Approaching the responsible use of nanotechnologies. The global trends

Nanotechnologies will be ubiquitous enabling technologies encompassing several fields of science, industry and economy. The first large-volume nanotechnology-based products include nanomaterials, that raise general safety concerns, because of the very peculiar interaction with living systems, related to their size. Very few assessed results on nano-safety are available today, after one decade of intensive research throughout the world, due to the complexity of the interactions between nanomaterials and biological systems.
This article will give a brief sketch of the intricate scientific discussions ongoing, to show in detail what are the efforts to gain certainties and regulations, and reach a full and responsible economical exploitation of nanomaterials

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Verso un uso responsabile delle nanotecnologie. Le tendenze globali

Le nanotecnologie saranno tecnologie abilitanti onnipresenti, e copriranno molti settori della scienza, dell'industria e dell'economia. I primi prodotti nanotecnologici di grande diffusione comprendono i nanomateriali, che suscitano preoccupazioni generalizzate sulla loro sicurezza, a causa della specifiche interazioni con i sistemi viventi, legate alle loro dimensioni. Ancora pochi risultati condivisi sono disponibili a tutt'oggi, dopo un decennio di intense ricerche in tutto il mondo, per la complessità della interazione tra i nanomateriali ed i sistemi biologici.
Questo articolo fornirà un rapido quadro delle complicate discussioni scientifiche in corso, per mostrare in dettaglio quali sono gli sforzi per acquisire certezze e regolamenti ed arrivare a uno sfruttamento economico pieno e responsabile dei nanomateriali

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Nanotechnologies - between science fiction and reality

The rising wave of nano*technologies*, that is now at the top of popularity and concerns, started to move with the concepts of the US scientist Richard Feynman (Nobel laureate in Physics in 1965) that in 1959 gave a lecture entitled "There's plenty of room at the bottom", introducing the first ideas to manipulate the matter at the atomic scale.

It is a fact that the study of matter at the scale of nanometer (1 billionth of a meter) was greatly accelerated by the invention of the scanning tunneling miscroscope (STM), that earned Gerd Binnig and Heinrich Rohrer at IBM-Zurich the Nobel Prize in Physics in 1986.

The ability to see individual atoms in materials, enabled by STM and many similar tools, has been the start-up of huge efforts worldwide to fabricate nano-objects, as the US National Nano*technology* Initiative[1].

The figures of NNI are impressive: total budget till 2012 is 16.5 billion dollars; for the fiscal year 2012, 2130 M$ are available, including 611 M$ reserved for energy-related nano*technologies*, managed by the US Department of Energy[2].

The word "nano" brings frequently the imagination close to science fiction scenarios.

As nano-things are smaller than cells of living systems, their organs, their vascular systems, scientists (and novelists) imagined to assemble "nanobot surgeons"[3], nano-robots that provide surgical operations inside the human body, controlled from outside, with the additional capability to work on individual cells, much beyond the skills of a real, human surgeon.

Small nano-agglomerates of few atoms have indeed outstanding properties.

Nano-particles (NPs) have a very large fraction of their atoms on the surface: this entails that the chemical reactivity is much higher than for bulky materials, and NPs are suitable, first of all, to speed-up chemical reactions of any kind.

The high specific surface areas enhance any reaction depending on the number of exposed sites (up to fine dust spontaneous explosions)[4].

Beyond dreams and science fiction, it is a matter of fact that nowadays the only nano*technologies* on the market are the silicon chips (composed of silicon nanostructures much less than 100 nm in size) and a few types of nano*materials*.

During their lifetime, the former are tightly assembled together and sealed with encapsulants preventing any release of matter outside the chip; the latter are often used in the free form, and can penetrate inside living systems. Due to the superior potential impact on the biosphere, in terms of safety, we will focus our attention here only on nano*materials*.

Nanomaterials are in our lives

Nanomaterials have been in our everyday life for centuries.

The brightest example comes from the world of light and colors. In the stained glass that are ubiquitous in medieval churches (Figure 1), often colors were obtained by adding gold or silver nanoparticles to the glass. Silver was used to obtain yellow; gold for red (Figure 2). Also in Deruta ceramics, during the Renaissance period, copper and silver NPs were used to give an iridescent look to ceramic glazings.

FIGURE 1
Stained glass of medieval churches
Source: http://nano—tech.blogspot.com/p/history.html

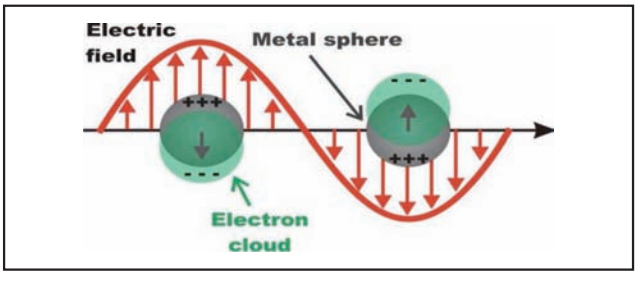


FIGURE 2 The resonant oscillation of surface electrons of metal NPs excited by light reflects colored light; color is a function of NP size
Source: http://willets.cm.utexas.edu/LSPR.html

In the third millennium, scientists aware of the peculiar properties of nanomaterials, equipped with tools to see them, try to obtain almost any material in bids, powders, fibers of nanometric size, with top-down methods (e.g., by ball-milling of large-size powders) or bottom-up methods (e.g.. by direct chemical synthesis from molecular precursors). Such nanomaterials, that can be obtained in a more or less reproducible way (one of the prerequisites for industrial exploitation), are generally termed "engineered nanomaterials" (ENMs), to distinguish them from other anthropogenic nanoparticles and aero-dispersed Particulate Matter (PM).

