toxicological characteristics of the substances	safety advice concerning dangerous substances and preparations: S28 (after contact with skin, wash immediately with water - to be specified by the manufacturer) S38 (in case of insufficient ventilation, wear suitable respiratory equipment)	safety advice concerning dangerous substances and preparations: S22 (do not breathe dust) S26 (in case of contact with eyes, rinse immediately with plenty of water and seek medical advice) S36 (wear suitable protective clothing) S37 (wear suitable gloves) S39 (wear eye/face protection)	risk phrases: R36 (irritating to eyes) R37 (irritating to respiratory system) R40 (limited evidence of a carcinogenic effect)
I	Risk of fire or explosion	No considered	Improbable	Probable
J	Suitability of workplaces and installations	≤ class 100 clean room use of chemical fume hood (nanomaterials in the gas or aerosol phase) use of dry box (dusty nanomaterials)	1000 ≤ class clean room ≤ 10000	10000 ≥ class clean room

Table 6.2

Event	Nanomaterial	State of particles	Conditions (*)	Exposure route	Work activity and/or event
a	SWCNTs	dry powder or liquid suspension	N	inhalation/dermal	water dispersion
b	SWCNTs	liquid suspension	N	dermal	deposition and fixing on plate
c	TiO_2	dry powder	A	inhalation/dermal	dry box partial failure of the air treatment system
d	TiO_2	aerosol dry powder	E	inhalation/dermal	uncontrolled spill due to an accidental overturning of the package

(*) N - normal • A - abnormal • E - emergency

Table 6.3

Event	A	B	C	D	E	F	G	H	I	J	Σ A ÷ B	Corrective factor (**)	Risk assessment	Risk level (***)
a	1	2	2	3	1	1	2	3	1	1	17	2	34	medium
b	1	2	2	3	1	1	2	3	1	1	17	2	34	medium
c	1	1	2	2	1	1	2	2	1	3	16	2	32	medium
d	1	1	3	2	1	1	3	2	1	3	18	2	36	high

(**) 0.5 - good scientific knowledge • 1 - sufficient scientific knowledge • 2 - insufficient scientific knowledge
(***) 5 ÷ 15 - "low" level risk • 16 ÷ 35 - "middle" level risk • 36 ÷ 60 - "high" level risk

6.4.2 Engineering measures

If potentials hazards cannot be eliminated or the substance substituted with a less hazardous material (which appears quite unlikely in the case of NMs with unique properties), appropriate engineering measures must be taken. In the case of fluid aerosols, control techniques such as the source enclosure (e.g. isolating the generation source from the worker) or local exhaust ventilation systems should be effective to capturing airborne nanoparticles, provided they are properly designed, installed and maintained according to the manufacturer's instructions. Dimensions of ventilation systems should be determined using good knowledge-based technique on aerosols generation, transport and capture recommended by the American Conference of Governmental Industrial Hygienists (ACGIH, 2011). These control techniques should be effective for controlling airborne exposures to nanometer-scale particles (Sainfeld JA and Pandis SN, 1998). It is worth noting that, during processing, some

types of NMs may form agglomerates that are difficult to disperse in the workplace. In this case, the protection measures must be taken during material sampling and equipment cleaning/maintenance. Carbon black, titanium oxide, carbon nanotubes and metal oxide nanoparticles require total containment.

6.4.3 Dust collection efficiency of filters

According to the current state of knowledge, a properly designed exhaust ventilation system equipped with a High Efficiency Particulate Arresting (HEPA) should effectively remove nanoparticles (Hinds, 1999). The use of HEPA filters must be coupled to a well-designed filter housing. An improper use of the filter may lead to filter efficiencies which are much less than predicted. An unventilated enclosure which is effective in controlling the emission of larger particles may not be effective in controlling NMs due to their greater penetration ability.

6.4.4 Work Practices

The incorporation of good work practices in a risk management programme can help to minimize workers' exposure to nanomaterials. Examples of good practices include the following:

- cleaning work areas at the end of each work shift (at a minimum) using HEPA vacuum pickup and wet wiping methods. Dry sweeping or air hoses should not be used to clean work areas. Cleanup and disposal should be conducted in a manner that prevents worker contact;
- preventing the storage and consumption of food or beverages in workplaces where nanomaterials are handled;
- providing hand-washing facilities and encouraging workers to use them before eating, smoking or leaving the worksite;
- providing facilities for showering and changing clothes to prevent the inadvertent contamination of other areas (including take-home) caused by the transfer of nanoparticles on clothing and skin.

6.4.5 Cleanup of nanomaterial spills

No specific guidance is currently available on cleaning up nanomaterial spills; in any case, the recommendations put forward by the pharmaceutical industry on treatment and cleaning up of pharmaceutical residuals could be applied in the workplaces using NMs (wood JP, 2001).

Until relevant information is available, it would be prudent to base strategies for dealing with spills on current good practices, together with available information on exposure risks (safety sheets).
Standard approaches to cleaning up powder and liquid spills include using HEPA filters, wetting powders down, using dampened cloths to wipe up powders and applying absorbent materials.
When developing procedures for cleaning up nanomaterial spills, consideration should be given to the potential for exposure during cleanup. Inhalation and dermal exposure will likely pose the greatest risks. Consideration will therefore need to be given to appropriate levels of personal protective equipment.

6.4.6 Personal protective clothing

Currently, no guidelines are available on the selection of clothing or other apparel for the prevention of dermal exposure to nanoparticles (overalls, gloves and protective clothing). A research has shown that penetration efficiencies for 8 widely different fabrics ranging from 0.0 % to 31%, with an average of 12% (Shalev et al. 2000). Even though little is known on the efficiency of clothing in protecting against NM exposure, due to the paucity of data available, it is worth noting that, despite the ability of NMs to penetrate the skin, very few studies have demonstrated their adverse effects on human health.
Recent investigations have shown that, based on conventional occupational hygiene practices, approximately 84% of employers encourage exposed workers to wear protective clothing (ICON, 2006). This practice is particularly recommended in research labs (US DOE, 2007) and regulatory guides (ASTM, 2007).
However, even for powders in the macro scale, it is recognized that skin protective equipment is very limited in its effectiveness to reduce or control dermal exposure (Schneider et al. 2000).
In any case, although nanoparticles may penetrate the epidermis, there has been little work to suggest that penetration leads to disease.
Existing clothing standards already incorporate testing with nanometer-sized particles and therefore provide some indication of the effectiveness of protective clothing with regard to nanoparticles. For instance, ASTM standard F1671-03 specifies the use of a 27 nm bacteriophage to evaluate the resistance of materials used in protective clothing to penetration by bloodborne pathogens (ASTM, 2003).

