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Nanomaterials: Environmental pollution, ecolological risks and adverse health effects

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ABSTRACT

Nanotechnology ant its applications has emerged as one of the central new technologies in the 21st century. In the last decade thousands of scientists and technologists are employed in this area, a great number of patents and scientific publications have been published A large number of new nanotechnology products have flooded the market of the developed world and inevitably large amounts of money are invested in Research & Development in the most advanced technological nations. Future prospects in different fields of nano-applications seem unlimited and its high potential will affect our daily life, our health and the environment in the years to come. Nanomaterials found applications in the fields of consumer products (cosmetics, textiles, diagnostic materials, personal care products, paints, etc), food, energy, medicines, computers, portable telephones and a great variety of other scientific fields. But in recent years, scientists and environmentalists are thinking about possible hazards to human health resulting from nanoparticulate exposures in the working environment, after contact with consumer products and through environmental pollution. The requirements for appropriate health risk assessment and safety regulations of nanomaterials are being explored. Also, environmental pollution and the fate of nanomaterials in the natural environment, especially in the aquatic environment, are some of the great concerns to scientists and environmentalists. In this paper we present an overall review of the current state of knowledge related to toxicity and human health risk of the engineered nanoparticles. The review presents the latest research papers on new challenges facing scientists and technologists with nanaomaterials. Furthermore, the review examines the future requirements for making nanotechnology safe for the consumer, the industrial worker and less polluting for the environment and the ecosystems. Based on the current toxicological results, scientists provide a proposal on how risk assessment in the nanofield could be achieved and how it might look like in the near future.

KEYWORDS

Nanotechnology;
Nanomaterials;
Toxicity;
Cytotoxicity;
Human health;
Environmental pollution;
Nanoecotoxicity.

INTRODUCTION

Nanotechnology is the technological revolution of the last decades that deals with the manipulation of matter on an atomic and molecular scale for fabrication of macroscale products[1,2]. Nanoscale technologies refer to the broad range of research and applications whose common trait is extremely small size (1 to 100 nanometers, nm) and products with special physicochemical characteristics and quantum mechanical effects[3,4].

Nanotechnology became an important industrial sector in all industrialized countries. In 2000 for the first time the USA government established the National Nanotechnology Initiative (NNI) to serve as the central point of communication and collaboration for all Federal agencies engaged in nanotechnology research, bringing together the expertise needed to advance this broad and complex field[5]. The USA through NNI has invested 3.7 $ billion in nanotechnology research, the European Union countries 1.2 $ billion and Japan 750 $ million (in the period 2004-2005). In 2009 Russia announced that will channel 318 billion rubles ($10.6 billion) into development of nanotechnology by 2015[6]. The Annual Global Nanotechnology Research Funding report estimated that the world's governments currently spend $10 billion per year, with that figure set to grow by 20% over the next three years, while China (2011) will spend up to US$2.25 billion in nanotechnology research. Worldwide statistics showed that the total government funding for nanotechnology and nanomaterials' research will be $65 billion, rising to $100 billion by 2014[7]. A recent conference report showed that corporations and institutional investors for nanotechnology R&D spent 9,2 and 9,7 US$ billions respectively, and governments spent 8,4 and 8,2 US$ billions (2009-2010). The sectors that are leading the nanotechnology R&D are transportation and aerospace, nanomedicine, electronics, energy, materials, food and food packaging, etc[8].

In the last decade, nanotechnology applications and nanomaterials continue to evolve rapidly and the overall market for new nanoproducts is growing. The Woodrow Wilson International Center for Scholars (WWICS) in the USA established a Project on Emerging Nanotechnologies (April 2005). The Project was dedicated to helping ensure that as nanotechnologies advance, possible risks are minimized, public and consumer engagement remains strong. The Project identified a list of more than 1,000 nano-enabled products currently on the market containing information on products from over 20 countries[9].

Engineered Nanomaterials (ENMs) can enter the marketplace as materials themselves, as intermediates that either have nanoscale features or incorporate nanomaterials, and as final nano-enabled products[10]. Nanomaterials can generally be organized into four types: a) *Carbon-based materials*: composed mostly of carbon, spherical (fullerenes), elliptical, or tubular in shape, b) *Metal-based materials* include nanoscale gold (Au), nanoscale silver (Ag), and metal oxides, such as titanium dioxide (TiO_2). Also quantum dots, c) *Dendrimers*. nanoscale polymers built from branched units, which can be tailored to perform specific chemical functions with interior cavities into which other molecules can be placed, such as for drug delivery, d) *Composites*. Combine nanoparticles with other nanoparticles or with larger, conventional-scale materials[3].

Various fields of consumer products have been affected by introducing ENMs in: cosmetics, textiles, food contact materials, improved diagnosis and treatment (drug delivery systems) of all kinds of diseases. In addition, novel technologies applying ENMs are expected to be instrumental in waste remediation, in the production of efficient energy storage and advances in computer sciences[11,12].

Carbon nanomaterials have a wide range of uses in vehicles, sports equipment and integrated circuits for electronic components. Nanoscale cerium oxide (CeO_2) is used in electronics, biomedical supplies, energy and fuel additives. Nano titanium dioxide (TiO_2) is used in sunscreens, cosmetics, paints and coatings. Nano silver is being incorporated into textiles and other materials to eliminate bacteria and odor from clothing, food packaging, and other consumer products where antimicrobial properties are desirable. Nano-scale iron is used as optics polishing and as a better-absorbed iron nutrient supplement, whereas one of its more prominent current uses is to remove contamination from groundwater[13].

ARE NANOMATERIALS TOXIC? CONCERNS FOR INCREASED RISKS TO HUMAN HEALTH

The sudden rise of a vast range of applications of nanomaterials in the last decade prompted scientists to debate the future implications of nanotechnology in environmental pollution. As any new technology the concerns were about environmental pollution, especially the toxicity in aquatic organisms and their environmental impact in ecosystems.These concerns led to a debate among advocacy groups, consumer protection agencies and governments on whether special health and safety regulation of nanotechnology is warranted for workers and consumers[14,15].

