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Review

Hazards and Risks of Engineered Nanoparticles for the Environment and Human Health

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Abstract: The objectives of this article are to: (1) investigate the current state of knowledge of the risks of engineered nanoparticles for the environment and human health, (2) estimate whether this knowledge is sufficient to facilitate their comprehensive and effective risk assessment and (3) provide recommendations on future research in the field of risk assessment of nanomaterials. In order to meet the objectives, the relevance of each of the four steps of the risk assessment methodology (*i.e.*, hazard identification, dose-response assessment, exposure assessment and risk characterization) was evaluated in the context of the current state of knowledge of the risks of nanomaterials, limitations were identified and recommendations were given on how to overcome them.

Keywords: engineered nanoparticles; risk assessment; hazard identification; dose-response assessment; exposure assessment; risk characterization; environmental sustainability; human health

1. Introduction

1.1. Background

In contrast to the small size of the nanoparticles, the scale of their application is tremendous. Nanotechnology influences virtually all industrial and public sectors, including healthcare, agriculture, transport, energy, materials, information and communication technologies.

Both the potential benefits and the risks, associated with the application of engineered nanoparticles (ENPs) have been widely debated in recent years. In contrast to the dominating optimistic projections that nanotechnology will bring significant technological development and well-being to society, it is considered that exposure to certain ENPs may cause environmental problems and/or do harm to human health. Since the early discussions about the risks of ENPs, the chemical risk assessment (CRA) has been put forward as the most relevant approach to understand, evaluate and quantify these risks. Currently, a variety of methodologies are being internationally discussed and evaluated with great vengeance with the idea that, in the near future, it will be possible to perform complete and scientifically sound risk assessment of ENPs.

1.2. Objectives

The objectives of this article are to:

1. Investigate the current state of knowledge of the risks of ENPs for the environment and human health.
2. Estimate whether this knowledge is sufficient to facilitate comprehensive and effective risk assessment of ENPs.
3. Provide recommendations on future research in the field of risk assessment of ENPs.

1.3. Methodology

This article is based on an extensive review of literature published in the period: January 1992–September 2009. The selected literature consisted mainly of scientific publications, but also books, information from conferences and patent data were used.

2. Nanotechnology and Its Applications

2.1. Nanotechnology and Nanoparticles

"Nanotechnology" is a field of applied science and technology, dealing with the organization and control of matter on the nano-scale (*i.e.*, between 1 and 100 nm) and the manufacturing of products and devices with dimensions, lying within this size range. A nanometer (nm), from the Greek "nanos" for "dwarf", equals one billionth of a meter.

"Nanomaterials" are all materials with sizes on the nano-scale in at least one of their dimensions [1], while "nanoparticles" are materials, nano-sized in at least two dimensions [2]. The nomenclature "nanoparticles" encompasses particles as well as fibrous materials and tubes, but it excludes materials, such as coatings, films and multilayers.

Two types of nanoparticles (NPs) can be distinguished: (1) naturally occurring NPs (e.g., produced naturally in volcanoes, forest fires or as combustion by-products) and (2) engineered nanoparticles (ENPs), deliberately developed to be used in application (e.g., carbon black, fumed silica, titanium dioxide (TiO_2), iron oxide (FO_x), quantum dots (QDs), fullerenes, carbon nanotubes (CNTs),

dendrimers). Naturally occurring NPs do NOT fall in the scope of this article. The paper encompasses only ENPs. The main reasons why materials built of ENPs have different optical, electrical, magnetic, chemical and mechanical properties from their bulk counterparts are that in this size-range quantum effects start to predominate and the surface-area-to-volume ratio (sa/vol) becomes very large [1]. The sa/vol of most materials increases gradually as their particles become smaller, which results in increased adsorption of the surrounding atoms and changes their properties and behavior. Once particles become small enough, they start to obey the quantum mechanical laws. Materials reduced to the nano-scale can suddenly show very different properties, compared to what they exhibit on the macro-scale, which enables unique applications. For example, opaque substances become transparent (copper); stable materials become combustible (aluminum); inert materials become catalysts (platinum); insulators become conductors (silicon); solids turn into liquids at room temperature (gold) [3].

2.2. Areas of Application

Today, nanotechnology is available on the market for great variety of applications. Some examples are: cosmetics and sunscreens, water filtrations, glare filters, ink, stain-resistant clothing, more durable tennis balls, more lightweight tennis rackets, dressings for burns or injuries [4].

Table 1. Nanotechnology areas of application (modified after [4]).

Areas	Applications
Automotive	Lightweight construction; Catalysts; Painting; Tires; Sensors; Windshield and body coatings
Construction	Materials; Insulation; Flame retardants; Surface coatings; Mortar
Electronics	Displays; Data memory; Laser diodes; Fiber optics; Optical switches; Filters; Conductive coatings; Antistatic coatings; Transistors
Engineering	Protective coatings for tools, machines; Lubricant-free bearings
Food and Drink	Packaging; Storage life sensors; Additives; Juice clarifiers
Medicine	Drug delivery systems; Contrast medium; Rapid testing systems; Prostheses and implants; Antimicrobial agents; In-body diagnostic systems
Textiles	Surface coatings; "Smart" clothes (anti-wrinkle, stain resistant, temperature controlled)
Chemical	Fillers for paints; Composite materials; Impregnation of papers; Adhesives; Magnetic fluids
Cosmetics	Sunscreen; Lipsticks; Skin creams; Toothpaste
Energy	Lighting; Fuel cells; Solar cells; Batteries; Capacitors
Environmental	Environmental monitoring; Soil and groundwater remediation; Toxic exposure sensors; Fuel changing catalysts; Green chemistry
Household	Ceramic coatings for irons; Odor removers; Cleaners for glass, ceramics, metals
Sports	Ski wax; Tennis rackets; Golf clubs; Tennis balls; Antifouling coatings for boats; Antifogging coatings for glasses, goggles
Military	Neutralization materials for chemical weapons, bullet-proof protection

3. Defining "Hazard" and "Risk"

The term "hazard" has many definitions. This paper uses the definition of the United States Environmental Protection Agency (EPA) which defines 'hazard' as the "inherent toxicity of a compound" [5]. According to this definition, if a chemical substance has the property of being toxic, it is therefore hazardous. Any exposure to a hazardous substance may lead to adverse health effects in individuals or even death.