6.4.7 Respirators

Respirators may be necessary when engineering and administrative controls do not

adequately keep worker exposures to an airborne contaminant below a regulatory limit or an internal control target. Currently, there are no specific exposure limits for airborne exposures to engineered nanoparticles although occupational exposure limits (e.g. OSHA PELs - Permissible Exposure Limits; NIOSH RELs - Recommended Exposure Limits; , ACGIH TLVs - Threshold Limit Values) exist for larger particles of similar chemical composition. Scientific evidence indicates that nanoparticles may be more biologically reactive than larger particles of similar chemical composition and thus may pose a greater health risk when inhaled.

The decision to institute respiratory protection is recommended after the outcomes of the risk assessment and the following implementation of prevention and protection measures. Hence, the use of respiratory protection is required if, despite risk reducing interventions, the worker exposure remains elevated.

To date, different guides on how to select the appropriate respirators are available (NIOSH, 2004).

In any case, respirators must not hinder the worker's activity and create further exposure conditions. The decision to institute respiratory protection, as a consequence, should include:

1. an evaluation of the worker's ability to perform the work while wearing a respirator;
2. regular training of personnel;
3. periodic environmental monitoring;
4. respirator fit testing;
5. respirator inspection, cleaning and maintenance.

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chapter 7

Prospects for policies and communication strategies

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7.1 Regulatory framework for nanotechnology development

7.1.1 introduction

The strategic relevance of nanotechnologies is nowadays widely acknowledged and the commitment from both industrialized and developing countries has increased constantly. In addition, the expectations about the benefits of these technologies are great, even though a widespread opinion is that such benefits can be fully realized only if their development is responsible and minimizes potential risks related to their use.
There are concerns about the effects on Environment, Health and Safety (EHS) as well as on the ethical, legal and social issues (ELSI) that nanoscience and nanotechnologies (N&N)[1] and their applications might have. The attention paid to these issues is higher and higher as the amount of nanotechnology-derived products on the market is constantly increasing. Nowadays, European Commission, national governments, entities and organizations devoted to establish legislations and regulations consider it top priority to define a regulatory framework to ensure a safe and responsible development of nanotechnologies. At present, more attention is paid to the so-called engineered nanomaterials (*Engineered NanoMaterials - ENMs*), i.e. intentionally produced, "free engineered" nanomaterials and to their effects on Environment, Health and Safety (EHS)[2].

[1] N&N is henceforth referred to nanoscience and nanotechnologies in their widest meaning, including nanomaterials and nano-related products

[2] Nanoparticles and nanomaterials produced by natural processes are generally considered as ultrafine particles and have been under investigation for a long time. Engineered nanomaterials which are not aerodispersed but are part of macroscopic materials have usually proven not to directly interact with biological systems, at least during their use, thus raising much less concern.

The ethical, legal and social issues (ELSI), although relevant, are currently less urgent. However, in the medium to long term, when the most revolutionary nanotechnology applications become true, these issues will be of great interest and adequate attention will be required to address them appropriately.

N&Ns are usually regulated by current legislations and regulations. At the institutional level, stakeholders are deeply debating about the adequacy of such approach, due to:

- the wide variety of materials and applications;
- typical characteristics and behaviours of nanomaterials at the nano-scale;
- lack of characterization data on nanomaterials;
- lack of technical regulations on nomenclature, metrology and materials;
- proprietary nature of information;
- multidisciplinarity and difficult communications among different disciplines.

To overcome such difficulties, strictly interconnecting instruments have been identified with a view to involving N&N stakeholders. These are:

- Knowledge development. Different countries are more and more engaged in research on Environment, Health and Safety (EHS) risks and, to a less extent, in the ethical, legal and social issues (ELSI) associated with nanotechnologies (see details in Chapter 3).
- Legislation ("hard regulation"). National and international authorities and institutions, in particular at the European level, but also US countries, such as Australia and Canada, have started to promote *ad hoc* studies, create working groups and develop technical abilities to ascertain the applicability of the current regulatory framework on nanomaterials.
- Voluntary measures ("self regulation"). In order to support and help the implementation of the existing regulation with a view to minimizing risks, Governmental institutions, industries and stakeholders have developed different self regulations such as codes of conducts (CoCs), good practices and methods for the risk management and nanomaterials research programmes (reporting schemes/stewardship programs).
- Standards (technical rules) and international cooperation. The main international/regional organisations for standardisation have launched initiatives on N&Ns. Some supranational organisations (in particular OECD) promote, with specific activities, the harmonization, knowledge-sharing and coordination at the international level on N&N safety and regulatory issues.

The lack of standard protocols in the measurement and characterization of nanomaterials as well as risk and exposure levels assessment pose the main challenge to the creation of a regulatory framework in this field. These gaps (sometimes) make it difficult to implement and improve the existing regulations and, at the same time, hin-

der the definition of new regulatory/control mechanisms. Although much has been put in place in the last few years, the complexity and multidisciplinarity of these technologies take a long time to develop universally accepted and shared methods.
To date, the first goal to achieve is the definition of some major building blocks for ENM risk assessment which provide a classification of nanomaterials according to their typology, properties and relevant characterization parameters and potential risks related (at least) to the most used ENMs (or to those with the highest potential level of exposure).
The following paragraphs provide a brief overview as context for the regulatory framework, self regulations, technical rules and international cooperation.

7.1.2 Legislation ("hard regulation")

As just mentioned, most of countries involved in the N&Ns have set out studies and investigations to verify the applicability of the existing regulations to such technologies. Some priorities and measures have been identified in order to enhance the effectiveness of existing regulatory schemes; in most cases, the need to increase commitment to EHS research has been also highlighted with a view to overcoming current limits of scientific knowledge about N&Ns and their characterizations.
Specific regulatory measures have been taken as amendments or modifications to the technical specifications of the in force legislation.
It is useless to say that this is a complex and relatively long activity, considering that, due to the multidisciplinarity of nanotechnologies, there is a very wide range of legislations and directives covering the various N&N applications all along the product life cycle (over 90 community legislative, regulatory instruments having special relevance for N&Ns have been estimated) (European Economic and Social Committee, 2008).
European Commission (EC) technical committees and agencies are now active in this field. In June 2008, the outcomes of various initiatives have been collected in the "*Regulatory Aspects of Nanomaterials*" review (European Commission, 2008a).
The main conclusion of the review is that existing regulation may be applied to N&N, but all supporting instruments (technical specifications, guidelines, etc.) must be improved in order to ensure their implementation and adequacy.
Following a review published by the "Committee on the Environment, Public Health and Food Safety", the EU Parliament (April 2009) (Schlyter C, 2009) approved a resolution which appears to be in partial contrast to the view of the Commission.
The (non-binding) document asks the EC to review the existing framework (by 2011) and makes sure the application of the "*no data, no market*" principle. It is too early to know which modifications will be accepted following the resolution, also consid-