In the last decade health and safety issues with ENMs are a new big issue with regulatory bodies. In the past few years, several kinds of opinions or recommendations on the nanomaterial safety assessment have been published from international or national bodies. Among the reports, the first practical guidance of risk assessment was published from the European Food Safety Authority (EFSA, May 2011), which included the determination of exposure scenario and toxicity testing strategy. The EFSA guidance document is the first of its kind to give practical guidance for addressing potential risks arising from applications of nanoscience and nanotechnologies in the food and feed chain. The guidance covers risk assessments for food and feed applications including food additives, enzymes, flavourings, food contact materials, novel foods, feed additives and pesticides[16].

Recently (July 2012) the Scientific Committee on Consumer Safety (SCCS) of European Commission released guidance for assessment of nanomaterials in cosmetics. A series of activities in EU marks an important step towards realistic safety assessment of nanomaterials. The Commission published the "*Guidance on the Safety Assessment of Nanomaterials in Cosmetics*". The document was drafted by the SCCS to help the cosmetics industry comply with article 16 of Regulation (EC) No 1223/2009 on cosmetic products (into force on July 2013)[17].

In the USA, the Food and Drug Administration (FDA) regulates nanotechnology differently. It established a draft guidance for industry in June 2011 for both "Cosmetic Products" and "Food Ingredients and Food Contact Substances" in April 2012. These documents do not restrictedly define the physical properties of nanomaterials, but when manufacturing changes alter the dimensions, properties, or effects of an FDA-regulated product, the products are treated as new commercial products[18].

Nanomaterials are used in a variety of FDA-regulated products because of their unique properties, imparting potential advantages to products. In the USA the law does not subject cosmetic products and ingredients to pre-market approval by FDA. Rather, firms and individuals who market cosmetics have a legal responsibility to make sure their products and ingredients, including nanoscale materials, are safe under labelled or customary conditions of use, and that they are properly labelled. FDA monitors the use of nanoscale materials in cosmetics and keeps abreast of research into their safety[19].

Non-Gonvermental Organizations (NGOs) that are in the forfront of environmental protection have issued their own studies on nanotechnology. Safety issues of nanotechnology products are in the agenta of concern for environmental organizations. For example, in 2006, the organization Friends of the Earth (FoE) released a report, "*Nanomaterials, Sunscreens and Cosmetics: Small Ingredients, Big Risks*."[20]. Since then, FoE have released annual reports sharing updating research studies on ENMs and alarming risks, which could affect consumers, workers and the environment. FoE gathered a number of scientific evidence showing that nanomaterials have the potential for adverse health effects. FoE efforts were focused on ensuring that at the end of the day (someday) consumers will be granted the rights and safe products they deserve. These changes will allow consumers to have more information and make healthier and more informed choices. Since there are an yet no definite epidemiological studies, the precautionary principle is used by NGOs for the ENMs. This approach put emphasis on future assessment and update regulations of new products by most of national authorities, although the approaches are still case by case because of the specialized features and multiple ENM applications[21-23].

NGOs in developed industrial countries of Europe tend to be more cautious than their North American counterparts about the concentration of extensive power to nanotechnology industries. Also, the main concerns of NGOs revolve around the concentration of power as a result of intellectual property regimes, the misuse of nanotechnology for destructive purposes, the disruption of existing economic systems, some ethical issues related to human improvement and privacy, and negative impacts on human health and the environment by unregulated use of ENMs[24].

The recommendations by OECD (Organisation for Economic Co-operation and Development) on the safety assessment of nanomaterials is part of the OECD system for the Mutual Acceptance of Data (MAD) in the Assessment of Chemicals. MAD is a multilateral agreement which saves governments and chemical producers around €150 million every year by allowing the results of a variety of non-clinical safety tests done on chemicals and chemical products, including nanomaterials, to be shared across OECD and other countries that adhere to the system. For example, Argentina, Brazil, India, Malaysia, Singapore, South Africa as well as all OECD countries are full adherents to the MAD system[25].

The safety of ENMs has become the concern of Indian research institutions. Over the last decade the Indian state has strived to establish an adequate foundation for advancing nanotechnology resulting in the expansion of R&D and commercialization of nanoproducts. A greater focus on technology development has meant that India's engagement with the overdue but not less significant risk debate appears to be far behind global discourses.

India too has witnessed the emergence of nanomaterial use in products such as water filters and other household appliances such as washing machines air conditioners and fridges, textiles, cosmetics, personal care, etc. This has direct ramifications on the intensity of efforts that would need to be undertaken to address risks of ENMs at the national level[26-28]

OCCUPATIONAL HEALTH AND SAFETY ISSUES OF WORKERS IN THE MANUFACTURING PROCESSES OF NANOMATERIALS

Engineered nanomaterials (ENMs), as with all new technologies and manufacturing processes in the past, the earliest and most extensive exposure to hazards is most likely to occur in the working environment. Manufacturing workers in nanotechnology-related industries and small workshops are the ones to be exposed to nanoengineered materials with novel sizes, shapes, and physical and chemical properties. Occupational toxicologists are the first to discover adverse effects in occupational health and safety of ENMs.The scientific information that is currently available on exposure routes, potential exposure levels, and material toxicity of nanomaterials is very limited. The first studies focused on the low solubility of nanoparticles because of their higher toxicity potential. Nanoparticles during manufacturing processes can penetrate into the respiratory system and through the blood circulation can move into other organs. Studies showed strong indications that nanoparticles can penetrate through the skin. Survey of the scientific literature indicates that the available information is incomplete and many of the early findings have not been independently verified. Current recommendations for ENMs in the working environment, in order to minimize exposure and hazards to workers are largely based on common sense, knowledge by analogy to ultrafine material toxicity, and general health and safety recommendations. There are strong indications that ENMs surface area and surface chemistry are responsible for observed responses in cell cultures and animals[29].

Studies showed that most airborne Carbon NanoTubes (CNTs) or Carbon NanoFibers (CNFs) found in workplaces (during the manufacturing processes) are loose agglomerates of micrometer diameter. However, due to their low density, they linger in workplace air for a considerable time, and a large fraction of these structures are respirable. So, industrial workers are the first to be exposed to nanomaterials at high concentrations[30].