EPA defines "risk" with respect to the above definition of "hazard" as "a measure of the probability that damage to life, health, property, and/or the environment will occur as a result of a given hazard" [5]. According to this definition, if the probability of an exposure to a hazardous material is high and the consequences for the health or environment are significant, then the risk is considered to be high. It is important to consider both the frequency of the event and the degree of the hazard to estimate risk [2].

Usually two categories of risk are distinguished in literature: "known risks" and "potential risks". When the relation between a cause and an effect is established, we talk of "known" risks. The responsibility for such risks can generally be attributed. When the causal relationship is established, prevention is possible. When the relationship between a cause and damage is not well known, we talk of "potential" risks. In case of potential risks, it is unclear whether there is a danger, how significant the damage can be or what is the probability of its occurrence [2, after 6]. This situation is characterized by a state of suspicion (not awareness) and it is generally admitted that a precautionary approach can be applied in order to prevent potential damage [2, after 6]. The risks of ENPs for the environment and human health fail in the second category: potential risks. It is very important to assess the risks of hazardous agents. The likelihood that a hazardous substance will cause harm (the risk) is the determinant of how cautious one should be and what preventative or precautionary measures should be taken.

4. Risk Assessment of ENPs

Since the early debates about the potential hazards of ENPs, the risk assessment of chemicals (CRA) has been put forward as the most relevant approach to understand and quantify the related risks [7]. CRA is a process, in which scientific and regulatory principles are applied in a systematic fashion in order to describe the hazards, associated with the environmental and/or human exposure to chemical substances. It is defined as "a process, intended to calculate or estimate the risk to a given target organism, system or (sub)population, including the identification of attendant uncertainties, following exposure to a particular agent, taking into account the inherent characteristics of the agent of concern, as well as the characteristics of the specific target system" [8]. The CRA is a four-step process, consisting of: (1) hazard identification, (2) dose-response assessment, (3) exposure assessment and (4) risk characterization. Its main outcome is a statement of the probability that when humans or other environmental receptors (e.g., plants, animals) are exposed to a chemical agent, they will be harmed and to what degree.

The CRA methodology is internationally recognized and employed by major actors, such as the World Health Organization (WHO) and the Organization for Economic Co-operation and

Development (OECD), as well as by several European and U.S. agencies [9]. It is considered a valuable tool, very important for the regulation of chemicals. CRA is also a fundamental ingredient of the new European Union (EU) chemical regulation policy, known as Registration, Evaluation and Authorization of Chemicals (REACH).

In order to achieve the objectives of this study, the current state of knowledge of the risks of ENPs for the environment and human health were summarized and evaluated in relation to each of the four elements of the CRA framework, as more important scientific findings were highlighted and limitations were identified and discussed.

4.1. Hazard Identification

"Hazard identification" (HI) is defined as the "…identification of the adverse effects, which a substance has an inherent capacity to cause" [10, after 11]. Until recently, much of the discussion about the environmental and health risks of ENPs was considered to be rather speculative than realistic. In the last few years, however, a number of experimental studies found that exposure to certain ENPs can lead to adverse health effects in living organisms. In 2007, Hansen *et al.* identified 428 studies reporting on toxicity of ENPs [12]. In these studies, adverse health effects of 965 tested ENPs of various chemical compositions were observed [12].

4.1.1. Current state of knowledge

The following sections shortly describe some of the most important scientific findings, relevant for HI of ENPs. Their purpose is to summarize the current state of knowledge of the hazards of ENPs, based on experimental studies. For simplification, the studies are divided into two categories—*in vivo* and *in vitro* studies.

In vivo studies

Carbon nanotubes (CNTs)

A study, performed by Lam *et al.* [13], demonstrated that single-walled carbon nanotubes (SWCNTs) are able to cause dose-dependent effects of interstitial inflammation and lesions in *mice* and *rats* (0–0.5 mg kg^{-1} for 7 to 90 days). Warheit *et al.* [14] observed pulmonary grandulomas in *rats* after exposure to SWCNT soot (1 and 5 mg kg^{-1} for 24 hours to 3 months). In contrast to Lam *et al.* [13], however, the effects, observed by Warheit *et al.* [14] were not dependent on dose. Smith *et al.* [15] tested the ecotoxicity of SWCNTs, dissolved in sodium dodecyl sulphate (SDS) and sonication on *juvenile rainbow trout* (0.1, 0.25 and 0.5 mg L^{-1} for 24 hours to 10 days) and they observed a dose-dependent rise in ventilation rate, gill pathologies (oedema, altered mucocytes, hyperplasia), and mucus secretion with SWCNT precipitation on the gill mucus. They also observed a significant dose-dependent decrease in thiobarbituric acid reactive substances (TBARS), especially in the gill, brain and liver, which is an indication of oxidative stress.

Multi-walled carbon nanotubes (MWCNTs) were shown by Carrero-Sanchez *et al.* [16], to exhibit acute toxicity in *rats* with LD_{90} of 5 mg kg^{-1}. Long MWCNTs were shown by Poland *et al.* [17] to cause significant inflammation and tissue damage in *mice*, while shorter MWCNTs caused less inflammation, which suggests that CNT toxicity is influenced by the particle morphology. In addition, they concluded that water-soluble components of MWCNT do not produce strong inflammatory effects in *mice*.