ering that sometimes this principle is substantially already included in the existing framework. Among interventions are the review of the current substance registration and identification methods (i.e. REACH and some occupational safety and environmental directives) and the drawing up of an inventory of the different types and uses of nanomaterials on the European market, before June 2011.
Hence, an intensification of initiatives (detailly described below) aimed at monitoring the use of ENMs is expected, including binding measures.
Even though member states tend to follow the EC instructions in terms of regulation, some countries have put in place specific initiatives in this area.
France, Germany, the Netherlands, England, Austria, some Scandinavian regions and **Switzerland** are institutionally engaged to explore EHS issues and give special attention to N&Ns with the support of regulatory agencies and auditors from different application sectors. At international level, **United States, Canada** and **Australia** are among the most committed countries in this field. These nations clearly address EHS and regulation issues in strategies and policies to promote national nanotechnology development and the interested institutional bodies are becoming proactive (e.g through working groups) in coping with them.
Canada and **Australia** have explicitly expressed the need for a precautionary (although not completely binding) approach to the manufacturing and use of nanomaterials. It is worth noting that the precautionary principle is part and parcel of the REACH directive for chemical substances, even if its implementation is still widely debated. Currently, main attention is paid to the regulatory framework concerning the following nanotechnology application sectors:

- Chemistry and Materials
- Cosmetics
- Food
- Occupational Health and Safety
- Environmental Safety
- Medical and Pharmaceutical Instrumentation

While the existing framework appears sufficiently adequate for some sectors like medicine or pharmacy industry, legislation concerning other fields like cosmetics and food is still appropriate.
Within the framework of the N&N, much attention has been given to the **regulation of chemicals and materials**. Various agencies (mainly in Europe, United States, Canada and Australia) have included indications for nanomaterials in their technical specifications with a view to monitoring the marketing of such substances.
The REACH ("*Registration, Evaluation, Authorisation and Restriction of Chemicals*") legislation which regulates production, use and marketing of chemicals in Europe is

one of the most adequate and restrictive legislation in this field; though, many issues still remain open, such as the use of mass thresholds levels or the exemption of some materials (resulting in the exemption of the same material at nano-scale) (European Commission, 2008b).

In particular, specific measures have been already put in place for nanomaterials. As an example, in order (*inter alia*) to adequately control the use of carbon nanotubes, carbon- and graphite-based products have been excluded from the list of substances exempt from registration under REACH[3].

In March 2008, the *European chemicals Agency* (ECHA) established a subgroup on the enhancement of the applicability of REACH to nanomaterials (*Competent Authorities Sub Group on Nanomaterials - CASG Nano*[4]). In the United States, nanomaterials are regulated under the *Toxic Substances Control Act* (TSCA) which is main legislative instrument of the *Environmental Protection Agency* (EPA). This statute is the equivalent to the European REACH (EPA, 2007; EPA, 2008); however it is worth noting that these two instruments are deeply different.

In Europe, the manufacturer has the obligation to demonstrate that the chemicals are safe before placing it in the market (under REACH); conversely, in the U.S. the regulator has the responsibility to demonstrate that the chemicals adversely affect human health or the environment, prior to limit its use or remove it from the market (under EPA-TSCA). Shared approaches or, at least, clear agreements are key to prevent obstacles and misunderstandings on the marketing of nanomaterials (this issue, still under debate, is valid also for chemicals)[5].

The limits of the existing **cosmetics** and **foods** regulations have recently called for changes to the European directives on these fields[6] [7]. Under both regulations, the definition of engineered (insoluble) nanomaterials and specific requirements regarding risk assessment approaches for all products containing nanomaterials have been included in the legislation.

With regard to **safety at work**, the commitment was mostly centred upon the assessment and adaptation of existing risk management practices and upon the development of appropriate guidelines for handling and disposal of ENMs. Among

[3] http://chemicalwatch.com/788

[4] http://ec.europa.eu/environment/chemicals/nanotech/index.htm

[5] The development of a shared and coherent regulatory framework both at the European and American level has been addressed in a joint project whose final report is now available (Breggin L et al, 2009).

[6] http://www.europarl.europa.eu/news/expert/infopress_page/067-52498-082-03-13-911-20090324IPR52497-23-03-2009-2009-false/default_en.htm and http://www.euractiv.com/en/science/meps-back-tougher-rules-nanotechnology/article-181695

[7] http://www.europarl.europa.eu/news/expert/infopress_page/066-52333-082-03-13-911-20090323IPR52331-23-03-2009-2009-true/default_en.htm

institutions which have made substantial contributions in this area (NIOSH, 2009a; BAuA, 2007; FIOH, 2008; Ostiguy C et al, 2009; AFFSET, 2008) are:

- *National Institute for Occupational Safety and Health* (NIOSH), United States;
- *Federal Institute for Occupational Health and Safety* (BAuA), Germany;
- *Federal Office of Public Health* (FOPH), Switzerland;
- *Institut de recherche Robert-Sauvé en santé et en sécurité du travail* (IRSST), Canada;
- *Agence Française de sécurité sanitaire de l'environnement et du travail (AFFSET),* France.

A review of this issue has been recently issued by the *European Agency for Safety and Health at Work* (EU-OSHA, 2008).

It is clear that the lack of adequate ENM measurement and monitoring instruments and the paucity of detailed information concerning risks and levels of exposure makes it difficult to provide exhausting data in this filed.

As already stated, the regulatory framework for medical devices and pharmaceutical products has proved to be adequate even if associated with nanotechnologies, due to the sufficient accuracy of the authorisation procedures. Yet, many technical analyses suggest that a "case-by-case" approach should be adopted to evaluate the authorisation procedures for these products and consider the peculiar characteristics of nanomaterials.

As is the case for European legislations, the classification of some nano-related medical devices having functions and characteristics of medical devices, drugs and biomaterials is a critical issue.

In conclusion, as evidenced by the above mentioned data, the monitoring and regulation of N&Ns all along their life cycle still need to be defined and further information on the main critical issues in this area is envisaged. In particular, efforts should be devoted to:

The lack of **validated and standardized methodology and tools for measuring, monitoring and evaluating the potential (eco) toxicity of ENMs** is the main challenge to both determine the real levels of exposure and set out threshold values, like NOEL (no observed effect levels) and OEL (occupational exposure limits). This makes the definition of adequate regulatory technical specifications more complex.