The first toxicological studies for ENMs were performed in rat and mouse models. Pulmonary exposure to single-walled carbon nanotubes (SWCNTs), multi-walled carbon nanotubes (MWCNTs), or CNFs caused the following pulmonary reactions: acute pulmonary inflammation and injury, rapid and persistent formation of granulomatous lesions and progressive alveolar interstitial fibrosis at deposition sites. Furthermore, pulmonary exposure to nanoparticles can induce oxidative stress in aortic tissue and increases plaque formation in an atherosclerotic mouse model. Pulmonary exposure to MWCNTs depresses the ability of coronary arterioles to respond to dilators. These cardiovascular effects may result from neurogenic signals from sensory irritant receptors in the lung. In addition, pulmonary exposure to MWCNTs may induce levels of inflammatory mediators in the blood, which may affect the cardiovascular system. Intraperitoneal instillation of MWCNTs in mice has been associated with abdominal mesothelioma (this was a typical disease of asbestos fibers exposure of workers in the past decades). However, further studies are required to determine whether pulmonary exposure to MWCNTs can induce pleural lesions or mesothelioma[31].

The adverse health effects in workers of nanotechnology industries became recently a concern. to NIOSH (National Institute of Occupational Safety and Health, USA), which is the leading federal agency conducting research and providing guidance on the occupational safety and health implications and applications of nanotechnology Additionally, NIOSH recommended that engineering controls and personal protective equipment can significantly decrease workplace exposure to CNTs and CNFs. Considering the available data on health risks, it appears prudent to develop prevention strategies to minimize workplace exposure, such as enclosure, exhaust ventilation and respiratory protective masks or respirators and worker training for good handling practices[32]. NIOSH has also created a field research team to assess workplace processes, materials, and control technologies associated with nanotechnology. But, much research is still needed to understand the impact of nanotechnology on health, and to determine appropriate exposure monitoring and control strategies. At this time, the limited evidence available suggests caution when potential exposures to nanoparticles may occur[33].

The Health and Safety Executive (HSE) which deals with the UK regulatory framework for occupational health and safety in the workplaces is covering the safe use and handling of manufactured nanomaterials. The Report "Using Nanomaterials at Work" is a new guidance prepared in response to emerging evidence about the toxicity of these materials. It is specifically about the manufacture and manipulation of all nanomaterials including carbon nanotubes (CNTs) and high aspect ratio nanomaterials (HARNS). It has been prepared in response to emerging evidence about the toxicity of these materials[34].

In Germany (2011) the Federal Institute for Occupational Safety and Health (BAuA, Bundesanstalt fur Arbetsschutz und Arberitsmedizin) together with the German Chemical Industry Association (Verband der Chemischen Industrie/VCI), the Federation of German Industry (BDI) and the Federal Ministry of Education and Research (BMBF) started a second survey on occupational health and safety in the handling and use of nanomaterials. The awareness to the topic and the scientific and pragmatic approach led to high number of answers from industry, research organisations, universities and state institutions[35]. Also, in 2011 a working group consisting of the Institute of Energy and Environmental Technology e.V. (IUTA), the Federal Institute for Occupational Safety and Health (BAuA), the German Social Accident Insurance Institution for the Raw Materials and Chemical Industry (BG RCI), the Institute for Occupational Safety and Health of the DGUV (IFA), the Technical University Dresden (TUD) and the German Chemical Industry Association (VCI) published the document "*Tiered Approach to an Exposure Measurement and Assessment of Nanoscale Aerosols Released from Engineered Nanomaterials in Workplace Operations"*[36].

Also, the competent authorities for protection of workers and the environment in Germany with the Federal Environment Ministry developed the idea for amending the REACH regulation of the EU because there is need for better identification and assessment for potential hazards arising from nanomaterials in the future[37].

CONSUMER PRODUCTS WITH ENMS AND POTENTIAL FOR EXPOSURE AND TOXIC EFFECTS TO HUMANS

The development and arrival of novel nano-based consumer products in the last decade has raised concerns over consumer health and safety. The main nanoproducts are food materials, innovative food packaging, intelligent delivery mechanisms of nutrients and bioactive materials. In the interesting Report of RIVM (National Insitute for Public Health and the Environment, Bilthoven The Netherlands): "*Exposure to Nanomaterials in Consumer Products*" (Letter Report 340370001/2009, www.rivm.nl) there is an extensive catalogue of nanomaterials in the various types of consumemer products. The nanomaterials are mainly in the form of particles, composites, capsules, fullerenbes, carbon nanotubes, coatings, nanoporous materilas, quantum dots, nanofibres, nanowires. The most important ENMs are used: a). food, beverages, food containers, food supplements, b) electronic and computers (electronic parts, display, ink, paper, hardware, recording), c) household products (cleaning substances, coatings, adhesives, lighting, filtration, sanitation, air purification), d) motor vehicles (catalytic convertors, fuel, energy-batteries, paints, air filtration, etc), e) clothing, textile coatings, shoes, sporting goods, f) medical products, wound dressing, skin care, biomedical applications, g) personal care products (oral hygiene, etc), cosmetics, sunscreens However, in the pursuit of delivering more and more patentable technologies and a great variety uses of nanoparticles in foodstuffs alarmed toxicologists. Food regulators and other consumer products respond to the potential threat of nanomaterials guided by toxicity studies[38].

Widespread application of nanomaterials for consumer products confers enormous potential for human exposure and environmental release. Technological developments in nanoproducts and applications are outpacing research of human health and environmental risks from pollution. Many decades ago the world had the example of. genetically modified organisms and the risk assessment problems related to their use., the future of nanotechnology will depend on public acceptance of the risks versus benefits from the nanomaterials. Consumers using ENMs can be affected by *inhalation exposure*. Especially, with ENMs that are smaller than 100 nm diameter and can potentially become airborne particles. These "nanostructured particles" are potentially of concern if they can deposit in the respiratory system of the consumer (nanoparticles have high surface area and surface activity). Classes of nanoparticles can cause respiratory toxicity to consumers especially for discretenanometer-diameter particles, agglomerates of nanoparticles, and droplets of nanomaterial solutions, suspensions, or slurries[39].