The size of ENMs falls in the range of the smaller fraction of colloidal particles (10^{-9} m to 10^{-6} m), thus presenting a different behavior from both molecular particles and bulk particulate matter; in fact, in environmental media they lack significant settling under normal gravitational conditions, whilst exhibiting significantly lower diffusivity than truly dissolved species.

The leading classes of ENMs entering our daily lives are: fullerenes, carbon nanotubes, metallic and metal-oxide nanoparticles, carbon black, polystirene, dendrimers, nanoclays, some macromolecules and nano-polymers[5].

The expected size of the nanotechnologies markets is huge.

According to *Electronics.ca Publications*, the global market value for nanotechnology is expected to increase to nearly $ 27 billion in 2015, for a 5-year compound annual growth rate (CAGR) of 11.1%[6]. Concerning U.S. – a leading country in nanotech - the production volume is estimated in the range of 7800-38000 tons/yr for TiO_2 down to 2.8-20 Tons/yr for nano-Ag (nano-CeO_2 and carbon nanotube volumes are in between)[7].

The Woodrow Wilson Research Institute shows that the absolute number of "nanotechnology-based consumer products" is 1317 for 2010[8], with a relative increase of such products of 20-30 % per year (Figures 3 and 4).

Similar surveys and findings for Europe are provided by ANEC and BEUC, two leading consumer associations in this area[9].

In some products, commercial ENMs are embedded in other materials, as in composite materials with nano-sized fillers, but they can also be used in free form for: environmental remediation, medical diagnostics and therapeutics, cosmetics, food and feed additives, etc.

Due to the very large specific surface area, NPs can easily interact with air, water, soil and all chemical species they meet. Similarly, once NPs have entered the human organism from some "entry portal" (nose, mouth, skin), they are immediately covered by biological substances of many kinds. The surface of nanopar-

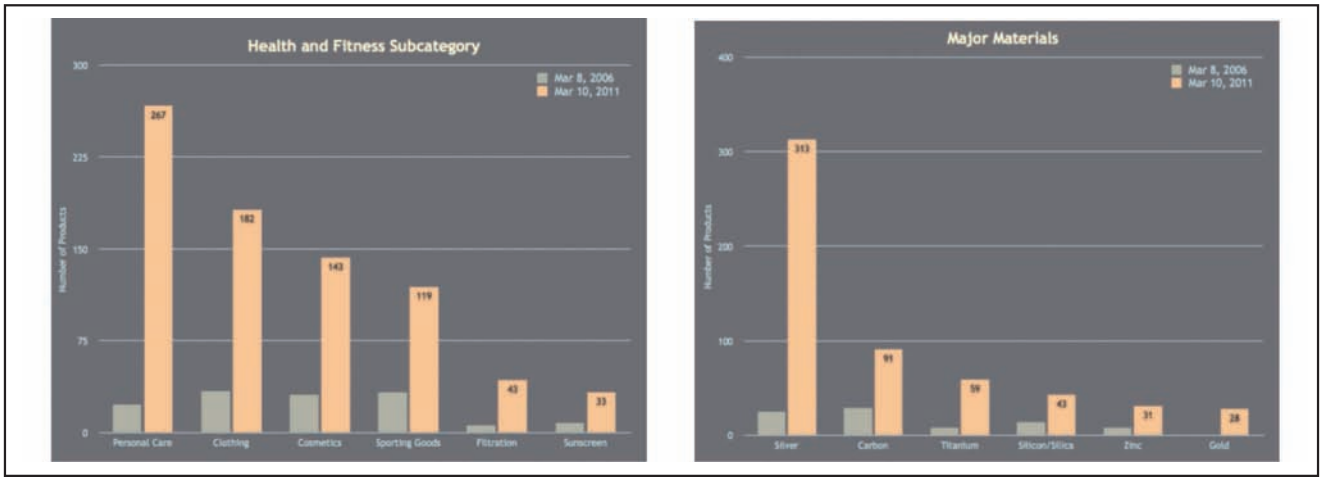


FIGURE 3 Nanotechnology-based products in the world markets, listed by application
Source: Woodrow Wilson Research Inst.

FIGURE 4 Nanomaterials incorporated in nanotechnology-based products
Source: Woodrow Wilson Research Inst.

ticles readily interacts with any chemical environment, releasing ions and atoms, but also favors the re-clustering of nanoparticles into larger agglomerates. During their journey from the synthesis reactor to the human body and back to the environment, NPs can "react with everything".

Environmental fate of engineered nanomaterials

In order to assess the environmental hazards posed by ENMs, it is necessary to estimate potential exposures through the comprehension of potential fate, transport and persistence of ENMs in environmental media. However, at present, there is a lack of ecotoxicological guidelines and validated models to assess potential ENM environmental exposures.

Multiple environmental matrices are traditionally evaluated to assess human exposures by different routes: atmosphere, gaseous, aerosol for inhalation exposures; aqueous, atmosphere, soil for dermal exposure; food, water for oral intake (Figure 5).