Threshold levels based on mass (or concentration), used by different legislation, are very likely not adequate for the identification and evaluation of ENMs. This hampers the registration, control and implementation of specific procedures in relation to the regulated product or material.

The definition of a substance or product provided by a regulatory scheme may not be sufficiently detailed to **properly distinguish the nanomaterial from the same**

material in a macro-form. ENMs, as a result, is subjected to the same specifications as the macromaterial (although the different characteristics and properties). This may influence the ability to regulate and control the introduction and use of nanomaterials into the market.

7.1.3 Voluntary measures ("Self regulation")

Knowledge gaps and the lack of a specific regulatory framework led to the definition of voluntary measures to provide common references, principles and guidance and help increase the level of trust and confidence among stakeholders. Three different intervention levels can be identified and some reference example are also reported (Mantovani et al, 2009):

- Monitoring systems/database (knowledge gathering)
  - Nano-scale Materials Stewardship Program (NMSP) - EPA, USA (first period: 2007-2010, second period: on-going)
  - Voluntary Stewardship Programs (VSP) - DEFRA, UK (2006-2008)
- Codes of conduct (increase in the level of trust)
  - EC CoCs on responsible research (February 2008, reviewed every 2 years)
  - Organizational or business association codes of conduct: BASF Europa, IG-DHS- "Retailers" Association , Switzerland (2007-2008)
- Risk Management Systems (increased safety levels)
  - NanoRisk Framework - DuPont, Environmental Defense, USA, BUHLER, Switzerland (2007-2009)
  - CENARIOS - TÜV SÜD, Innovation Society, Switzerland (2008)
  - Responsible Production and Use of Nanomaterials - German Chemical Industry Association - VCI, Germany (2008)

The monitoring systems of ENMs for industries and entities which use, manufacture, process or import these materials aim to increase regulators' knowledge about the extent of the use of nanomaterials and about different types and manufacturing methods used at the industrial level, in order to facilitate the application of regulatory measures for a responsible development of nanotechnologies.
Reporting has been conducted with respect to regulatory schemes on materials and chemicals and required specification and characterization on materials, production volumes, risk management and assessment, etc. Participation in the initiatives launched from 2007 to 2009 was scarce and their effectiveness was so reduced that in some countries, such as Canada and France, the adoption of mandatory reporting schemes is under consideration.
Codes of conduct aim to define values, principles and practices for a safe and re-

sponsible development of nanotechnologies. The main purpose, however, is to provide a common reference and increase the level of trust and confidence among stakeholders. Although they generally have a non-binding character, there could be a degree of liability related to the subscription to such documents.
The European Code of Conduct (February 2008) (European commission, 2008c) is based on seven principles, comprising *precaution, inclusiveness and sustainability* and provides a series of guidelines on actions, priorities, prohibition, restrictions or limitations to assure the safe development of nanotechnology. All Member States have been formally asked to adopt it and to report to the EC the state of implementation and any updates/modifications proposals.
Risk management, accreditation and certification systems are generally adopted at the industrial level to increase the level of safety in relation to the manufacturing, production and use of nanotechnologies. They provide guidelines and best practices in risk management and EHS issues They do not have a regulatory role, and, as in the case of accreditation, can work similarly to product quality certification systems (even if with a lesser degree due to the lack of internationally validated standards).
Of particular interest are the results of a study on occupational safety conducted in Germany and Switzerland.
In 2006, the VCI (representing over 90% of the entire German chemical industry), based on the results of the survey conducted in the industry, with the "*German Federal Institute for Occupational Safety and Health*", published in 2007 the *"Guidance for Handling and Use of Nanomaterials at the Workplace"* (BauA) and, in March 2008, the more general report *"Responsible Production and Use of Nanomaterials"* (VCI).
Analogously, in Switzerland, a recent initiative involving institutions, organisations and research centres led in 2009 to the publication of the first version of the "*Precautionary Matrix for Synthetic Nanomaterials*" (Schweizerische Eidgenossenschaft, 2008) which provides a very practical and interesting structured method to assess risks associated to ENMs at the industrial level.
Self-regulation initiatives have an important role in coping with current uncertainties about the regulatory scenario of nanotechnologies. As for institutional initiatives, in particular, one of the main critical issues is represented by their voluntary nature which is usually coupled with a scarce involvement, thus undermining its effectiveness.

7.1.4 Standards (technical standards) and international cooperation

As previously pointed out, the need for appropriate standards to name, describe, specify, measure and characterise nanomaterials is also well recognised and is actively pursued to ensure the implementation of a regulatory framework for nanotechnologies.

Currently, it is the International Standards Organization (ISO) Technical Committee (TC) 229 "*ISO TC 229: Nanotechnologies*", in conjunction with the International Electrotechnical Commission (IEC) TC 113 "IEC TC 113 - *Nanotechnology for electrical and electronic products and systems*", that is the most relevant authority for the development of technical standards in these specific fields.
National bodies of more than 32 countries are members of the Nanotechnology Technical Committees of ISO and IEC, and specific national committees for nanotechnology have been established in most of these countries. The most active national bodies devoted to this field are the BSI/NT1 set up in UK, SAC/TC279 in China and ANSI-NSP in the US.
Several other "liaisons" have been established within ISO Technical Committees dealing with issues related to N&Ns such as ISO TC 201 (*surface and chemical analysis*), ISO TC 202 (*micro-beam analysis*), ISO TC 94 (*personal safety, protective clothing and equipments*) and ISO TC 213 (*biological evaluation of medical devises*)[8].
CEN, CENELEC and ETSI were given a specific mandate by the European Commission on N&Ns (European commission, 2007a). In particular, CEN TC 352, in conjunction with ISO TC 229, is developing standards devoted to nanotechnologies.
At international level, some *Standard Developing Organisations* such as ASTM (with TC ASTM E56 Committee) and IEEE (*International Electrical and Electronics Engineers*) are particularly active in this field.
Within ISO TC 229, the wide spectrum of issues to be faced for the definition of a regulatory framework for nanotechnologies has been organised in 4 working groups (WGs) (ISO, 2007):

- J-WG 1 - Terminology and Nomenclature
- J-WG 2 - Measurement and Characterization
- WG 3 - Health, Safety and Environment
- WG 4 - Material specification

JWG 1 and JWG 2 are joint ISO/IEC working groups.
IEC TC 113 has also established a third WG (not linked to ISO) specifically devoted to the assessment of performances of components and electrical systems. ISO TC 229 is the UNI (Italian National Unification Body) Technical Committee while the Italian Electrotechnical Committee (CEI) has established the CEI TC 113, analogous to the work of IEC.