Skin or dermal penetration

Skin (or dermal) penetration is another form of exposure that concern toxicoilogists for the variety of consumer products with nanaomaterials. Skin can be exposed to solid nanoscale particles in cosmetics through either intentional or nonintentional means. Intentional dermal exposure to nanoscale materials may include the application of lotions or creams containing nanoscale TiO_2 or ZnO as a sunscreen component or fibrous materials coated with nanoscale substances for water or stain repellent properties. Nonintentional exposure could involve dermal contact with anthropomorphic substances generated during nanomaterial manufacture or combustion[40]. Despite the recent advances, it is unclear whether nanoparticles can penetrate the human skin and have any toxicological impact. Concerns regarding dermal penetration include skin or other organ cytotoxicity, accumulation, metabolism and ptotoactivation on skin. An example of dermal contact with nanoparticles, is the nanoscale TiO_2 and ZnO (<100 nm) which are included in sunscreens because of their ability to block ultraviolet (UV) light. It is known that TiO_2 particles below approximately 200 microns do not scatter visible light but will still scatter some UVA radiation. Thus the inclusion of nanoscale TiO_2 (anatase, rutile) or ZnO in sunscreens has the consumer-desired goal of a clear sunscreen with UV-absorbing properties. The surfaces of nanocrystals of TiO_2 can generate ROS which have the potential for cytotoxic reactions[41-43].

New scientific developments provide an alternative by avoiding the generation of ROS in commericial nanoproducts. For example, anatase TiO_2 nanoparticles are covered with inert oxides SiO_2, Al_2O_3 or zirconium[44]. But in general, studies showed that under UV irradiation sunscreens with TiO_2 produce ROS that significantly depend on their composition. The continuous in situ irradiation of TiO_2 powder, recommended for cosmetic application, investigated in different solvents (water, DMSO, isopropyl myristate) resulted in the generation of oxygen-centered reactive radical species (superoxide anion radical, hydroxyl radical, etc)[45]. Skin penetration by nanoscale TiO_2 is another issue of concern for toxicologists. Researchers applied TiO_2 into human skin either as an aqueous suspension or oil-in-water emulsion and evaluated skin penetration. They observed that TiO_2 apparently penetrated skin when applied as an oil-in-water emulsion, and that penetration was greater when applied to hairy skin, suggesting surface penetration through hair follicles or pores[46]. Another study in human skin provided compelling evidence that nanoparticles can achieve epidermal and dermal penetration (microsphere 0.5-1.0 μm)[47]. Recent studies showed that nanoparticle skin studies display, increasingly, a multidisciplinary character (penetration, toxicity studies) but their results are often contradictory. Toxicologists recommend standardisation of available test systems and focusing on the correlating physicochemical nanoparticle properties to penetration potential[48].

A study showed that UVB-damaged skin slightly enhanced TiO_2 nanoparticles or ZnO penetration in sunscreen formulations but no transdermal absorption was detected[49]. A recent review on the subject of skin penetration and dermal or percutaneous absorption of metal nanoparticlers and their effect on skin (especially TiO_2 and ZnO) presented results from various studies that contain contradictory data[50].

Pulmonary toxicity by inhalation

Pulmonary toxicity of carbon Nanotubes (single-walled NT are graphite sheets rolled into tubes, 1 nm in diameter and 1000 nm or more in length) have been studied in recent years. Some NT are capped at either end by half-fullerene domes to achieve great strength. Some nanotubes have a strong tendency to agglomerate by van der Waals forces into tattered ropes, whereas, others remain as a fine powder (much like carbon black.) is of great concern for exposure of many workers (in aerospace and other industries) and for consumers using miniature electronics. Under some conditions the NTs can reach the respiratory system and can penetrate deep into the lung. The NTs toxicity will also depend on whether they are persistent or cleared from the lung and whether the host can mount an effective response to sequester or dispose of the NT particles[51]. Nanotubes were proved to be at least as toxic as quartz (SiO_2) and much more toxic than carbon black (a form of amorphous carbon from incomplete combustion of fuels that has a high surface-area-to-volume ratio), with some indication of the effect of metal content on toxicity. The redox properties of iron in SWNT were implicated in oxidative stress and cytotoxicity in cell cultures of human keratinocytes[52].

These studies and other experimental findings implicate ENMs with respiratory human risks in the working environment. Nanomaterials inhaled into the lungs (depending on their content) are capable of eliciting an inflammatory, granulomatous, and fibrogenic response. Scientists suggest that permissible exposure level (PEL) for respirable graphite dust (legislated many decades ago) may be inadequately protective for exposure to SWNTs (single wall nanotubes). *In vivo* experiments with mice that were exposed to airborne nanotubes at a concentration of 5 mg/m^3, the PEL for respirable graphite dust, and 40% of the respired nanotubes deposited in the pulmonary region, the lungs would accumulate a mass of nanotubes equivalent to the low dose within 4 working days and a mass equivalent to the high dose within 17 working days. Moreover, because SWNTs were more toxic than quartz based on histopathology, assuming similar relative toxicity in humans, a PEL below that for quartz dust (0.05 mg/m^3) is suggested until further characterization of nanotube toxicity[53]. Some toxicological *in vivo* studies used rats, mice and hamsters that were exposed to fine-sized TiO_2 particles (300 nm), TiO_2 nanoscale rods or TiO_2 nanoscale dot particles (10 nm) were placed intratracheal instillation doses (1 to 5 mg/kg) in the body of experimental animals. Results have demonstrated no significant differences among any of the particle-exposed groups compared to vehicle controls with regard to inflammatory or cytotoxic lung responses at any postexposure time periods[54].

A recent review on toxicological data of titanium dioxide (TiO_2) nanoparticles focused on the respiratory system, showing the importance of inhalation as the primary route for exposure in the workplace and for consumer products. Oral exposure mainly occurs through food products containing TiO_2 -additives. Most dermal exposure studies (*in vivo* or *in vitro*) report that TiO_2 do not penetrate the stratum corneum (SC). In the field of nanomedicine, intravenous injection can deliver TiO_2 nanoparticulate carriers directly into the human body. Upon intravenous exposure, TiO_2 can induce pathological lesions of the liver, spleen, kidneys, and brain (at high concentration exposures). There is also an enormous lack of epidemiological data regarding TiO_2 nanoparticles. Long-term inhalation studies in rats have reported lung tumors[55].