C_{60} fullerenes

Most studies on the toxicological effects of C_{60} fullerenes suggest that these materials tend to induce oxidative stress in living organisms [18-21]. Lai *et al.* [18] observed a significant increase in lipid peroxidation (LP) products (a sign of oxidative stress) after intravenous administration of 1 mg kg^{-1} C_{60} $(OH)_{18}$ in male *mongrel dogs*. Oberdörster [19,20] studied the effects of C_{60} fullerenes in the brain of *juvenile largemouth bass* and observed high LP levels (0.5 and 1 ppm for 48 h). Elevated LP was also observed by Zhu *et al.* [21] in the brain and gills of *daphnia magna* after exposure to hydroxylated C_{60} fullerenes (C_{60} $(OH)_{24}$) and tetrahydrofuran (THF)-dissolved C_{60}, as it was shown that THF did not contribute to the effect. Sayes *et al.* [22] detected an increase in the numbers of bronchoalveolar lavage (BAL)-recovered neutrophils (*i.e.*, white blood cells) after intratracheal instillation of C_{60} and C_{60} $(OH)_{24}$ in *rats*, 1 day after the exposure. They also observed a significant increase in LP values 1 week after the exposure. Accute effects of functionalized C_{60} were also reported. Zhu *et al.* [21] estimated LC_{100} in *fathead minnow* after exposure to 0.5 ppm of THF-dissolved C_{60} for 6–18 hours. Chen *et al.* [23] observed a LD_{50} of 600 mg kg^{-1} polyalkylsulfonated C_{60} in female *rats* after intraperitoneal administration (0–2,500 mg kg^{-1} for up to two weeks). Oberdörster [24] tested uncoated, water soluble, colloidal C_{60} fullerenes and estimated a *Daphnid* 48-hour LC_{50} of 800 ppb.

Metal and metal oxide ENPs

Li *et al.* [25] found that metal ENPs induce more severe lung toxicity in *mice* than bulk particles from the same materials. Gordon *et al.* [26] tested the effects on *humans* of exposure to zinc (Zn) ENPs. After 2 hours of exposure to 5 mg m^{-3} of Zn ENPs, the exposed individuals started feeling sore throat, chest tightness, headache, fever and chills. Beckett *et al.* [27] repeated that test in three trials, 2 hours each, but at lower concentration (*i.e.*, 500 μg m^{-3}), and found no indication of adverse effects. The latter two studies suggest that Zn ENPs toxicity is concentration-dependent and the most probable uptake path is through the respiratory system. A study of Sayes *et al.* [22] concluded that environmental exposure to Zn ENPs causes pulmonary (lung) inflammatory response in *mice*. Wang *et al.* [28] found that Zn ENPs can cause severe symptoms of lethargy, anorexia, vomiting, diarrhea, loss of body weight and even death in *mice* when gastrointestinally administered, whereas they observed limited effect for micro-scale Zn at equal concentrations. Yang and Watts [29] tested the effect of Aluminium (Al) ENPs on the relative root growth (RRG) in *Zea mays* (corn), *Glycine max* (soybean), *Brassica oleracea* (cabbage), and *Daucus carota* (carrot). The study found that the ENPs significantly inhibited the growth of the plants after administration of 2 mg mL^{-1} for 24 h.

Oberdörster [30] and Oberdörster *et al.* [31] observed that smaller TiO_2 ENPs tend to cause more severe pulmonary damage in *mice* than larger particles. In addition, Warheit *et al.* [32] found that smaller silicon dioxide (SiO_2) particles cause stronger lung inflammation in *rats* than larger ones. Wang *et al.*, [33] noticed that the smaller the TiO_2 particle size is, the greater the concentration in the liver of *mice* is. Bourrinet *et al.* [34] reported hypoactivity, ataxia, emesis, exophthalmos, salivation, lacrimation, discolored and mucoid feces, injected sclera, and yellow eyes in *dogs* after single-dose intravenous bolus administration of 20 and 200 mg kg^{-1} FeO ENPs and a significant increase in fetal skeletal malformations in *rats* and *rabbits*.

In vitro studies

Carbon nanotubes (CNTs)

A number of cytotoxicity studies with SWCNTs were reported in the literature. Shvedova *et al.* [35] observed oxidative stress and cellular toxicity in human epidermal keratinocytes, after 2 to 18 hours exposure to unrefined (iron containing) SWCNTs in concentrations, ranging from 0.6 to 0.24 mg mL^{-1}. Cui *et al.* [36] observed dose-and time-dependent inhibition of cell proliferation and a decrease in cell adhesive ability in human embryo kidney cells after exposure to SWCNTs in concentrations between 0.8 and 200 μg mL^{-1}. Sayes *et al.* [37] found that the surface functionalization of SWCNTs plays an important role in their cytotoxicity towards human dermal fibroblasts. Bottini *et al.* [38] noticed that MWCNTs were more cytotoxic when oxized towards Jurkat T leukemia cells, whereas Monteriro-Riviere *et al.* [39] observed a decrease of the viability of human osteoblastic lines and human epidermal keratinocytes after exposures to 0.1, 0.2, and 0.4 mg mL^{-1} of MWCNTs for 1 to 48 hours. Kang *et al.* [40] compared the cytotoxicity of commercially obtained MWCNTs in bacterial systems before and after physicochemical modification and they observed highest toxicity when the nanotubes were uncapped, debundled, short, and dispersed in solution. Kang *et al.* [40] concluded that there is need for careful documentation of the physical and chemical characteristics of CNTs, when reporting their toxicity.

C_{60} fullerenes

Adelman *et al.* [41] observed a reduction of the viability of bovine alveolar macrophages after exposure to sonicated C_{60} and increased levels of cytokine mediators of inflammation (*i.e.*, IL-6, IL-8 and TNF), while Porter *et al.* [42] found that C_{60} and raw soot were not toxic towards bovine-and human alveolar macrophages. The reason behind the discrepancy between the results of Adelman *et al.* and Porter *et al.* can be attributed to the fact that they used very different methods. Porter *et al.* used transmission electron microscopy (TEM) to image the distributions of the fullerenes within the macrophages, while Adelman *et al.* used a viability assay, based on metabolic activity as primary parameter.

Studies on the effects of ENPs on alveolar macrophages are very important because the alveolar macrophages are the first line of cellular defense against respiratory pathogens [11, after 43]. Yamawaki and Iwai [44] observed dose-dependent cytotoxicity of C_{60} $(OH)_{24}$ (1–100 μg mL^{-1}

for 24 hours), resulting in decreased cell density and lactate dehydrogenase (LDH) release in human umbilical vein endothelial cells cavity (a sign of increase in non-viable cell numbers). Rouse *et al.* [45] observed a dose-dependent decrease in the viability of human epidermeal keratinocytes after exposure to C_{60}-phenylalanine, as no contribution to the effect was attributed to the phenylalanine groups.