[8] For a complete list of "liaisons" implemented by ISO TC 229, visit http://www.iso.org/iso/standards_development/technical_committees/list_of_iso_technical_committees/iso_technical_committee.htm?commid=381983.

The work is intense and concrete results have already been obtained. In 2008, two documents were created:

- ISO/TS27687: Terminology and definitions for nano-objects - Nanoparticle, nanofibre and nanoplates (*technical specification providing terms and definitions concerning nanoparticles*);
- ISO/TR 12885: Health and safety practices in occupational settings relevant to nanotechnologies (*technical report to prevent adverse effects on human health and safety during production, handling, use and disposal of engineered nanoparticles*).

At present, more than 30 standards documents related to the above themes are under development, but due to the lengthy process, it will take some time before the matter is thoroughly addressed[9].

At international level, from 2006 the OECD coordination efforts led to the creation of two working groups devoted to nanotechnologies with a view to promoting international cooperation in the following sectors[10]:

- Responsible research, development and commercialisation of nanotechnologies (*Working Party on Manufactured Nanomaterials - OECD WPMN)*
- Implications of ENMs in EHS issues, development of compelling systems for evaluating the safety of ENMs (*Working Party on Manufactured Nanomaterials - OECD WPMN).*

30 OECD Member Countries, the European Commission, non-members (Brazil, China, Singapore, Thailand, Russia), ISO, WHO, UNEP and other relevant stakeholders participate in the activities of the two WGs.

Among other initiatives, the OECD WPMN has launched a "sponsorship programme" for the assessment and testing of a (representative) list of ENMs. As outcome of the sponsorship programme (launched in 2007), the compilation of a list of 14 nanomaterials for testing (based on materials which are on or close to the market) as well as a list of 61 endpoints for which they should be tested are envisaged[11]. The results of this activity have not been published yet.

In March 2009, a database was created on the initiatives and research projects launched at international level on EHS issues and is now available on the OECD website[12].

[9] For all regulatory references, visit http://www.iso.org/iso/iso_catalogue/catalogue_tc/catalogue_tc_browse.htm?commid=381983&development=on

[10] http://www.oecd.org/department/0,3355,en_2649_37015404_1_1_1_1_1,00.html

[11] Nanomaterials indicated by OECD WPMN are: Silver nanoparticles, Iron nanoparticles, Carbon black, Titanium dioxide Aluminium oxide, Cerium oxide, Zinc oxide, Silicon dioxide, Polystyrene, Dendrimers, Nanoclays (OECD, 2007)

[12] http://www.webnet.oecd.org/NanoMaterials/Pagelet/Front/Default.aspx

7.1.5 Conclusions

As confirmed by data provided, the contribution and the commitment for a responsible development of nanotechnologies involve the most active countries in this sphere, all convinced that they represent an essential tool for their success. This need is shared by all relevant stakeholders, including the industry, who understand that benefits and the commercial success of nanotechnologies may be realised only minimising the risks potentially associated with them. The availability of appropriate regulatory schemes, assuring the safe and proper use of nano-related products, without limiting their development, is essential.
Current regulatory framework and voluntary measures, accompanied with vigilant and proactive attitudes, may provide a temporary solutions; however, the demand to build a specific regulatory scheme which overcomes current limits is mounting. The way is still long to go and it is fundamental to bear in mind that "nanoregulation" requires a dynamic approach: it must adapt to the evolution of the scientific knowledge, to the increase of applications, to the concern and attitude of current and potential stakeholders. A continuous commitment, collaboration and open dialogue are key elements of the regulatory process.

7.2 Nanotechnology and the insurance industry

Nanotechnology is part and parcel of everyday's life and is spearheading developments in many productive sectors. Nano-related risks, however, are still raising big concerns. Some private risk management insurance companies are providing tools to address these concerns from evaluating and underwriting nanorisks to taking risk management ideas to the nanotechnology community.
In Zurich, an insurance company has developed a web-based software product tailored toward users of nanotechnologies for the identification of potential risk levels. Another company, Lexington Insurance Company, has launched a specific policy covering general and products liability for "nano" clients, especially those who may need special assistance in gauging the level of risk management in the field.
Other companies, such as Lloyd's, note insurers could totally exclude coverage for nanotechnology businesses. This is mainly due to the results of recent scientific works observing that carbon nanotubes can produce effects very similar to those of asbestos (Kim H, 2010).
From an economic point of view, the stakes are very high. As already stated in previous chapters, Lux Research has recently carried out a comprehensive survey in nanotechnology businesses and estimated that nanotechnology market is forecasted to

grow to 3.100 billion US Dollars by 2015, and approximately 10 billion new job will be created. According to the European Commission, the global revenue for nanotechnologies, currently estimated at 100 billion Euros, is forecasted to grow to 1.000 billion Euros and employ approximately 2 billion people (Castellet y Ballarà G and Marconi A, 2008; Castellet y Ballarà, 2009).

7.2.1 The role of Public insurance

From more than one decade, INAIL - National Workers' Compensation Authority - has been playing an important social role in protecting workers against labour accidents and work-related diseases, and besides performing its ordinary functions of workers compensation authority, it is committed to providing a global protection system. With this respect, the 1999-2000 INAIL Health Plan reflects this evolution: it outlines not only the provision of health and economic benefits, but also deals with medical treatment, rehabilitation, reintegration and prosthesization of victims of physical damage consequent to a work related accident or professional disease.
According to comments contained in a report by Senator Carlo Smuraglia with respect to occupational medicine, the Health Plan stressed the importance of gaining more knowledge about occupational diseases in terms of compensation and protection, thereby enhancing the epidemiological analysis of "forgotten" professional diseases.
The Leg. Decree 38/2000[13] and the Leg. Decree No. 81 of 9 April 2008, subsequently modified by the Leg. Decree of 3 August 2009 n. 106 and by Law n. 122[14] of 30 July 2010, have broaden the scope of INAIL's competences.
Due to the extensive changes undergone in the regulatory and institutional framework, some remarks concerning occupational diseases must be made.
The doctrinal framework defines the occupational diseases as "any morbidity that can be correlated with the performance of work activity" (A. Fiori): current INAIL social-insurance coverage is based on the so-called "mixed system" that covers any work-related morbidity. Paragraph 4 of article 10 of the Leg. Decree 38/2000, as provided in the sentence n. 179 of 1988 of the Constitutional Court, states that "extension of protection must be provided to any other disease that could be proved to have been caused by a working activity".
Art. 10 of the Leg. Decree 38/2000, confirming the validity of art. 139 of the Decree of the President of the Italian Republic No. 1124/1965[15] on the obligation of any