Some other toxicological studies investigated the effects of various surface treatments (0–6% alumina [Al_2O_3] and/or 0–11% amorphous silica [SiO_2]) on the toxicity of commercial TiO_2 particle formulations. Pulmonary bioassay data from instillation exposures in rats to TiO_2 particle-type formulations (compared to reference base TiO_2 particle types). The TiO_2 particle formulations with the largest concentrations of both alumina and amorphous silica surface treatments produced mildly enhanced adverse pulmonary effects[56].

As noted from the various toxicological studies, the relevant inhalation dosimetry in risk assessments of nanoparticles may be surface area or particle number rather than mass per volume or per body weight, although the complexity of other properties preclude generalizations to all nanoparticles[57]. Despite this complexity, some patterns are emerging for the more studied nanomaterial substances. The primary mechanism of action by inhalation or dermal routes appears to be free radical generation and oxidative stress associated with surface reactivity. Oxidative stress associated with TiO_2 nanoparticles, for example, results in early inflammatory responses such as an increase in polymorphonuclear cells, impaired macrophage phagocytosis, and/or fibroproliferative changes in rodents[58].

Although most toxicological studies with nanomaterials have been *in vitro*, or short-term *in vivo* studies involving unnatural delivery (e.g., intratracheal instillation) in limited species and types of nanoparticles, the National Toxicology Program is planning short and long-term studies, including oral, dermal, and inhalation exposures for some nanoparticles (http://ntp-server.niehs.nih.gov/files/nanoscale05.pdf). Nanomaterial research and risk assessments will ultimately need to address multiple potential health effects including cardiovascular, carcinogenicity, reproductive/developmental, immunological, and neurological[59]. A review (2010) summarised the main concerns of adverse effects to health caused by acute or chronic exposure ENMs in the respiratory system. The lung is one of the main routes of entry for ENMs into the human body and once enter the interstitial air spaces are quickly taken up by alveolar cells. The review highlighted the different aspects of lung toxicity resulting from ENM exposure, such as generation of oxidative stress, DNA damage and inflammation leading to fibrosis and pneumoconiosis, and the underlying mechanisms causing pulmonary toxicity[60].

Another review (2013) summarized scientific evidence for the potential toxicity and safty issues of Enginered NanoParticles (ENPs) for the respiratory system (workers and consumers) and risk for lung cancer after prolonged exposure. Most studies showed that ENPs can cause pulmonary oxidative and pro-inflammatory gene expression in the lungs after chronic exposure at low doses stress[61].

RECENT TOXICOLOGICAL STUDIES: ARE ENMS CARCINOGENIC?

There is a constant stream of scientific papers in the last 5 years on the cytotoxicity of engineered nanomatrials using *in vitro* and *in vivo* studies and other methodological approaches. Alhough there are physicochemical differences of ENMs compared to particulate matter, fibrous materials and amorphous dusts, the mechanisms of toxicity and cytotoxicity must be very similar. *In vitro* studies were performed a standardized *in vitro* screening of 23 engineered nanomaterials (ENM) by adapting three classical *in vitro* toxicity assays to eliminate nanomaterial interference. Nanomaterial toxicity was assessed in ten representative cell lines. Six ENM induced oxidative cell stress while only a single nanomaterial reduced cellular metabolic activity, but none of the particles studied affected cell viability. Results suggested that surface chemistry, surface coating and chemical composition are likely determinants of nanomaterial toxicity. Scientists suggested that accurate identification of nanomaterial cytotoxicity requires a matrix based on a set of sensitive cell lines and *in vitro* assays measuring different cytotoxicity endpoints[62].

Scientists studied the cytotoxicity *in vitro* of gold nanoparticles since they are used in many products. These were 12 nm spherical gold nanoparticle coated or not with hyaluronic acid. Toxicological results ranging from the effects of a 10-days exposure in an *in vitro* model with BALB/c 3T3 fibroblast cells show how 12 nm spherical gold nanoparticles are internalized from 3T3 cells by endo-lysosomal pathway. Other results showed that gold nanoparticles, though not being a severe cytotoxicant, induce DNA damage probably through an indirect mechanism due to oxidative stress. Coating the gold ENP with hyaluronic acid reduces cytotoxicity and slows their cell internalization. These results will be of great interest to medicine. Gold ENP (with or without coating) are suitable for therapeutic applications due to their tunable cell uptake and low toxicity[63].

A recent review (2012) summarised many *in vitro* investigations about the toxicology of engineered nanoparticles. The evaluation of nanoparticles toxicity by *in vitro* studies gave toxicologists important information, especially in terms of toxic mechanisms. Some studies showed that some ENP induce oxidative stress, apoptosis, production of cytokines, and cell death. There are also studies of different results, some with low and some with high influences, for the same type nanoparticle. The aggregation state and metal ion release ability of nanoparticles affect its toxicological cellular effects. This inconsistency prompted scientists to want standardised methodologies[64].

In vivo studies for nanotoxicity are steadily emerging in the scientific literature of the last decade to evaluate biological impact of nanomaterial exposure in experimental animals. Over the last decade nanotoxicology methods have mostly relied on *in vitro* cell-based characterizations that do not account for the complexity of *in vivo* systems with respect to biodistribution, metabolism, hematology, immunology, and neurological ramifications. Efforts in standardizing methodology to study the *in vivo* safety of these materials are currently undertaken by various government agencies and research organizations[65]. Some other *In vivo* studies on ENMs were focusing on nanomaterials for wide applications in medicine, biological sensing, drug delivery and biomedical imaging. Experimental animals were used in these studies to evaluate the toxicity but also the biodistribution of ENMs and their in *vivo* pharmacokinetics pathways, depending of the surface chemistry, shape and sizes. Data are summarised in a recent review, including the toxicological debates on administrations routes, doses and surface functionalization which are critical to the *in vivo* toxicity studies[66].