Several studies have been carried out in the field, possibly leading to valuable information to understand the environmental fate of ENMs. With regard to the atmospheric ENM emissions, results of several studies suggest that ENMs are likely to rapidly aggregate in particles ranging between 0,1-1 μm, with an atmospheric residence time of 10 to 20 days[10],[11]. In relation to the transport of ENMs in aquatic systems, the modeling might be more problematic due to several issues. Literature suggests that colloids mobility might be greatly influenced by water ionic strength and chemical composition; in addition, there are still doubts on whether the introduction of ENMs in aquatic systems may lead to the formation of stable aquatic colloidal suspensions[12],[13],[14]. With regard to the transport into natural soils and sediments, ENMs are likely to display relatively slow kinetics in the adsorption onto environmental solid phases, if compared to truly dissolved species. Therefore, equilibrium conditions in solid/water partitioning phenomena seems to be reached only after prolonged duration.

In any case, it is necessary to develop adequate models describing the intermedium transport behaviour of ENMs with an acceptable degree of uncertainty and it is, therefore, necessary to assess the air/water, air/solid and solid/water partitioning behaviour of ENMs with adequate accuracy upon their introduction in environmental systems. Traditional approaches rely on the adoption of partition coefficients which are theoretically based on an equilibrium partitioning concept. However, in systems where the assumption of equilibrium cannot be considered valid, it might be necessary to follow alternative kinetic approaches. Kinetic models might represent a solution to predict ENM fate, transport and exposure to the biosphere.

In conclusion, for the ENM environmental fate and risk assessment, a lot of work has still to be carried out, in order to keep pace with the nowadays commercialization and ubiquitous diffusion of these new materials.

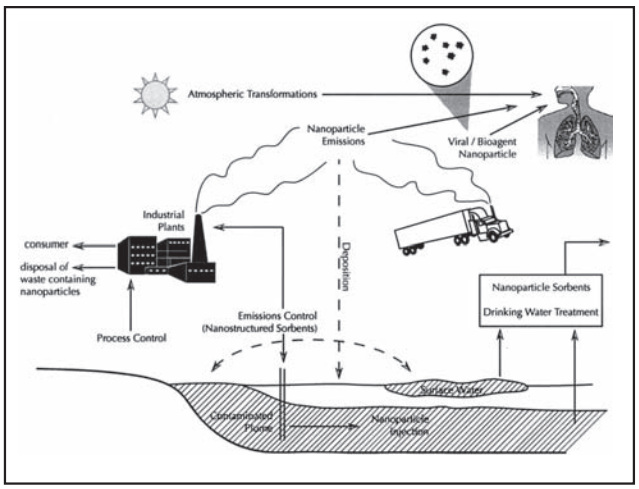


FIGURE 5 Dispersion routes of NPs in the environment
Source: J. Air & Waste Management Association[15]

Health hazards of engineered nanomaterials

To assess the potential health hazards of ENMs, it is necessary to understand their mechanisms of interaction with biological targets (Figure 6). This deals with questions like intracellular uptake of nanoparticles by endocytosis or, conversely, the indirect induction of damage to cells beyond a cellular barrier, by cell-to-cell signalling. Intracellular uptake is definitely important and is being considered a read-across parameter to compare responses of different cell types, or *in vitro* vs *in vivo* experimental results, but "un-targeted" effects (of CoCr nanoparticles) have also been shown in cultured cells and in mice[16].

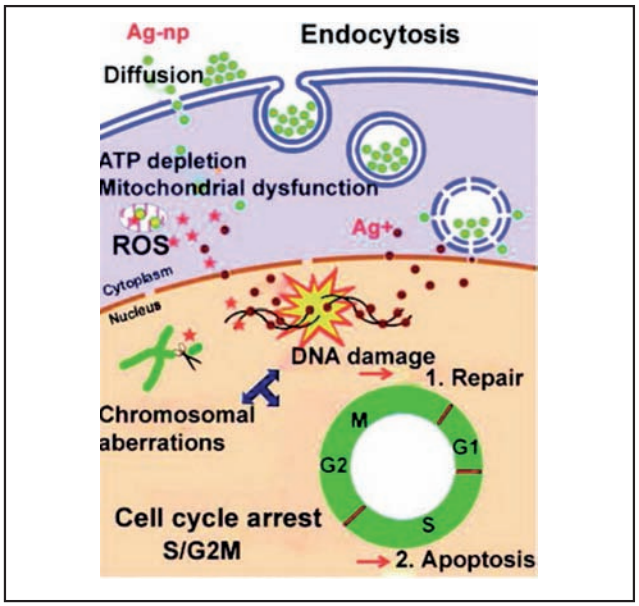


FIGURE 6 Hypothetical mechanisms of silver nanoparticle (Ag-np) cytotoxicity.
Ag-NPs can enter into the cell by diffusion or endocytosis. Once inside the cytoplasm, they can interfere with energy production in mitochondria and promote the generation of reactive oxygen species (ROS). ROS and Ag+ ions released from Ag-NPs may cross the nuclear membrane and cause DNA damage. DNA damage can be either repaired or lead to irreversible chromosome damage or cell death (apoptosis)
Source: ACS Nano[19]