[13] Leg. Decree No. 38/2000 on insurance against employment injury and occupational diseases, pursuant to art. 55, par. 1 of the Law No. 144 of 17 May 1999.
[14] Leg. Decree. No. 81/2008 and subsequent integrations and modifications on occupational health and safety.
[15] Decree of the President of the Italian Republic No. 1124/1965 on mandatory insurance against employment injury and occupational diseases.

physician to detect and report any professional disease provided in a specific list, stated that also "probable and potential work-related diseases" must be included in such list and a National Registry of work-related diseases has been recently instituted in the INAIL's database.
Through the creation of this Registry, INAIL provides key services to any public entities involved in occupational health and safety protection; it represents a national observatory providing all information concerning diseases included in the list provided in art. No. 139 of the Decree of the President of the Italian Republic No. 1124/1965. Following the approval of the Ministerial Decree of 11 December 2009, this list has been broken into three sub-lists: List I (diseases that are very likely to be work-related); List II (diseases that are poorly correlated with the job activity: scarce or no knowledge exists about such diseases and they cannot be included in List I); List III (diseases that are likely to be work-related; probability cannot be calculated due to scarce or imprecise scientific evidence).
The National Registry of work-related diseases is an essential tool to collect data in terms of prevention, health surveillance, epidemiology and insurance coverage.
Moreover, INAIL has drawn up a LIST IV that includes all notifications of diseases that the doctor may have decided to report, pursuant to the art. No. 139 of the Decree of the President of the Italian Republic No. 1124/1965, but they are not expressly provided for in the three above mentioned lists.
The evidence of the so-called "forgotten" diseases is so guaranteed.
Under art. 9 of Leg. Decree No. 81 of 9 April 2008, subsequently modified by the Leg. Decree No. 106 of 3 August 2009 provides the definition of Public Entities involved in occupational health and safety and states that INAIL - which, under art. 8, is responsible for the technical management of a national information system for prevention (SINP) - "actively participates in investigations and research activities on professional injuries and diseases, in cooperation with the Ministry of Labour, Health and Social Policies and ISPESL".
The coordinated activities of Entities and Public Organisms, under Law No. 122 of 30 July 2010, is certainly leading to the enhancement of all those competences, also in the field of scientific research and investigations for the prevention of professional injuries and diseases, already attributed to ISPESL under art. 9.
In particular, the future exposure scenario for nanomaterials will be characterized by the following points:

- an increasing number of people will be exposed;
- the occupational exposure is one of the main concerns;
- the potential harmful effects do appear until long after the exposure, thus making it more difficult to reconstruct the exposure history, to assess the causal efficiency of pathogens and the complex interaction of different risk factors.

Provided that relevant scientific literature must be taken into serious consideration in order to provide a correct judicial orientation, in the absence of information all stakeholders should take adequate risk management measures (Allianz - OECD, 2007).
Attention must be paid to the management of nano-related risks both in the field of insurance and prevention.
To ensure an adequate nano risk management, it is necessary:

- to develop a National Governmental Plan that guarantees sufficient funding to endorse independent research on nano-related risks:
- to guarantee transparency to get free access to research findings;
- to promote a continuous dialogue between risk assessors and industry;
- to develop a nomenclature for international regulatory schemes;
- to create an adequate risk regulatory framework;
- to develop a global risk governance approach.

7.2.2 Conclusions

With a dramatic increase of manufacture and use of nanomaterials and a growing number of workers who are potentially being exposed to them, a number of regulatory tools are available today to enable Welfare officers to regularly and rigorously evaluate the health effects of nanoparticle exposure.
The applications of nanotechnology have "gone beyond" safety and health research issues and represent today an emerging risk which requires new preventive approaches in facing it.
Drawing up a risk management schedule which takes into account all potential risks for workers, users and environment is the main challenge.
The current identification and implementation of control strategies may have relevant implications for future workers' health protection.

7.3 Need for the development of the Risk Communication

Risk communication must be seen as an interactive process that enables the information exchange among people, groups and institutions and that implies the transfer of multiple messages concerning the assessment and management of risks. Risk communication to stakeholders may be realised only through a detailed and comprehensive risk assessment and characterization carried out in accordance with a specific setting.
Risk acceptance relies on the trust in the risk management capabilities rather than

on quantitative risk analysis. The study of concrete and better known conditions compared to those related to nanotechnologies shows that there are three four major risk communication difficulties and they relate to the message, source, channel and receivers.

Message problems relate to uncertainties about risk assessment processes (due to the paucity of certain scientific data) and risk analysis which are excessively technical and cannot be understood by the lay public.

Source problems consist in lack of trust in the source (scarce credibility), disagreement among scientific experts (confusion), the objective limitations of responsible authorities or resources, the lack of reassuring data (insufficient knowledge), inability to explain the limits of the risk assessment and subsequent uncertainties and, finally, the use of bureaucratic, legalistic, and technical language. Channel problems can include biased media reporting which emphasizes critical issues, inefficiencies, disagreements, conflicts of interest, premature disclosures of scientific information, over simplifications and inaccuracies in interpretation.

Receiver problems cover a host of items which include inaccurate perceptions of levels of risk (overconfidence in one's ability to avoid harm), lack of interest in the problem and its technical implications (probabilistic evaluation), overconfidence in the validity and accuracy of regulatory authorities and reluctance to make distinctions among different types of risk or among risks, cost and benefits.

Currently, the definition of tailored strategies for risk communication in this field nanotechnology is still premature. Knowledge shortages in characterization and harmfulness of nanomaterial exposures do not permit to identify occupational and environmental risks, but the time is ripe to bridge the gap between science and nanosafety issues.

By and large, risk communication, seen as the transfer of the acquired knowledge, is based on the public's own perceptions about risks. Lay public usually does not understand what nanotechnology really is, what makes nanomaterials so peculiar and which are the risks associated with their use. Nanotechnology processes and modes of action are largely unknown to observers, users and consumers. This may engender skepticism and mistrust, especially when a public debate is launched on their risks and this is exactly what is happening in Italy. As a result, a public and open dialogue with citizens and consumers is more than ever necessary in order to build an objective opinion on nanotechnologies and prevent unjustified concerns.