Another important health risk of nanaoparticles that concerned scientists for a long time was their potential for carcinogenicity. A critical review (2011) for ENMs carcinogenicity was contacted by a working group of the German Federal Environment Agency and the German Federal Institute for Risk Assessment. The working group concluded that the potential carcinogenic risk of nanomaterials can be assessed only on a case-by-case basis. There is certain evidence that different forms of CNTs and nanoscale TiO_2 particles may induce tumours in sensitive animal models. The scientists of the working group assumed that the mode of action of the inhalation toxicity of asbestos-like fibres and of inhalable fractions of biopersistent fine dusts of low toxicity is very similar to nanoparticles. For example, it is known that nano-TiO_2 is linked to chronic ROS generation and inflammatory processes (pathways for the initiation of carcinogenicity). All epidemiological studies on carcinogenicity for a variety of manufactured nanomaterials are not sufficiently conclusive. The existing database is not adequate for risk assessment. Some studies provide evidence of a nano-specific potential to induce tumours, other studies did not (possibly due to insufficient characterisation, difference in the experimental design, use of different animal models and/or differences in dosimetry). An assessment of the carcinogenic potential and its relevance for humans are currently fraught with uncertainty. On the other hand, certain nano-properties such as small size, shape and reactivity, retention time and distribution in the body.as well as subcellular and molecular interactions may play a role in determining the carcinogenic potential of the nanomaterial. All of these factors leave no doubt about the carcinogenicity of ENPs need more research and more detailed epidemiological procedures[67].

Furthermore, in another review (2012) scientists analysed a series of studies for the potential of lung cancer for exposures to airborne manufactuted nanoparticles (MNPs). The reviewers concluded that low toxicity and low solubility MNPs are unlikely to pose a substantial lung cancer risk as they are not very biologically active. Probably nanoparticles with a more reactive surface can generate ROS and promote inflammation more readily. Inflammation could be sufficiently intense to lead to secondary carcinogenesis via the oxidants and mitogens produced during inflammation. There is some evidence from in vitro experiments that some MNPs can gain access to the DNA of the nucleus cause oxidative damage. MNPs that are fibre-shaped and have properties similar to asbestos fibers might pose a special cancer hazard to the lungs, pleural and peritoneal mesothelium[68].

NANOMATERIALS AS ENVIRONMENTAL POLLUTANTS

The dramatic rise of applications of ENMs and their use in electronic devices, consumer products, medicines and personal care products inevitably generated an emerging class of environmental pollutants. Some scientists suggest that existing regulations for chemical environmental pollutants are sufficient to predict ENMs distribution between environmental compartments (air, soil and water), some others believe that we need new rules to account for the specific properties of ENMs[69].

Over the past decade, researchers have made significant progress in understaning factors that influence the fate and transport of ENMs in environmental compartments, especially waste products from manufacturing in the aquatic environments. Environmentalists from the beginning employed the basic rules of toxicological monitoring, such as octanol–water partition coefficients, solid-water partition coefficients, rate constants describing reactions such as dissolution, sedimentation, and degradation. The first studies showed that ENMs appear to accumulate at the octanol–water interface and readily interact with other interfaces, such as lipid–water interfaces. However, ENMs probably do not behave in the same way as dissolved chemicals, and therefore, researchers need to use measurement techniques and concepts more commonly associated with colloids. Only a few structure–activity relationships have been developed for ENMs so far, but such evaluations will facilitate the understanding of the reactivities of different forms of a single ENM. The establishment of predictive capabilities for ENMs in the environment would enable accurate exposure assessments that would assist in ENM risk management[70].

This was an explroratory decade after 2000 of research efforts as far as environmental problems of ENMs are concerned. Scientists concentrated on the environmental methodologies and the analytical techniques of ENMs, especially fate, transport and toxicolological effects. The results forced them to realise that their studies were hampered by a lack of adequate analytical techniques fot he detection and quantification at environmental relevant concentrations in complex media. The traditional analytical techniques proved inadequate for the physicochemical forms of ENMs. The majority of ENMs in the environment are presented as colloidal systems and the surrounding environment affects their properties, making analysis susceptible to artefacts. The most pressing research needs at present for ENMs are the development of techniques for extraction, cleanup, separation, and sample storage that introduce minimal artefacts, increase sensitivity, and add specificity of analytical techniques. Scientists are also interested to develope techniques that can differentiate between abundant, naturally occurring particles, and manufactured nanoparticles[71].

Experimental analytical techniques showed that ENMs exhibit significant settling under normal gravitational conditions and they are also likely to exhibit significantly diminished diffusivities (when compared to truly dissolved species) in environmental media. It is known that air/water, air/soil, and water/soil intermedium transport is governed by diffusive processes in the absence of significant gravitational and inertial impaction processes in environmental systems. For example, in the case of significant atmospheric ENMs, nanoparticles exhibit an atmospheric residence time of ten to twenty days and atmospheric aggregates (range 10^{-6}-10^{-7} m) are the least likely to deposit in human respiratory system. Also, ENMs colloidal particles suspensions showed stability in water and aquatic exposure assessment models produce great uncertainty in their results[72].

Scientists agreed that there are at present scarse data on ENMs emissions and environmental concentrations. One of the few available ENMs studies investigated TiO_2 particles that are used in large quantities in exterior paints as whitening pigments. The TiO_2 particles were traced (roughly 20 and 300 nm) to the discharge into surface waters. Analytical electron microscopy revealed that TiO_2 particles are detached from facade paints by and are then transported by facade runoff and are discharged into natural waters. By combining results from microscopic investigations with bulk chemical analysis the researchers calculated the number densities of synthetic TiO_2 particles in the runoff[73].

Some experimental evidence is available on the release of nanosized materials from commercial textiles during washing. In one study researchers observed that dissolution of Ag-NPs occurs under conditions relevant to washing (pH 10) with dissolved concentrations 10 times lower than at pH 7. However, bleaching agents (H_2O_2, peracetic acid) can greatly accelerate the dissolution of Ag. The amount and form of Ag released from the fabrics as ionic and particulate Ag depended on the type of Ag-incorporation into the textile. These results have important implications for the risk assessment of Ag-textiles and environmental fate studies[74]. Another important aspect of silver nanoparticles is transport from antibacterial fabrics into sweat. After incubation of the fabrics in artificial sweat, silver was released from fabrics to varying extents, ranging from 0 to about 322 mg/kg of fabric weight.The quantity released dependent on the amount of silver coating, the fabric quality and the artificial sweat formulations including its pH[75].

Environmental pollution by ENMs is not very well researched. There is a handful of modelling studies that have investigated ENM release to the environment. Sewage sludge, wastewater, and waste incineration of products containing ENM were shown to be the major flows through which ENMs end up in the environment. However, reliable data are particularly lacking on release during ENM production and applications. Quantitative data linking occupational exposure measurements and ENM emission flows into the environment are almost completely missing[76].