Another critical issue is the biodistribution of nanoparticles in mammals following the various possible exposure routes and the identification of target organs. While, initially, the prevailing view was inspired by the experience of inhalation toxicology with particulate matter and aerosols, and identified the respiratory system as the main target to be concerned about, more recently, experiments with mice showed also the translocation of intravenously injected nanoparticles across the placenta[17] and the blood-testis barrier[18], raising interest on their possible reproductive effects. It is increasingly clear that biodistribution is largely influenced by surface modifications of nanoparticles produced by their first contact with cells and biological fluids, and this makes experiments with laboratory animals necessary. *In vivo* experiments are also mandatory to investigate the potential for nanoparticle accumulation after chronic exposures, which is most likely the case for human populations.
Whereas chemical toxicology is based on a more or less solid understanding of interactions between toxic compounds and biomolecules, leading to chemical changes in the latter with functional consequences, the field of nanotoxicology is still largely building this knowledge, and is still trying to ascertain the specific roles of NPs compared to their constituent chemical species. At present, the mostly claimed mechanism for nanoparticle-induced cytotoxicity is the elevation of the cellular levels of reactive oxygen species (ROS). ROS are molecules produced endogenously by normal cell metabolism, the level of which is strictly controlled because they can damage macromolecules such as DNA, proteins and lipids. The transition metal ions released from certain nanoparticles and, more generally, the high surface area associated with nanomaterials, can promote the generation of ROS. When the increase in ROS levels overrides the cellular antioxidant defense mechanisms, an inflammatory response can be initiated leading to the perturbation and destruction of mitochondria and, eventually, to programmed cell death.
Cell death is not the only effect to be concerned about, because more subtle cellular alterations, like DNA damage, can lead to carcinogenesis. Hence another key area governing health risk assessment of chemicals is genotoxicology, involving the study of genetic damage following the exposure to test substances. For many nanomaterials, the evidence being accumulated suggests that they may have genotoxic potential[20]. However, a mechanistic understanding of the effects observed is still lacking: it has been shown that nanoparticles of titanium dioxide and silica can cross the nuclear barrier, and there is always the possibility they might access DNA during mitosis, when the nuclear membrane is broken down. Yet, so far a direct interaction of nanoparticles with the double helix has not yet been shown. The possible direct interference with the cytoskeleton and microtubules essential for a variety of cell functions, including the correct chromosome distribution at mitosis, has been postulated, but not demonstrated[21]. Notably genotoxic effects of nanoparticles can also be indirectly elicited because of oxidative DNA damage induced by ROS.

The emblematic case of nano-silver

About the difficulty to overcome formidable scientific issues in nanosafety, emblematic is the case of nano-silver. Nano-Ag is already in the markets, but is still waiting for the complete assessment of the possible hazards. Instead, it is already known that dissolved Ag+ ions in water can be toxic for aquatic organisms, and nano-Ag in water is shown to be acutely toxic to *Daphnia magna*, due to the dissolution of Ag nanoparticles (NPs) in the form of free Ag+ ions[22].
In their review of the toxicity of Ag and Au NPs, H. J. Johnston et al. conclude[23] that "there is a limited understanding into the potential detrimental outcomes of human exposure to silver NPs" and whether they are related to the small size of NPs, or their dissolution into Ag ions, or both.
R. Arvidsson et al. evaluated the quantities of Ag NPs that can be released from wound dressings, textiles and electronic circuitry, and conclude that the most abundant source are textiles, due to the large areas involved (mainly underwear, for a large number of individuals), the use (large number of washing cycles), the high specific release[24]. Nano-Ag in textiles ranks first in the list, with a very broad range of $6x10^{28}$ – $6x10^{32}$ particles released per year in the environment.
Experiments on the pulmonary exposure of rats demonstrate[23] that Ag NPs accumulate in the lungs, are able to translocate in other organs like liver, kidney, spleen, brain but they are also cleared by excretion. This entails that: Ag NPs can cross the air-blood and blood-brain barriers, the blood carries the particles in the circulatory system, the particles accumulate in the filter organs, especially in the liver. Again in general, smaller particles have a larger ability for a widespread distribution, but also an increased possibility to pass through filter organs like liver and spleen, and be excreted.
Most of the comments to the research results analyzed in Ref. 23, conclude that further investigation is required to assess the results, and end up with unquestionable conclusions that must be the basis of the risk assessment of nanoparticles.
As reported in a recent investigation on nano-Ag environmental fate and exposure[25], data on the effective release during product use are useful to estimate the quantity of nano-Ag that is potentially present in environmental compartments. In particular, it was demonstrated that nearly 100% of silver content was lost by nanosilver-impregnated socks within four washings[26]. In any case, one of the limited issues related to nano-Ag environmental fate seems to be the assessment of the chemical form (speciation) which is taken by nano-Ag in the environmental compartments.

The lack of consistency in the dose metrics used, the limited physico-chemical characterization in most reports, the scattered experimental conditions tested lead to inconsistencies in the literature results that make it very difficult to come to firm conclusions, and further efforts should be dedicated to produce reports that are more informative.
Unfortunately, the fast progress to certainties in nanosafety is hampered by toxicological tests that are time-consuming and resource intensive, especially considering the variety of nanomaterials to be tested. This is why researchers are trying to develop computational models to predict the behaviour of nanomaterials in biological systems, and regulatory agencies are looking for ways to "group" nanomaterials by their toxic potential.