This dialogue should be bidirectional. Scientists, entrepreneurs and the public sector must understand the legitimate concerns on this issue among different categories of the population, including workers. At the same time, people should improve their knowledge about nanotechnologies and be more active in this area in order to pro-

vide a right dimension to nanotechnologies. A tight dialogue should be established with the industries involved. Analogously, relevant stakeholders should exploit their experiences for the exchange of scientific information, including toxicologic and ecotoxicologic data obtained in the research centres.
Groups should be identified according to their involvement and state of knowledge in the field of nanotechnologies. This could be made through the financing of *ad hoc* activities with the aim to make the audience familiar with experts' opinions, raise questions, unveil concerns and highlight needs for in-depth analysis. These approaches are based on more general criteria than those provided by communication studies.
Stakeholders may communicate different contents and act in different ways. The industry, as example, may communicate that the risk assessment is adequate and that everything is under control because all guidelines and operating procedures have been implemented. Small enterprises, notoriously reluctant to address safety issues, may conclude that the risk assessment process is too expensive and, therefore, decide not to perform it, while awaiting new regulations or decrees. Authorities usually focus on regulatory and risk management issues and call for further legislative precautions. Insurance companies tend to improve the dialogue about risks and warn people against them. Communication channels hunt for sensational news and mislead the public oscillating between the magic and demonization of (nano)technology.
Finally, researchers, who are expected to be clear-headed and independent, ask more funding to deepen knowledge about those issues that still remain uncertain.

7.3.1 EU Risk communication policy

The communication of risks associated with the development of nanotechnologies is part and parcel of the EU policy devoted to the promotion of an integrated, safe a socially acceptable approach to the development and use of nanoscience and nanotechnology. The main objective is to provide adequate information aimed to properly affect attitudes, promote the social dialogue and commitment.
The European Community has put in place a strategy on health and safety at work (European Commission, 2007b), even though such development may create new risks. Commission underlines the need for an integrated technological development with research activities (European Commission, 2004). In particular, the Commission has also highlighted the need to deal with safety issues, either real or perceived, at a very early stage; to favour the integration between health, environment and risks issues and research and development; to sustain the production of toxicologic and ecotoxicologic data, preferably based on dose-response or quantitative structure ac-

tivity relationships (QSARs), the latter being a helpful tool when estimating impacts of nanotechnologies on human health and environment.
In this scenario, document elements such as consensus documents, reports, technical manuals and reviews concerning nanosafety are also provided (OECD, 2007)[16].
Communication is a key element for the EU development strategy: sustainability, safety and health would be empty words without the implementation of a risk communication policy among stakeholders in the potential exposure scenarios. In this phase still marked by knowledge gaps on risks and assessment processes, communication is widely used by media to provide sensational information scarcely based on scientific observations: the need for an adequate communication strategy is, therefore, as great as ever.
As early as 2005, the European Commission adopted the "Nanoscience and nanotechnologies: an Action Plan for Europe 2005-2009" resolution (European Commission, 2005). This Plan of Action outlines a series of articulated and interconnected activities to immediately put in place a safe, integrated and responsible approach to nanoscience and nanotechnology. The key objective of the Action Plan is to ensure that risk assessment related to human health, environment, consumers and workers is responsibly integrated at all stages of the life cycle of the technology, starting at the point of conception, R&D, manufacturing, distribution, use and disposal or recycling (European Commission, 2008d).
The European Commission issued a code of conduct to ensure a safe and socially acceptable nanotetchnology development including seven guiding principles (European Commission, 2008e):

a) Meaning: N&N research activities should be comprehensible to the public. They should respect fundamental rights and be conducted in the interest of the well-being of individuals and society in their design, implementation, dissemination and use;
b) Sustainability: N&N research activities should be safe, ethical and contribute to sustainable development. They should not harm or threaten people, animals, plants or the environment, at present or in the future
c) Precaution: N&N research activities should be conducted in accordance with the precautionary principle, anticipating potential environmental, health and safety impacts of N&N outcomes and taking due precautions, proportional to the level of protection, while encouraging progress for the benefit of society and the environment;

[16] www.oecd/env/nanosafety

d) Inclusiveness: governance of N&N research activities should be guided by the principles of openness to all stakeholders, transparency and respect for the legitimate right of access to information. It should allow participation in the decision-making processes of all stakeholders involved in or concerned by N&N research activities;
e) Excellence: N&N research activities should meet the best scientific standards, including integrity of research and good laboratory practices;
f) Innovation: governance of N&N research activities should encourage maximum creativity, flexibility and planning ability for innovation and growth;
g) Accountability: researchers and research organisations should remain accountable for the social, environmental and human health impacts of their work.

These policy documents have been collected and synthesized by the Commission (European Commission, 2008f).
Analogously, an English Consortium (Responsible NanoCode, 2008) and a multinational company (BASF, 2008), as well as some countries (NNI, 2008; Schweizerische Eidgenossenschaft, 2008; IRGC, 2008) and trade unions (ETUS, 2008), have developed codes of conduct in order to underline health and safety issues.
Policy activities implemented by OECD and member states (OECD, 2007) have been synthesized in a document including the following actions: i) R&D programmes and strategies; ii) analysis of regulatory issues; iii) establishment of guarantee committees and working groups; iv) voluntary stewardship programmes; v) good practice documents; vi) stakeholders' information and consultation programmes.

7.3.2 Risk communication: contents and strategies

An appropriate communication ensures the availability of certain information to increase awareness among specific categories of the population. Practically, it must answers to the following questions: i) to whom?, (receivers); ii) What? (the object); iii) How? (the most adequate tools to face issues appropriately).
The information is appropriate when it is conveyed in a transparent and non-passive way and directly involves workers and their representatives or labour inspectorates. The European Trade Union Confederation (ETUC) has recently urged the enterprises to be more transparent in dealing with nanosafety issues (ETUC, 2008).
Communication contents, appropriate strategies, recipients and the in-depth knowledge of target audiences have been highlighted within the workshop "*Communication Outreach in Nanotechnology: from recommendations to action*", held in Brazil on 24 and 25 October 2007.
The communication of research outcomes to populations or groups potentially at

risk should be realised following a precise logic approach which includes specific questions about the current situation of public perceptions and media reporting (conveying enthusiasm about benefits of nanotechnology developments, but also concerns about potential risks associated with them) or about the emotional attitudes of all interested parties ranging from curiosity to concern, from prudence to unconditioned trust. This is why a deep knowledge of the receivers is a key pre-requirement for providing an either accurate and easily comprehensible information.
It is also essential to identify innovative communication strategies thorough transparent approach in order to open new communication channels to new generations, involve citizens and other categories, such as workers and their representatives in compliance with the ethical principles governing society and human values, and meet real needs.
The term "nanotechnology" encompasses a wide range of multidisciplinary applications and products. It is evident that stating that "*nano* is dangerous/risky" would be misleading and counter-productive; it is worth underlying, though, that R&D in this field could have a positive impact on our everyday life and that benefits and problems associated with them need to be promptly addressed.
The message that should be conveyed is that "nano" is not magic and nanotechnology is a new phase of technology that enhances nano-scale effects and this new opportunity must be faced consciously and attentively. This is "sustainability". Safety and risk management issues of nanomaterials all along their life cycle cannot be separated from the ethical and legislative implications, potential impact on the health system and risk management processes in companies that produce or use nanomaterials.