Quantum dots (QDs)

The toxicity of QDs was found to be influenced by several factors: (1) composition, (2) size, (3) surface charge and (4) coating of the QDs [7,46-48]. Jaiswal *et al.* [46] found that CdSe/ZnS QDs (*i.e.*, CdSe QDs in a zinc sulfide (ZnS) matrix), coated with dihydrolipoic acid (DHLA) had no effect on mammalian cells, while Hoshino *et al.* [47] reported adverse effects on *mouse* lymphocytes after exposure to CdSe/ZnS QDs, coated with albumin. In addition, Lovrić *et al.* [48] observed that smaller (2.2 ± 0.1 nm), positively charged QDs exhibit stronger cytotoxicity than larger (5.2 ± 0.1 nm), equally charged QDs under the same conditions. It was also found that the cytotoxicity of QDs is influenced by the exposure to light and by temperature [49,50]. Green and Howman [49] observed 56% damaged DNA after exposure to CdSe/ZnS together with UV light versus only 29% after exposure to CdSe/Zn in the absence of UV light. Chang *et al.* [50] found that CdSe/CdS (*i.e.*, CdSe QDs in a cadmium sulfide (CdS) matrix) were toxic to cancer cells at 37 °C, but at 4 °C they were not toxic at all.

Metal and metal oxide ENPs

Sayes *et al.* [51] found that anatase TiO_2 ENPs are able to kill human dermal fibroblast (HDF) cells at LC_{50} of 3.6 μg mL^{-1}, while Wang *et al.* [52] observed decrease in the viability of human lymphoblastoid cells due to exposure to TiO_2 ENPs (0–130 μg mL^{-1} for 6–48 h). Chen & Mikecz [53] found that SiO_2 ENPs do significantly inhibit replication and transcription in human epithelial HEp-2 cells (25 μg mL^{-1} for 24 h). Muller *et al.* [54] observed that Fe_3O_4 ENPs, coated with dextran, decrease the viability of human monocyte macrophages. Alt *et al.* [55] found that nano-particulate silver (Ag) is an effective bactericide against *S. epidermidis*, while Baker *et al.* [56] noticed that it effectively kills *E. coli* bacteria too. Sayes *et al.* [57] observed an increase in the production of LDH levels (an indicator of inflammation) in immortalized rat lung epithelial cells after 1 hour exposure to Zn ENPs at 520 μg cm^{-2}.

4.1.2. Limitations to hazard identification of ENPs

It is very important to note that the vast majority of the reviewed studies demonstrate some degree of hazardous effects on the tested organisms. Toxicity has been reported for many ENPs, as shown in the previous sections, but for most of them further investigation and confirmation are needed before hazard can be identified. A lot of studies, relevant for HI, have been carried out with different ENPs, but most of them were obviously not meant to facilitate risk assessment; they use non-standardized tests, differing greatly from each other in regard to endpoints, tested species, methods of

administration, dose ranges and exposure periods [7]. The lack of standardized testing results in non-reproducible results and makes the univocal HI of ENPs impossible.

Another significant drawback for the HI of ENPs is the serious lack of characterization data, which makes it difficult to identify which physical and/or chemical characteristics (or combinations of characteristics) determine the hazards, documented in the (eco)toxicological studies [12,58,59].

4.2. Dose-Response Assessment

"Dose-response assessment" (DRA) is defined as "…an estimation of the relationship between dose, or level of exposure to a substance, and the incidence and severity of an effect" [10, after 11]. It is the process of characterizing the relationship between the dose of an agent, administered to or received by an individual, and the consequent adverse health effects.

4.2.1. The concept of "dose"

In toxicological studies a "dose" is the quantity of anything that may be received by or administered to an organism. The "dose" is normally measured in mass units (*i.e.*, μg, mg, g), as higher doses of the same compounds are expected to cause more severe adverse effects.

DRA studies with ENPs, however, suggest that the toxicity of some ENPs is not mass-dependent, but influenced by other physico-chemical characteristics (e.g., surface area, chemical composition, particle morphology) [7, after 60]. Oberdörster *et al.* [61] and Stoeger *et al.* [62,63] found that the toxicity of low-soluble ENPs was better described by their surface area than by their total mass [7, after 61-63]. Wittmaack [64,65] suggested the number of particles as the most appropriate dose metrics, while Warheit *et al.* [66,67] found that toxicity of some ENPs was associated with the number of their surface functional groups. Despite these findings, however, it is still largely unknown which properties influence the toxicity of most ENPs and this gap in knowledge is partly attributable to the fact that the tested ENPs are seldom well characterized.

4.2.2. Characterization of ENPs

Developing understanding about the physical and chemical properties of substances and materials is fundamental for their risk assessment [59]. Studying the standard properties (e.g., composition, structure, molecular weight, melting point, boiling point, vapor pressure, octanol-water partitioning coefficient, water solubility, activity, stability) is sufficient for the characterization of most chemical compounds. For ENPs, however, more profound investigation is needed and other properties, such as particle size distribution, sa/vol ratio, shape, electronic properties, surface characteristics, state of dispersion/agglomeration and conductivity need to be studied [5]. The high complexity and great diversity of ENPs, however, make their characterization very difficult [59].

As it can be inferred from the Table, most of the current research on the properties of ENPs is focused on the identification of metrics and associated methods for the measurement of ENPs and their properties. This type of research is fundamental in the sense that without reliable measurement methodology it would be impossible to develop good understanding of the physical and chemical

properties of the ENPs. Only few comprehensive studies on the development of standard, well-characterised reference nanomaterials were published so far. To facilitate the appropriate interpretation of testing results, it is essential to select representative sets of ENPs, characterize them and share them among laboratories worldwide.