Nanotechnological applications and their associated environmental problems are reflected in the numerous publications the last few years. Here we present a selection of books focusing on the environmental problems and health and safety issues by ENMs[77-82].

NANOECOTOXICOLOGY AND RISK ASSESSMENT

Nanotechnology applications raised many of the same issues as any new technology, including concerns about environmental impact of nanomaterials in ecosystems. It is estimates that over 1000 manufacturer-nanotechnology products are publicly available now and 4-5 new applications are coming into the market every week. The rapid application of nanotechnology products in the last decade formulate the need for a new subdiscipline of ecotoxicology that is called Nanoecotoxicology, with the first scientific papers starting in 2006. The ecotoxicological problems from ENMs and their presence in the natural environment and the ecosystems are challenging tasks for environmental toxicologists and ecological risk assessment speciailists[83].

In the decade, the scientific discipline ENM ecotoxicology faces two important and challenging problems: a) the analysis of the safety of nanotechnologies in the natural environment and b) the promotion of sustainable development while mitigating the potential pitfalls of innovative nanotechnologies. The most important concern inevitably is focused on the problems of pollution on the quatic environmental compartments. Nanoecotoxicology studies until now focused on the aquatic freshwater species and soil organisms[84-86].

Also, the new REACH regulation in the European Union for environmental safety of commercial chemicals (Registration, Evaluation, Authorization and Restricition, 2007) promoted a series of nanoecotoxicology studies. These studies focused on adverse effects of nanoparticles on fish, algae and daphnids, which are ecotoxicological model organisms for classification and labeling of chemicals. At present the ecotoxicological literature contains numerous studies which used a battery of selected test organisms at different food-chain levels. Unlike in the past, one single biotest can not predict ecotoxicological effects in complex ecosystems at different levels, so 3-4 tests are used. The initial nanoecotoxicology studies focused on ENMs of TiO_2, ZnO and CuO and other materials that proved toxic to several aquatic invertebrate test species[87].

For example, in laboratory experiments the impact of TiO_2 nanoparticles was observed in the population dynamics and production of biomass across a range of freshwater algae. Researchers exposed 10 of the most common species of North American freshwater pelagic algae for 25 days (phytoplankton) to five increasing concentrations of n-TiO_2 (ranging from controls to 300 mg n-TiO_2 L^{-1}). On average, increasing concentrations of n-TiO_2 had no significant effects on algal growth rates. Although titanium TiO_2 nanoparticles could influence certain aspects of population growth of freshwater phytoplankton, the effects are unlikely at environmentally relevant concentrations[88].

The zooplankton *Daphnia magna* is a typical test organism that is used in ecotoxicological studies. Scientists combined a chronic flow-through exposure system with subsequent acute toxicity tests for the standard test organism *Daphnia magna*. Their results showed that juvenile offspring of adults that were previously exposed to TiO_2 nanoparticles exhibit a significantly increased sensitivity, compared with the offspring of unexposed adults, as displayed by lower 96 hours-EC_{50} values. Researchers concluded that ecotoxicological research requires further development to include the assessment of the environmental risks of nanoparticles for the next and hence not directly exposed generation, which is currently not included in standard test protocols[89].

Soil eartworms test is another standard test in ecotoxicological studies for soil pollution. A recent study with earthworms (*Eisenia Andrei* and *Eisenia fetida*) tested the toxicity of TiO_2 nanomaterials. Three types of commercially available uncoated TiO_2 nano-materials were used (diameters 5, 10 and 21 nm). Exposure test were conducetd to field and to artificial soil containing between 200 and 10,000 mg nano-TiO_2 (mg/kg). Results showed no significant effect on juvenile survival and growth and adult earthworm survival However, earthworms avoided artificial soils amended with nano-TiO_2. Researchers concluded that earthworms can detect nano-TiO_2 in soil, although exposure has no apparent effect on survival or standard reproductive parameters[90].

Bivalve molluscs are established for biomonitoring of toxic contaminants and ecotoxicological studies (because of their abundance in freshwater and marine ecosystems as suspension feeders). Bivalve molluscs represent a particularly suitable aquatic model organism for investigating the effects and mechanisms of action underlying the potential toxicity of ENMs in marine invertebrates. As suspension-feeders, bivalves have highly developed processes for cellular internalization of nano- and micro-scale particles (endo- and phagocytosis), integral to key physiological functions such as intra-cellular digestion. Researchers have exposed in particular mussels *Mytilus spp* at different types of ENMs. The *in vivo* experimental results indicate that, due to the physiological mechanisms involved in the feeding process, ENMs agglomerates or aggregates are taken up by the gills and then directed to the digestive gland, where intra-cellular uptake of nanosized materials induces lysosomal perturbations and oxidative stress[91].

Another study examined the uptake of nanoparticles by two species of suspension-feeding bivalves (mussels *Mytilus edulis*, and oysters *Crassostrea virginica*), which capture individual particles with diameter <1nm with a retention efficiency of around 15% or more. Results indicated that aggregates of NP significantly enhance the uptake of 100-nm particles. Nanoparticles had a longer gut retention time suggesting that nanoparticles were transported to the digestive gland. Researchers suggested that their data tend to indicate a mechanism for significant nanoparticle ingestion by marine species. Inevitably, this wiil have implications for further toxicological effects and transfer of ENMs to higher trophic levels[92].

Marine bivalves were used for tests of toxicity of gold nanoparticles (AuNPs) that are used in many nanomaterials. The marine bivalve *Scrobicularia plana* was exposed to AuNPs of size (size 5, 15 and 40 nm, at concentrations 100 ìg Au L^{-1}) for 16 days under laboratory conditions. at 100 µg. Results showed that the clams accumulated gold in their soft tissues. The response was metallothionein induction (cysteine-rich, low MW proteins with capacity to bind metals and xenobiotics) and increased antioxidant enzyme activities of catalase (CAT), superoxide dismutase (SOD) and of glutathione S-transferase (GST). These responses are typical of increased oxidative stress However, the researchers underlined that these effects were observed at a dose much higher than expected in the environment[93].