Predictive nanotoxicity models: current challenges and future opportunities

The need for developing predictive nanotoxicity models has also been recently highlighted by the European REACH legislation[27] and by the US Environmental Protection Agency[28]. This might be the way to speed up the authorization process to commercialize any ENM proposed for the future markets.
Only a few efforts to build predictive models for the effects of NPs in toxicology exist[29]. They are mainly based on Quantitative Structure-Activity Relationship (QSAR) methods. QSAR modeling is based on the empirical hypothesis that similar compounds have similar chemical and biological properties. Briefly, QSAR is a statistical model that relates a set of structural or property "descriptors" of a chemical compound to its biological "activity". The "descriptors" include structural parameters which are typically related to steric and electronic properties and they can be computed or measured in experiments. The "activities" include physicochemical measurements and biological assays (i.e., cytotoxicity tests). However, the descriptors for building models will have to be substantially extended in the case of nanosafety, to take into account the interactions that take place at the nano-bio interface (Figure 7). The mechanisms of the inter-atomic interactions between NPs and biological molecules are not sufficiently understood and little is known about the possible effects on the molecular structure and function of biological molecules, specifically proteins, DNA and biological membranes[30].

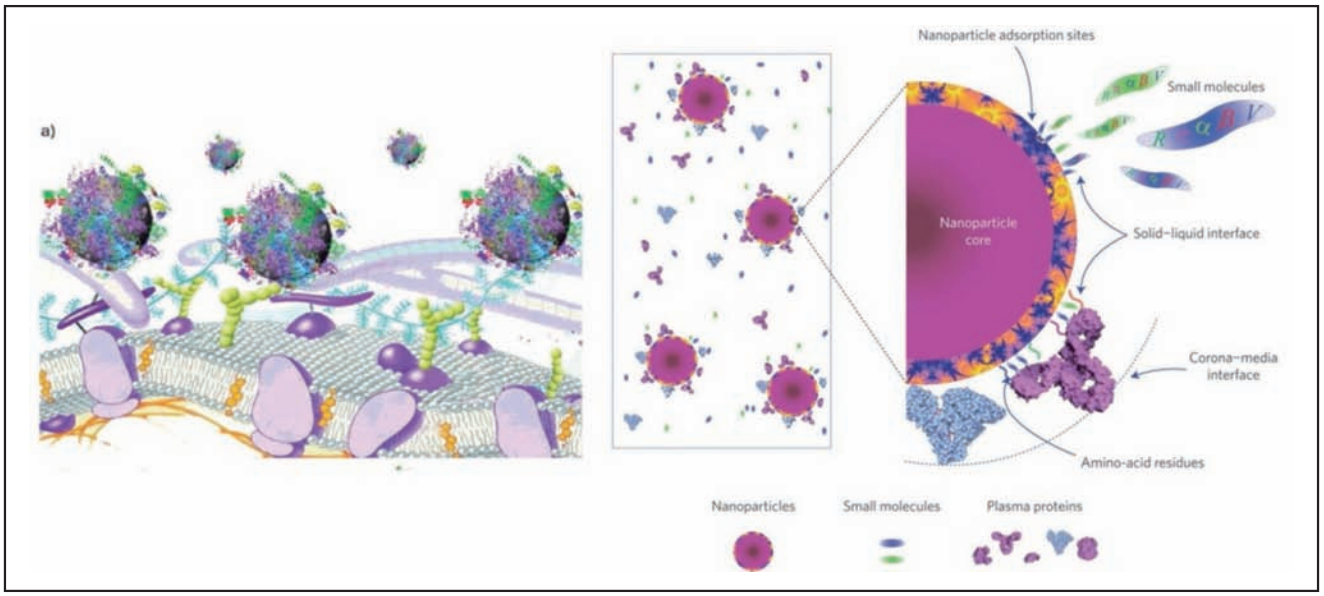


FIGURE 7 NPs exposed to biological fluids are rapidly covered by proteins and other biomolecules to form a 'protein corona' which can interact with biological membranes. The nano-bio interface consists of a NP surface, a solid-liquid interface and a corona-media interface

Source: http: // pubs.acs.org / action/ showImage?doi = 10.1021%2 Fja910675v&iName = master.img000.jpg&type = master
http: // www.nature.com / nnano / journal / v5 / n9 / fig_tab / nnano.2010.164_F1.html

Current computational techniques include electronic structure methods[31] and molecular dynamics methods[32], which have all been used to gain better understanding of the interactions and dynamics of NPs or ENMs within biological systems.

First-principle electronic structure methods, based on density functional theory, compute the total energy of molecules as a function of electron density. These methods have not been extensively used due to their computational load. This is a strong limit because alternative, very approximate computational approaches do not properly describe electronic states on the NP surface, and bulk-like properties are often used as input for QSAR methods.

Molecular dynamics (MD) methods enable to compute the time dependent behaviour of molecular systems and to investigate the structure-function relationship in biological systems with atomistic resolution. The simulation relies on the fundamental forces that govern atomic motion, which are derived from many-body inter-atomic interaction potentials. The potential, also known as force-field, describes bond stretching, bending and rotation as well as non-bonded interactions, including electrostatic and van der Waals interactions. From the MD trajectory of a system composed of NPs and biological molecule, the average values of physicochemical properties can be determined and used as descriptors for QSAR methods. However, computer simulations of organic/inorganic system are still at an early stage.