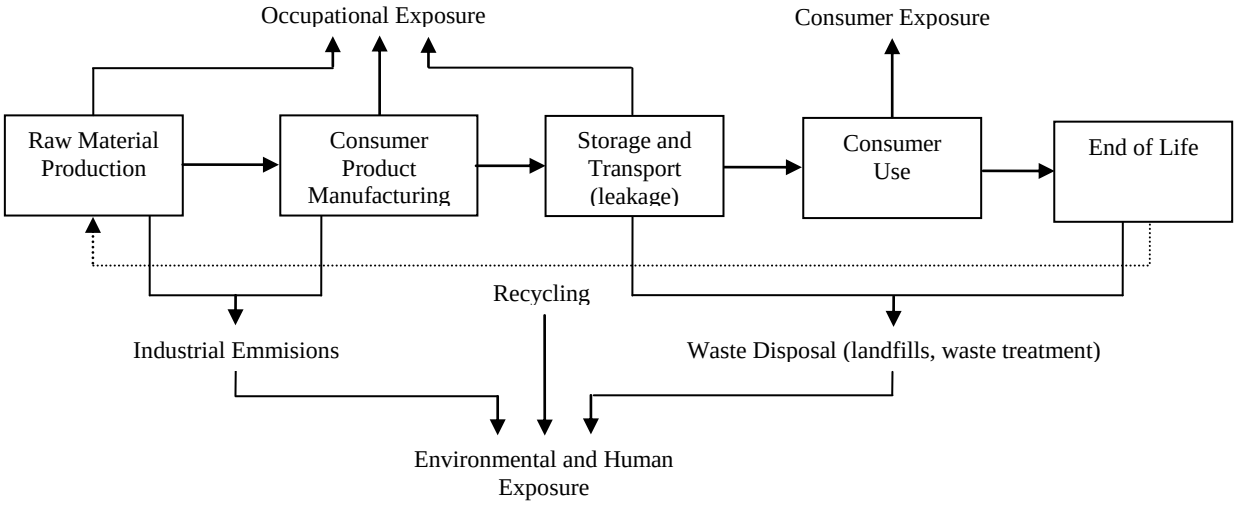
Table 2. A summary of studies on the metrology, characterisation and standardization of ENPs in the period 2004–2009 and their total funding value (modified after [68]).

Specific Research Field		**State of Progress**			**Total**
		Unknown	In Progress	Completed	
Identification of metrics and associated methods for the measurement of ENPs and their properties	Number of studies	4	12	12	28
	Funding value (mill. €)	-	16.23	6.80	23.02
Development of standardised, well-characterised reference ENPs	Number of studies	1	1	6	8
	Funding value (mill. €)	-	0.28	0.20	0.47
Understanding the properties of ENPs in the context of their ignition and explosion potential	Number of studies	-	1	2	3
	Funding value (mill. €)	-	5.57	0.32	5.89

4.3. Exposure Assessment

"Exposure assessment" (EA) is defined as "…an estimation of the concentrations/doses to which human populations (*i.e.*, workers, consumers and man exposed indirectly via the environment) or environmental compartments (aquatic environment, terrestrial environment and air) are or may be exposed." [10, after 11].

Figure 1. Possible pathways of occupational, environmental and human exposure to ENPs.



EA is a very important element in risk assessment of ENPs, since if no exposure to ENPs occur, it would be impossible that they cause any harm and there would be no risk at all. EA can be divided into three sub-areas: (1) occupational exposure assessment (OEA), (2) environmental exposure assessment (EEA) (including indirect human exposure from the environment) and (3) consumer exposure assessment (CEA).

4.3.1. Environmental exposure assessment

The environment may be exposed to ENPs during all stages of their life-cycles: raw material production, transport and storage, industrial use (incl. processing and/or trade), consumer use, waste disposal (incl. waste treatment, landfill and recovery) [11] (Figure 1).

A very important element of the EEA of ENPs is the study of their environmental fate. The fate of ENPs, released in the environment is determined by their mobility in the different media (*i.e.*, soil, water, air), as well as by their potential to biodegrade or undergo chemical transformation.

Environmental fate of ENPs

In order to determine the extent of environmental exposure to ENPs, it is necessary to understand their behavior in the environment. Until now, only a limited number of environmental fate studies with ENPs have been reported and the fundamental mechanisms behind their distribution are still not clearly understood (Table 3).

Table 3. A summary of studies on the source identification and environmental fate of ENPs, done between 2004 and 2009, and their total funding value (modified after [68]).

Specific Research Field		**State of Progress**			**Total**
		Unknown	In Progress	Completed	
Identification of sources of ENPs	Number of studies	1	11	13	25
	Funding value (mill. €)	-	13.35	2.37	15.72
Understanding the environmental fate, behaviour and interaction of ENPs in air	Number of studies		7	5	12
	Funding value (mill. €)	-	1.42	2.20	3.62
Understanding the environmental fate, behaviour and interaction of ENPs in soils and water	Number of studies		13	23	36
	Funding value (mill. €)	-	1.74	5.09	6.83

Fate of ENPs in air

The fate of ENPs in the air is determined by three main factors: (1) the duration of time particles remain airborne, (2) their interaction with other particles or molecules in the atmosphere and (3) the distance they are able to travel in the air [68]. The processes important to understand the dynamics of ENPs in the atmosphere are diffusion, agglomeration, wet and dry deposition and gravitational settling [68]. These processes are relatively well understood from studying the air-suspended ultrafine particles and that knowledge can be applied to ENPs as well [69]. In some cases, however, there can be considerable differences in behavior between ENPs and ultrafine particles, especially when the latter cannot agglomerate because they are coated [5].

With respect to the duration of time ENPs stay in the air, it is considered that they may follow the laws of gaseous diffusion [70]. The rate of diffusion is inversely proportional to the particle diameter and the rate of gravitational settling is proportional to it [70]. It is generally considered that particles in the nanoscale ($d < 100$ nm) have shorter residence time in the air, compared to medium-sized particles (100 nm $< d <$ 2,000 nm), because they rapidly agglomerate into much larger particles and settle on the ground [71]. Here again ENPs with anti-agglomerate coatings make an exception and their residence time cannot be predicted [71]. It is considered that deposited ENPs are usually not likely to be re-suspended or re-aerosolized in the atmosphere [70,72].

Many nano-sized particles are photoactive [72], but it is still unknown whether they are susceptible to photodegradation in the atmosphere. ENPs also show high absorption coefficients [69], and many of them can act as catalysts. However, no information is currently available on the interactions between ENPs and the chemicals they absorb, and how this interaction might influence atmospheric chemistry.