A recent critical review focused on the scientific literature on carbon nanotubes (CNTs) in environmental pollution, especially from polymeric products. The review included papers on transport through surface and subsurface media, aggregation behaviours and interaction with soil and sediment particles, potential transformations and degradation, and their potential ecotoxicity in soil, sediment, and aquatic ecosystems. The reviewers from the data concluded that one of major limitation of research is the quantification of CNT masses in relevant media[94].

Research showed that CNTs many influence the bioaccumulation and fate of other pollutants in environmental systems because they have strong sorptive capacities for metals and various hydrophobic organic chemicals. CNTs may act in a manner similar to charcoal or black carbon by sequestering such compounds and limiting their bioavailability and mobility. It is also possible that nanotubes could serve as concentrators, durable sources, and transporters of such chemicals into organisms, thus exacerbating bioaccumulation and food chain transfer[95].

Practical experiences on ecotoxicology research with ENMs are documented in another review and reviewers recommend changes in the challenging problems to assist researchers. The reviewers focused on nano-specific modifications of ecotoxicological protocols and the maintenance of exposure concentrations. Also, they considered generic practical issues, as well as specific issues for aquatic tests, marine grazers, soil organisms, and bioaccumulation studies. They recommend that current Invertebrate (*Daphnia*) ecotoxicity tests should account for mechanical toxicity of ENMs. Fish tests should consider semistatic exposure to minimize wastewater and animal husbandry. The inclusion of a benthic test is recommended for the base set of ecotoxicity tests with ENMs. The sensitivity of soil tests needs to be increased for ENMs and shortened for logistics reasons[96].

In May 2012 the European Network on the Health and Environmental Impact of Nanomaterisls (NonaImpactNet) released a report on nanomaterials and the collective experience of working at the research bench with ENMs. The researchers-reviewers in this report recommended modifications to existing experimental methods and OECD protocols. They provided details of experimental procedures on electron microscopy, dark-field microscopy, a range of spectroscopic methods, light scattering techniques and chromatographic techniques. The reviewers concluded that most ecotoxicity protocols will require some modifications[97].

From the presentation of the above selected ecotoxicological studies and reports we can conclude that for the moment there are many challenges and difficulties in interpretation of data from ecotoxciological tests concerning ENMs toxicity towards ecosystems. Ecotoxicologists propose reasonable modifications and adjustements of the standard ecotoxicity tests to the requirements of ENMs physicochemical characteristics.

FUTURE CHALLENGERS FOR RISK ASSESSMENT OF NEW NANOMATERIALS

Engineered nanomaterials in the last decade and their applications for various commercial products have advanced substantially. Novel materials at size of 100 nm or less has become one of the most promising areas of nanotechnology. Because of their intrinsic properties, nanoparticles are commonly employed in electronics, photovoltaic, catalysis, environmental and space engineering, cosmetic industry and in medicine and pharmacy. All these new products forced toxicologists and ecotoxciologists to deal with new challenges concering their toxicological assessment.

It has been largely recognised by many scientists that substantial limitations and uncertainties make the conventional risk assessment (RA) of chemicals unfeasible to apply to engineered nanomaterials. This fact offers new challenges in methodological toxicological apprioaches which leaves the health and safety regulators and environmental lawmakers with little support in the near future for regulating ENMs[98,99].

The future challenge facing scientists for ENMs toxicological assessmement have been collected in a recent study, with emphasis for cosmetics and textiles[100,101].

Nanomaterials from TiO_2 or ZnO	UV- protection
Silver nanoparticles	Anti-bacterial (e.g. in deodorants)
Fullerene (C_{60})	Antioxidants, radical scavenging creams
Pigments	Coloring
Silica nanoparticlers	Absorbance of oil, long-lasting cosmetics
Hydroxylapatite	Toothpaste (remineralizing)
Liposomes	Supply of e.g. vitamins

Cosmetics (nanomaterials and their applications)

Silver nanoparticles	Anti-bacterial properties
ZnO or TiO_2	UV- protection
TiO_2 or MgO	Self-sterilizing (chemical, biological protection)

SiO_2, Al_2O_3 with special coating	Water repelent
Cermaic nanoparticles	Abrasion resistance in textiles
Nanoclay	Electrical, heat, thermal resistance
Nanocellulose	Anti-wrinkle properties
Ferrum (iron, Fe, compounds) or others	Functional textilers (e.g. conductive properties)
Carbon nanotubes (CNTs)	Stronger fibers for textiles

Textiles (nanomaterilas and applications

CONCLUSIONS

The presentation of the most recent studies and reviews on the toxicity and ecotoxicity assessment of ENMs showed that there are not alerting human health and safety problems with nanotechnology applications. However, the emerging toxicological problems and uncertainties due to the special ENMs physicochemical characteristics give substantial new thoughts to regulators of national policies that guarantee the responsible development of nanotechnologies. The environmental pollution problems and impact to ecosystems by ENMs are at the forefront of concern of many national and international scientific and environmental organizations.

As far as to the reliable risk assessments of ENMs is concerned, until now there is still the remaining issue to be resolved of whether or not specific challenges and unique features exist on the nanoscale that have to be tackled and distinctively addressed, given that they substantially differ from those encountered with microsized materials or regular chemicals.

The safety evaluation and assessment of manufactured nanomaterials that ensure human health and environmental protection are overseen by the international Organization of Economic Co-operation and Development. The OECD's *Working Party on Manufactured Nanomaterials* (WPMN) is a scientific body that concentrates on human health and environmental safety implications of manufactured nanomaterials and aims to ensure that the approach to hazard, exposure and risk assessment is of a high, science-based, and internationally harmonised standard. Its programme seeks to promote international cooperation on the human health and environmental safety of manufactured nanomaterials, and involves the safety testing and risk assessment of manufactured nanomaterials. The future priorities for OECD for ENMs are: establishing an OECD database, testing ENMS for their health and safety evaluation, promoting alternative test methods for nano-toxicity, facilitating international co-operation, developing guidance on exposure measurements and promoting the environmental sustainable use of nanotechnology[102].

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We are independent researchers researching human health and environmental safety issues by emerging chemical hazards. We used the most reliable scientyific reports and peer-reviewed scientific research papers. We are publishing regularly (without financial support) reviews on scientific advances concerning environmental chemistry, toxicology and ecotoxicology issues on our website: www.chem-toc-ecotoc.org.

DISCLOSURE

The authors report no conflict of interest in this work

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