In conclusion, predictive models for NP risk assessment are still in their infancy. Even if physiologically-based-pharmacokinetics (PBPK) models are now commonly used in drug development and regulatory toxicology to predict the kinetics and metabolism of substances in the organisms, there is currently no established PBPK model for the distribution of NPs in the body, as acknowledged by the SCENIHR committee[33]. NPs are quite larger than molecules and the standard PBPK model transport equations need to be re-examined to assess their validity for NP[34].

Global trends and major players in nanosafety

The problem of balancing the desire to exploit the economic potential of ENMs with the need to protect

consumers and the environment, solving all the scientific questions that are in between, requires a global approach and coordination.
A particularly important role on nanosafety is played at worldwide scale by the Organisation for Economic Co-operation and Development (OECD)[35], and by the Joint Research Centre (JRC) of the European Commission, as they are boosting many initiatives that emphasize the standardization and assessment of scientific results.
OECD is committed to foster economic development in general, and its view about the business of ENMs is that: "Nanotechnologies are likely to offer a wide range of economic benefits. However, unlocking this potential will require a responsible and co-coordinated approach to ensure that potential safety issues are being addressed at the same time as the technology is developing".
OECD is active in the field of nanosafety since 2005, and OECD Council established the OECD Working Party on Manufactured Nanomaterials (WPMN) as a subsidiary body of the OECD Chemicals Committee, as well as the Programme on Safety of Manufactured Nanomaterials[36] in 2006. The Programme consists of specific projects: OECD Database on Manufactured Nanomaterials; Safety Testing of a Representative Set of Manufactured Nanomaterials; Manufactured Nanomaterials and Test Guidelines; Co-operation on Voluntary Schemes and Regulatory Programmes; Co-operation on Risk Assessment; The role of Alternative Methods in Nanotoxicology; Exposure Measurement and Exposure Mitigation; Environmentally Sustainable Use of Manufactured Nanomaterials.
The JRC is a very active partner of projects and networks in the field of nanosafety, through its Institute for Health and Consumer Protection (IHCP), and the current studies on nanosafety are a new implementation of the traditional efforts in the safety of chemical substances.
JRC-IHCP has a broad range of actions in this field: identification and characterization of nanomaterials, identification of new tools and methods for nanomaterial detection, testing methods alternative to the in-vivo experiments, standardization and regulatory actions, but also the management of the so-called "nanomaterials repository".
The nanomaterial repository offers qualified nanomaterials to researchers in the field of nanosafety and includes (as of 27 October 2011): Titanium Dioxide (rutile and anatase, mean particle size ranging from 67 to 267 nm), Zinc dioxide (mps = 140-150 nm), Silicon dioxide (mps=47-137 nm), Cerium dioxide (mps=28 nm and others), Silver (mps=15 nm and others), Multi-walled carbon nanotubes, Nanoclay (bentonite, mps 288 nm). Materials of this range have been distributed to many countries across Europe and the world.
What EC and JRC are putting into practice is the implementation of the requirements stated in the resolution of the European Parliament P6_TA(2009)0328 about the safety of nanomaterials. The resolution is working today as a sort of Agenda, with detailed requests of the Parliament to guarantee a responsible use of nanomaterials in the future in all EU Member States.

Nanosafety in Europe. Trends of regulations and research

Notwithstanding the strong generalized awareness of the importance of nanosafety, till now there are no general assessed testing procedures to measure the impact of ENMs on living systems and assess the related risks.
However, in Europe, there is a great effort to create new nano-specific tests and rules, or to include nanomaterials in the stream of the available regulations concerning chemical substances: the EC REACH Regulation that rules the "Registration, Evaluation, Authorisation and Restriction of Chemicals", in any form. Therefore the Regulation applies also to nanomaterials, which are covered under the definition of "substance" in REACH and may be considered to be distinct "substances" or "forms of a substance".
Moreover, it is a requirement of the regulation on Classification, Labelling and Packaging (CLP)3 that the classification and labelling of a substance is composition/form specific, thus, these are directly linked to specific compositions/forms.
Registrants can indicate that a dossier includes nanomaterials, which might be considered to be either distinct substances or forms of a substance. When a nanomaterial is considered to be a distinct substance, the registrant should complete a dossier as for any substance. When a nanomaterial is considered to be a

form of a substance, or when the dossier contains more than one listed composition, the registrant is encouraged to use labels for each composition/form to enable referencing a specific composition/form in a particular information requirement or data point with regard to the physico-chemical properties of the substance, environmental fate and behaviour, ecotoxicological information, toxicological information and specific information. In any case, classification of nanomaterials should be done on a case-by-case basis giving due consideration to the relevant available data; a separate classification and labelling notification may be required for the nanoform of a bulk substance if available data on intrinsic properties indicates a difference in hazard class.

Some authorities are going to provide sector-specific indications in the cases where the possible impact is greater: food and cosmetics.

The European Food Safety Authority, in 2011, issued the "Guidance on the risk assessment of the application of nanoscience and nanotechnologies in the food and feed chain"[38], that is explicitely a "scientific opinion". The guidance provides information about the physico-chemical characterization of the NPs involved in the food chain, and testing strategies to identify and characterize related hazards.

About cosmetics, the Cosmetic Products Regulation, that will enter into force in July 2013, sets some specific obligations concerning nanomaterials: manufacturers are obligated to mention the presence of nanomaterials in their products, the Commission can request the safety assessment, the labelling for "nano" ingredients is required on cosmetic products.

What is the trend in the field of EU research on nanosafety?