Fate of ENPs in water

The fate of ENPs in water is determined by several factors: (1) aqueous solubility, (2) reactivity of the ENPs with the chemical environment and (3) their interaction with certain biological processes [5]. Because of their lower mass, ENPs generally settle more slowly to the bottom than larger particles of the same material [5]. However, due to their high surface-area-to-mass ratios, ENPs readily sorb to soil and sediment particles and consequently are more liable to removal from the water column [73]. Some ENPs might be subject to biotic and abiotic degradation, which can remove them from the water column as well. Abiotic degradation processes that may occur include hydrolysis and photocatalysis [72]. Near to the surface ENPs are exposed to sunlight. It is likely that light-induced photoreactions can account for the removal of certain ENPs and for changing the chemical properties of others [72].

In contrast to the removal processes mentioned above, some insoluble ENPs can be stabilized in aquatic environments. Hoon *et al.* [74] investigated the aqueous stability of MWCNTs in the presence of natural organic matter (NOM). MWCNTs were readily dispersed as an aqueous suspension and remained stable for over 1 month. Hoon *et al.* [74] found that NOM is more effective in stabilizing the MWCNTs in water than a solution of 1% sodium dodecyl sulfate (SDS), a commonly used surfactant to stabilize CNTs in the aqueous phase [74]. The C_{60} fullerenes were found to spontaneously form insoluble, dense aqueous colloids of nanocrystalline aggregates and remain in the aqueous phase for

long periods [5]. Another known interaction, which can delay nanoparticle removal from the water column, is the absorption of humic acid. Sea surface microlayers, consisting of lipid-carbohydrate-and protein-rich components along with naturally occurring colloids, made up of humic acid, may attach ENPs to their surfaces and transport them over long distances [75].

Fate of ENPs in soil

The behavior of ENPs in soil media can greatly vary, depending on the physical and chemical characteristics of the material. Some ENPs can strongly sorb to the soil particles and become completely inert and immobile [5]. On the other hand, if ENPs do not sorb to the soil matrix, they might show even greater mobility than larger particles, because their small size might allow them to travel easily through the pore spaces between the soil particles. The possibility to sorb to soil and the respective sorption strength of ENPs is influenced by their size, chemical composition and surface characteristics [5].

Studies by Zhang [76], Lecoanet and Wiesner [77] and Lecoanet *et al*. [78] showed considerable differences in mobility of some insoluble ENPs in porous media. The properties of the soil, such as porosity and grain size, further influence the mobility of the particles. Just like the mineral colloids, the mobility of ENPs, agglomerated in colloid-like structures might be strongly affected by electrical charge differences in soils and sediments [76]. Surface photoreactions might induce photochemical transformations on the soil surface [72].

Biodegradation and chemical transformation of ENPs

In some cases, the biological processes in the environment can lead to the complete degradation of ENPs and sometimes they can only change their physical and/or chemical properties [5]. The mechanisms, which account for the biodegradation of ENPs are still not fully understood. The potential for biodegradation is strongly dependent on the material properties. Most of the ENPs in current use are composed of not easily biodegradable materials, such as ceramics, metals and metal oxides [5]. Despite this, however, a study of Filley *et al.* [79] found that C_{60} and C_{70} fullerenes can be completely metabolized by certain fungi species in medium time periods (12 days), which suggests that fullerene carbon is subject to biodegradation. In contrast, it was shown by Fortner *et al.* [80] that C_{60} fullerenes tend to form stable colloidal structures in water, which suggests a level of resistance to biodegradation. Some known biodegradable materials are certain polymer ENPs, used in drug delivery systems [81].

Certain ENPs undergo chemical transformation when released in the environment. An example are the zero valent iron (Fe^0) ENPs, used in environmental remediation [76], which are oxidized to FeO in the reaction path. Some other metal ENPs are converted to oxides in air or water (e.g., Zn, Cu, Si), which can be more toxic than their corresponding free metals [5]. Some types of QDs were shown to degrade due to photolytic and oxidative reactions, as the degradation of their coatings can reveal toxic metalloid cores [82].

4.3.2. Occupational exposure assessment

Workers may be exposed to nano-scale materials while manufacturing these materials, formulating them into products, transporting them or handling them in the storage facilities. Because higher concentrations of nano-scale materials and higher frequency of exposure to them are more likely to happen in workplace settings, occupational exposures require special attention.

General considerations

The primary route of exposure for workers, involved in manufacturing ENPs, is considered to be through inhalation and/or dermal contact after the manufacturing process is complete [7]. Exposure is less likely to occur during the manufacturing process itself, since most ENP manufacturing processes are performed in closed reaction chambers [7]. Contamination and exposure of workers are more likely to happen while handling and bagging the materials and also during cleaning operations [83].

In the product formulation phase, occupational exposures are most likely to occur while unloading the materials from shipping containers and cleaning the process equipment and vessels. During product manufacturing, exposures to ENPs are highly process-specific. For example, workers who manually apply spray coatings are often exposed to very high particle concentrations [5]. In contrast, particles, bound in nanocomposites are not likely to release and handling of composites would result in lower occupational exposure levels. High exposures are likely to occur during product machining (*i.e.*, cutting, drilling and grinding), repair, destruction and recycling [84].

Experimental results

A review by Aitken *et al.* [70], aimed to identify potential exposure scenarios, related to the manufacture and use of ENPs, studied the production processes of fullerenes, CNTs, metals and metal oxides. The review identified four main groups of ENP production processes: vapour deposition, gas-phase, colloidal and attrition processes [70]. According to the report, all production processes can potentially result in occupational exposure through inhalation, dermal or ingestion routes [70].

Maynard *et al.* [85] performed exposure measurements of airborne SWCNTs in production facilities to assess the propensity for aerosol particles to be released during agitation and to measure the size of particles released into the air while SWCNT material was removed from production vessels and handled prior to processing. Airborne concentrations of SWCNT were then estimated to be lower than 53 $\mu g\ m^{-3}$, while hand glove deposits of SWCNT during handling were estimated to be in the range 0.2–6 mg per glove. The study concluded that occupational exposures of SWCNTs are most likely to happen during handling and bagging of the materials and there is high risk of dermal uptake [85].