7.3.3 Communication channels

It goes without saying that strategies and tools for achieving communication objectives are influenced by the need and expectations of receivers. No specific rules to determine such instruments are available; however, the relevant characteristic of the public or interested parties should be considered.
According to the European Commission, the following approaches, treated in the above mentioned *Workshops*, may be used to this aim: i) active participation; ii) collaboration between education and science and between museums and laboratories; iii) development of an imaginative approach to get citizens closer to the world of nanotechnologies; iv) access to experiments; v) opening of research centres to people; vi) favouring communication on applications, benefits and, finally, potential risks of nanomaterials.
The following are some practical communication instruments now available: audio-

visual materials, electronic communications (bulletins, websites - *ICON Good Practices WIKI*, International Council on Nanotechnology, 2006 - mailing lists, etc.) tailored presentations and meetings such as the "nano forums" (i.e. meeting points of science and Industry; Nano&Nano, "Nanoweek", organized by Veneto Nanotech, etc.), networks of interested suppliers of scientific data (i.e. NanoImpactNet, networks of excellence, etc.), advanced training courses (i.e. "*Advanced Training Course on the Risks relating to Occupational Exposure to Nanomaterials - Institut National de Recherche et de Sécurité* - Paris, 11 -14 April 2006), press, newspapers, public press conferences, etc.
A relevant communication objective is to involve people in the field of nanotechnology and increase their awareness about scientific and technological development as well as potential positive and negative impact that nanotechnology may generate on the society and everyday life. Participation must be encouraged through the dialogue in order to build consensus between the private and public sector players, and between science/industry and consumers. Rendering science and technology "more democratic" is, certainly, the main goal.
For an efficient dialogue to be built, all elements of concern of the receivers must be preliminarily identified (i.e. expectations, concern in the way of acting of the industry or regulators, suggestions for scientists or politicians, receipt of potential suggestions) as they could be different from those of communicators and also the subjects' perceptions must be considered (state of consciousness of receivers, perceived risks and benefits); by so doing, (mutual) frustration would be reduced and communication would be more effective.

7.3.4 Occupational risk communication

Although the current state of knowledge is a challenge to appropriate assessment of potential occupational health and safety risks associated with the use of nanotechnologies and to an adequate choice of control standards, many efforts and initiatives are underway to overcome the precautionary principle and move towards the adoption of a proactive approach to the risk assessment which plays a key role in the implementation of a responsible development policy.
In the United States, Europe and Japan, many OSH (*Occupational Safety and Health*) Institutions have launched multidisciplinary research activities devoted to identify risks associated to the production and use of nanomaterials (i.e. UE funded projects such as NAnosafe, Impart, Nanotox, Nanoderm, Nanohealth, Nanocare, NanoImpactNet - see Chapter 3).
The increasing awareness among interested professionals concerning the specific

characteristics of nanomaterials and the peculiarity of their exposure scenarios is leading to the development of a conceptual framework and recommendation guidelines for risk management (NIOSH, 2007; Renn O and Roco MC, 2006; Schulte PA and Salamanca-Buentello F, 2007).
As authoritatively proposed by NIOSH (NIOSH, 2009b), an adaptation and a methodological development of such approach should be considered, instead of distorting traditional risk assessment approaches or legislative schemes (a regulatory framework has already be established by REACH). With this regard, various scientific research activities of Institutions and Regulatory Agencies have been launched with a view to unveiling the nature and the extent of potential risks related to the handling of nanomaterials and providing a solid scientific platform for health and safety risk management which takes into account the whole life cycle of nanomaterials (Thomas K et al, 2009; European commission, 2008g).

7.3.5 Conclusions

The assessment process of potential environmental and health risks associated with the nanotechnology development is at its preliminary stage and, as a consequence, what and how must be communicated in this regard is still primitive. Research on risk perceptions suggests that risk communication to lay public is filled with misunderstandings and emotional reactions. So far, relatively few studies have systematically explored the risk perception in the field of nanotechnology and how to constructively guide the public perception through a joint dialogue. The ever widening gap between the scientific knowledge on potential risks and our capabilities to communicate such notions might affect the development of those technologies that could have positive impacts on society. One of the main risk communication problem is that people tend to deeply change their views on the environmental and technological risks according to their cultural orientation (Kahan DM and BRaman D, 2006). In other words, the inability to efficiently communicate the potential risks of nanotechnologies is likely to waste all efforts to implement regulatory schemes or private sector investments projects that have been made so far. With this regard, nanotechnology could meet the same fate as other technologies; examples include the pacific use of the nuclear power or genetically modified organisms whose development has been mainly blocked by social and political controversy. This process has been regarded as "cultural polarization" (Kahan DM et al, 2008).
In the attempt to prevent an emotional approach to cope with a very difficult issue, a number of joint studies have been conducted by the "Cultural Cognition Process" (CCP) at Yale Law School and the "Projection on Emerging Nanotechnologies" (PEN)

coordinated by the "Woodrow Wilson International Center for Scholars" (Kahan DM and Rejeski D, 2009).

The outcomes of these studies have suggested some considerations and allowed to point out some recommendations on the communication approach. It is misleading to state that the public opinion and politics may be permeated by the scientific progress related to nanotechnologies; individual values influence the risk perception and so do nanotechnologies. Nevertheless, this makes it even more necessary to establish a communication which is comprehensible to socially and culturally diverse people. Individuals with different values tend towards approaching univocally to environmental risk issues ("cultural polarization"); this attitude appears unavoidable, unless communication strategies are attentively calibrated and communicators' identity considered. In fact, "experts" should reflect the social pluralism so that receivers may at least partly identify themselves and overcome cultural differences. Finally, CCP/PEN study highlighted how far the experimental science is from providing an efficient risk communication related to nanotechnologies. Recommendations to be shared concern: i) the urgent need for the scientific initiatives launched by research entities or institutions to address risk communication issues; ii) the need to use "message framing" to improve public receptivity to this topic and provide adequate scientific information; iii) the need to contextualize the message and adapt it to the real use of nanotechnologies and not to extremely specialist lab situations.

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