The Framework Programmes 6 and 7 allocated substantial funding to nanosafety. Starting a few years ago, a comprehensive list of projects, partners, research findings of the 25 projects funded by FP6 and FP7 is edited by the Directorate-General for Research: the "Compendium of Projects in the European NanoSafety Cluster"[39].

One other of the leading initiatives of EC in the field of nanosafety within FP7 is the creation of: "QNANO - A pan-European infrastructure for quality in nanomaterials safety testing". QNANO is a virtual infrastructure that allows transnational access of researchers to a qualified set of laboratories in Europe, working in coordination in the field of nanosafety. QNANO will push the efforts for standardization, round-robin testing and categorization of ENMs for nanosafety.

The definition of "nanomaterial" in Europe

In order to speed up the achievement of regulations about ENMs, on November 18, 2011, the EC adopted the definition of "nanomaterial"[37], here reported in brief:

"…2) Nanomaterial' means a natural, incidental or manufactured material containing particles, in an unbound state or as an aggregate or as an agglomerate and where, for 50 % or more of the particles in the number size distribution, one or more external dimensions is in the size range 1 nm-100 nm. In specific cases and where warranted by concerns for the environment, health, safety or competitiveness the number size distribution threshold of 50 % may be replaced by a threshold between 1 and 50 %.

3) By derogation from point 2, fullerenes, graphene flakes and single wall carbon nanotubes with one or more external dimensions below 1 nm should be considered as nanomaterial".

The situation in Italy

Obviously, also in Italy there are many R&D efforts in nanotechnologies, even though a real national strategic plan on nanotechnologies has never been set in place as in other countries. The fundings for nanotechnologies (and among them for nanosafety) are spread in a number of different financing tools and organisations.

Since 2004, the Italian Association of Industrial Research (AIRI) usually compiles a database of public and private expertise on nanotechnology in Italy: the Italian Nanotechnology Census (now at its 3rd edition). The number of organisations involved in nanotech approaches is around 200. All the leading universities and public and private research organisations have basic expertise in this field and can include nanosafety in more general institutional R&D activities.

The Census shows (2nd edition[40], 2006) that the Italian nanomaterial manufacturers are very few. According to the Census (and to additional contacts of the authors of this article), they are only Colorobbia (Tuscany) and Italcementi (Lombardy). Colorobbia produces a range of products (mainly nano-metals, -metal oxides and –ferrites); Italcementi produces

photocatalytic TiO_2 for building applications.
Many industries are end-users of nanomaterials produced elsewhere, embedded in composites, paints, cosmetics. Therefore, at present, the possible nanosafety risks for Italian workers are concentrated in a very few number of large-scale manufacturers' sites, and an appreciable number of small-scale research labs.
Due to the expected possible impact of nanomaterials manufacturing and application on workers' safety (both in industry and research labs), in previous years ISPESL - Italian National Institute for Occupational Safety and Prevention launched a timely initiative to study such issues, especially in workplaces.
Recently INAIL - Department of Occupational Health (the former ISPESL) issued a White Book[41] in July 2011, where the widening gap between the increasing commercial exploitation of nanotechnologies and the risk assessment in the workplace, as well the need for a rigorous communication of risk to citizen and workers are highlighted.
The search for a common regulatory framework in Europe is highly encouraged by the European Commission, also through the publication of the dedicated FP7 Call for Proposals: "Regulatory testing of nanomaterials". The most relevant Italian competences and leading research organisations in the nanosafety field, including ENEA, are participating in the project proposal preparation, under the national coordination of the Italian Ministry of Health. The main objectives of the project will be, on the short-medium term, to provide legislators with a set of tools for risk assessment and decision making for a selected number of nanomaterials and, on the long term, to develop new testing and evaluation strategies adaptable to a high number of nanomaterials.