In a recent study, Han *et al.* [86] measured occupational exposures in the production cycle of MWCNTs. Air samples were taken and the MWCNTs in the samples were counted using a transmission electron microscope (TEM). The results yielded that most of the MWCNT exposure levels (max. 0.43 $mg\ m^{-3}$) were lower than the current threshold limit value (TLV) for carbon black (*i.e.*, 3 $mg\ m^{-3}$).

Yeganeh *et al.* [87] measured the concentrations of airborne ENPs, released during manufacturing of carbonaceous nanomaterials, such as fullerenes and CNTs, in a commercial production facility. The mass concentrations (PM 2.5), the submicrometer size distributions and the photoionization potential (*i.e.*, an indicator of carbonaceous content) of the particles were measured at three locations: inside the fume hood where nanomaterials were produced, just outside the fume hood, and in the background. Average mass concentrations and particle number concentrations were not significantly different inside the facility versus outdoors [87]. However, large, short-term increases in PM 2.5 and particle number concentrations were associated with the physical handling of nanomaterials. In many cases, an increase in the number of sub-100 nm particles accounted for the majority of the increase in total number concentrations [87]. Photoionization results indicated that the particles suspended during handling, inside the fume hood, were carbonaceous and therefore likely to include ENPs, whereas those suspended by other activities, taking place outside the fume hood, were not. Based on the results of the study, the engineering controls at the facility were effective at limiting exposure to ENPs [87].

Fujitani *et al.* [88] compared the particle size distributions and morphology of aggregated/ agglomerated fullerenes at the production facilities of Frontier Carbon Corporation in Japan, during work and non-work periods. After this they compared the results to the nearby outdoor air. They found that the concentration of particles with diameters, shorter than 50 nm was not larger during the removal of fullerenes from a storage tank for bagging and weighing than prior to the activity [88]. It should be noted, however, that this size fraction is extremely difficult to quantify and it is really impossible to do it accurately, in spite of instrument manufacturer's reports.

Bello *et al.* [89] investigated the airborne exposures generated in a research lab during the dry and wet cutting of nanocomposites, consisting of advanced fibers and polymer matrces, containing CNTs. No significant difference in air concentrations during wet cutting, which is the usual procedure for such composites, was identified. Dry cutting, however, generated statistically significant quantities of nanoscale and fine particles, regardless of the composite type (e.g., CNT-carbon, CNT-alumina or their respective base composites) [89].

Biswas and Wu [90] concluded that there is linear dependence between the active operations in production and the concentrations of ENPs in the working settings, while several other authors suggested that the influences of background concentration (concentration of other particles than those of concern) as well as the potential spacial and temporal variations of exposure are very important and have to be taken into consideration [91-93]. Whereas the fraction of the total ultrafine particle number oncentrations generally decreases, fine particle number concentrations increases with time and distance from the point of emission [90].

Major limitations to the OEA are that official data on the number of workers exposed to ENPs are not available, the concentations of ENPs in the working settings are seldom properly measured and the occupational exposure pathways are still not well studied. [7,94].

4.3.3. Consumer exposure assessment

Today, nanotechnology is available on the market for great variety of applications and it is expected that widespread consumer exposure via direct contact with ENP-containing products will take place.

Since the spectre of the nano-products is very diverse, it is expected that the nature of consumer exposure will be disparate too.

Hansen *et al.* [95] divided ENP-containing products into several categories (*i.e.*, appliances, food and beverages, health and fitness, home and garden and goods for children). They found that the expected consumer exposure is highest for products in the categories "appliances", "health and fitness" and "home and garden". The following figure describes the distribution of ENPs versus product categories, based on the study of Hansen *et al.* [95]. The next figure compares between the probability of exposure and the types of ENPs used in the manufacturing of the products.

Figure 2. Material *vs.* product category [95].

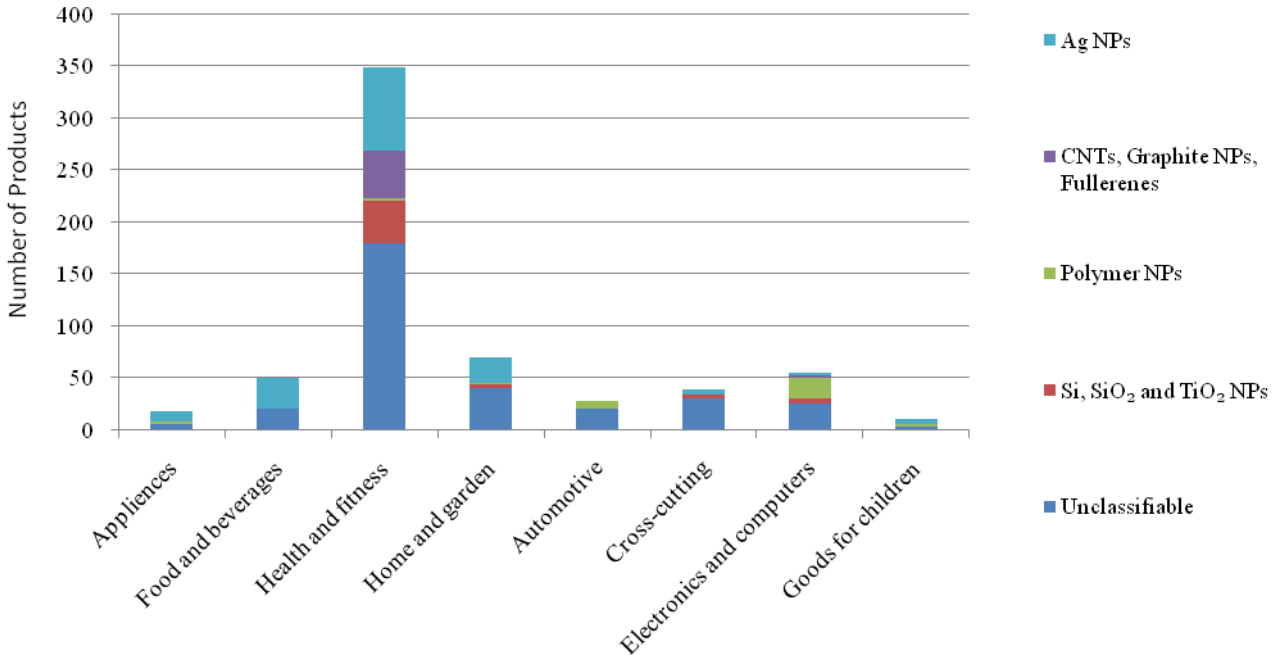
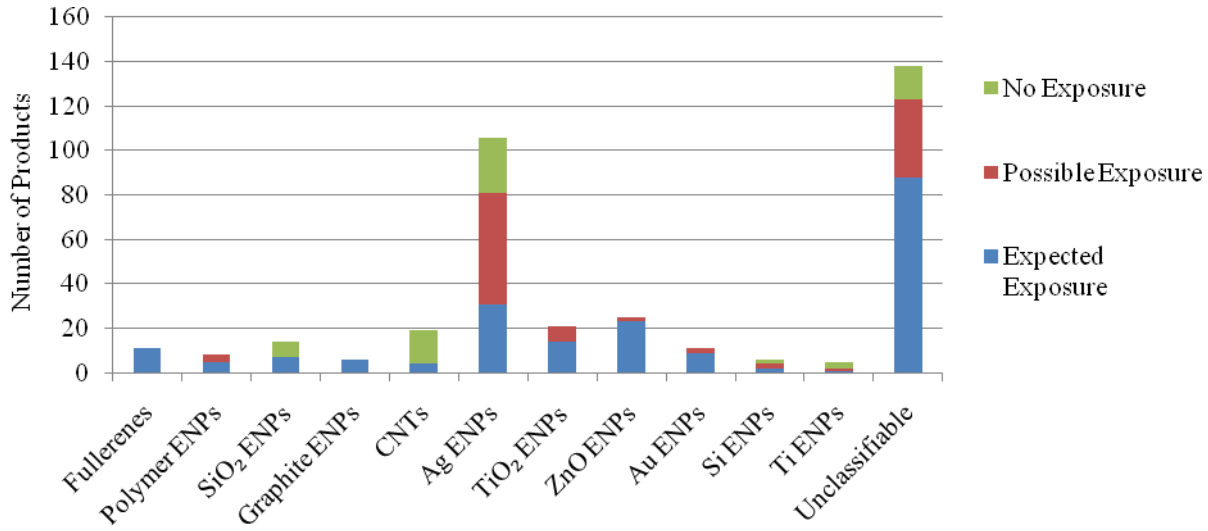