Efforts in nanosafety research in ENEA

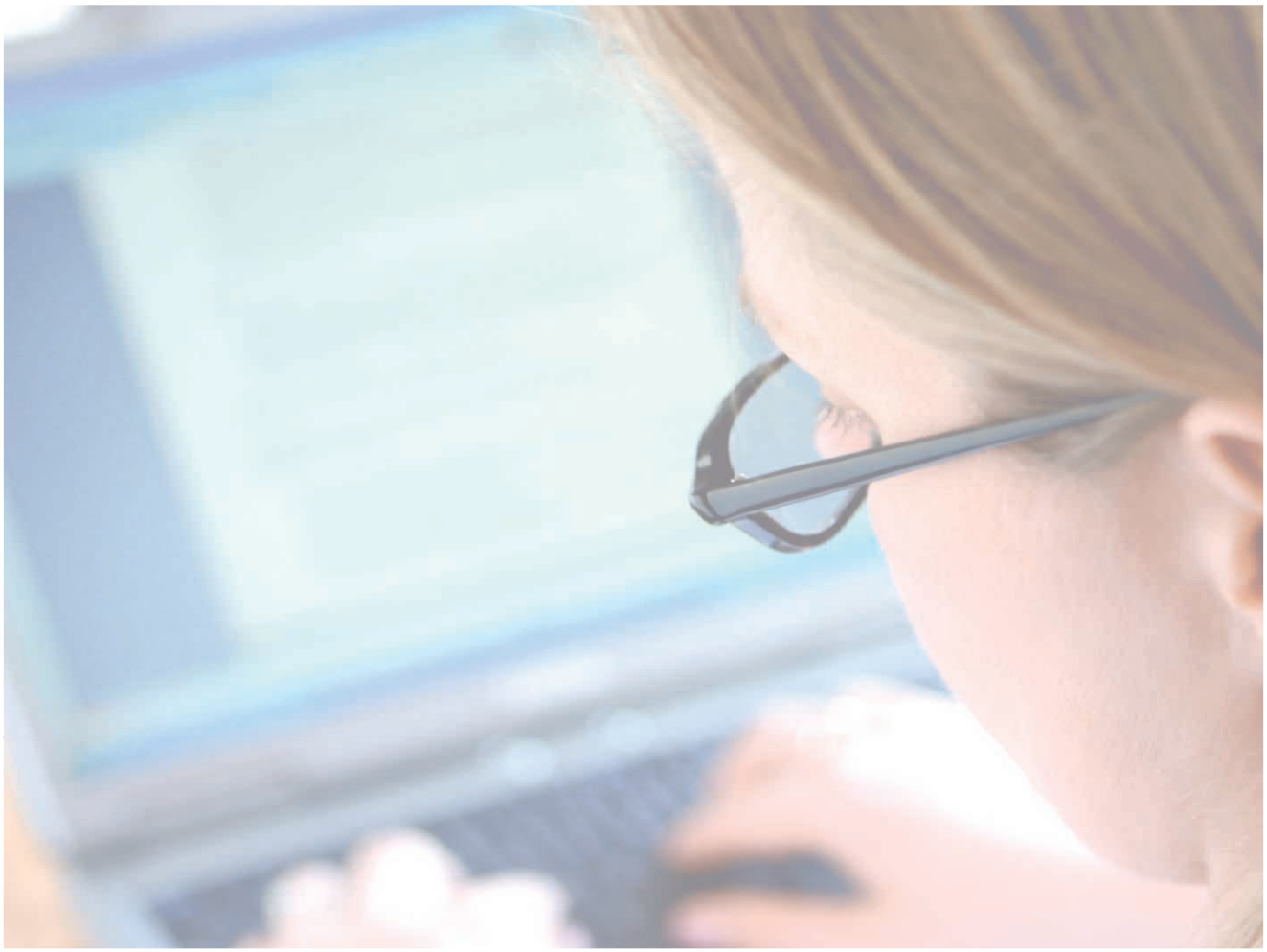
Recently, four ENEA labs belonging to the three Technical Units: "Materials Technologies", "Radiation Biology and Human Health", "Environmental Technologies", decided to integrate their competences in order to deal with the study of the impact of nanomaterials on living systems and the environment. First experiments are devoted to study the bio-interaction of home-made ferrofluids; next steps are planned towards in-silico modelling and bio-interaction of basalt fibres.
The main competences of the four labs, available for the ENEA nanosafety research, are:

1. Preparation, functionalisation and characterisation of engineered nanomaterials – an expertise on solid state and wet chemistry and material science with particular reference to synthesis and physico-chemical characterization of ferrite nanoparticles ($MnFe_2O_4$ and Fe_3O_4).
2. *In silico* modelling of biophysical/biological interactions with functionalized nanoparticles - focussed on the physico-chemical characterization of nanoclusters composed by SiO_2, ZnO_2, TiO_2 and carbon-based nanostructures in interaction with an ample range of biological systems, such as proteins, biological membranes and DNA.
3. Assessment of short- and long-term biological effects of engineered nanomaterials at molecular, cellular, organ levels by *in vitro* and *in vivo* experimental test systems – in particular, investigation of inflammatory, genotoxic, reproductive and carcinogenic effects, exploiting also the use of mouse models susceptible to specific pathogenetic mechanisms.
4. Definition of exposure scenarios, life cycle and risk assessment, Regulatory aspects (REACH regulation) - ENEA is active in the National implementation of REACH regulation by supporting the Italian Ministry of Economic Development in the National REACH Helpdesk management, in social and economic analysis for the substance restriction and authorization processes, and in the development and provision of supporting tools and solutions for SMEs facing REACH Regulation. ENEA has acquired extensive knowledge on exposure scenario elaboration for workers, consumers and the environment, risk assessment, life cycle analysis for chemical products, in compliance with the most recent European and national regulations concerning chemical substance use and associated risks.

The coordinated efforts of the four labs will be integrated with the research on ENM ecotoxicology, a recent expertise created in the ENEA Research Centre of Portici.

Conclusions

As technology is usually faster than regulations, also in the case of nanomaterials it happens that many NM-based products are arriving on the market, with an incomplete assessment of research results about nanosafety, and an insufficient translation into nano-specific regulations.
The achievement of these goals is slowed down by a still limited understanding of the intricate interactions of ENMs with living systems and the environment.
In general, the real risk assessment for nanomaterials during their whole lifecycle is still to be completed in most cases, and this requires an increased standardization of measurements, tests, procedures and a great advancement in the evaluation of the actual exposure, especially for consumers and the environment.
Speeding-up such evaluations will be necessary to boost the responsible development of the business for nanomaterials, compliant with the obligations related to the EC REACH Regulation and other EC Directives.
The JRC-EASAC joint report suggests to include in future research: the concept of "safety by design", similar to the current practice in the pharmaceutical sector (where hazard and risks are addressed at an early stage of research[42]), the sharing of scientific findings with the general public (as it is going on, for example, in UK, Switzerland, Germany, France), and new "nanospecific" training courses[43].



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