Figure 3. Material *vs.* exposure likelihood [95].



As it can be inferred from the study of Hansen *et al.* [95], the category of "unclassifiable" products, for which no information on the used materials is available, is the one, containing the highest number of products, which consumers are expected to be exposed to [95]. The lack of information about the ENPs, used in these products, is alarming since some of these materials might be potentially hazardous for their users.

The assessment of the consumer exposure to ENPs is significantly restricted by the lack of access to information about which commercially available products contain ENPs, the exact nanomaterial content of these products and the consumer behavior towards them [7]. For many products the number of users is also unknown [96]. The industry-derived data is obscured from public knowledge and this is to the detriment of all stakeholders (*i.e.*, the governments, the public and the private sector).

4.4. Risk Characterization

Risk characterization (RC) is the final step of the risk assessment procedure. RC is defined as "… estimation of the incidence and severity of the adverse effects likely to occur in a human population or environmental compartment due to actual or predicted exposure to a substance, and may include risk estimation" [10, after 11]. In this phase, all information, gathered during the first three steps of risk assessment is taken together, weighted and the risk is quantified.

4.4.1. Completed risk characterization studies with ENPs

The quantitative RC compares the predicted environmental concentration (PEC) of a chemical agent with its predicted no-effect concentration (PNEC). The PNEC is the concentration, below which the exposure to the substance is not expected to cause adverse any effects, while the PEC is the prognosticated concentration of a chemical in the environment. The PEC/PNEC ratio is called risk quotient (RQ). If the RQ is lower than 1, it is considered that no further testing or risk reduction measures are needed [11]. If it is greater than 1, further testing can be initiated to lower the PEC/PNEC ratio [9]. If that is not possible, risk reduction measures should be implemented [11].

In 2008, Müller and Nowack [97] reported the first fully quantitative environmental risk assessment of ENPs. They used nano-particulate Ag at threshold concentrations of 20 mg L^{-1} and 40 mg L^{-1} and exposed *B. subtilis* and *E. coli* bacteria to it. The results showed that, at the above concentrations, Ag ENPs did not affect the integrity of the microorganisms (*i.e.*, both concentrations were equivalent to NOEC). In addition, Müller and Nowack [97] calculated the PNEC values of nano-particulate Ag, TiO_2 and CNTs in water, which were 0.04 mg L^{-1}, <0.001 mg L^{-1} and <0.0001 mg L^{-1}, respectively. Combining these PNEC-values with the predicted exposure, they calculated the environmental concentrations of the above ENPs in Switzerland, stemming from different industries (*i.e.*, textiles, cosmetics, coatings, plastics, sports gear, electronics). Assuming worst-case exposure scenarios levels, Müller and Nowack [97] found that the RQs for Ag ENPs and CNTs were lower than 0.001, and concluded that there was little or no risk that these materials would do harm to aquatic organisms. Exposure to TiO_2, however, might possibly pose risks, since its RQs were ranging from 0.7 to 16.

Park *et al.* [98] assessed the risk of cerium oxide (CeO_2) to cause lung inflammation. First, they estimated an internal dose of 3.8^{-7} cm^{-2} cm^{-2} by converting the retained dose into surface area units

and then dividing by the area of the proximal alveolar region of the lung. Then they compared this value to the highest No Observed Effect Level (NOEL), found in *in vitro* toxicity studies (*i.e.*, 26.75 cm^{-2} cm^{-2}). Assuming that *in vitro* exposure data was reliable, Park *et al.* [98] concluded that it was highly unlikely that exposure to CeO_2 at the monitored and modeled environmental levels would elicit pulmonary inflammation.

4.4.2. Limitations to risk characterization of ENPs

Each of the elements of risk assessment holds certain limitations and challenges. RC, being at the end of the line, sums all of these limitations [7]. Toxicity has been reported on for many ENPs, but for most further investigation and confirmation are needed before hazard can be identified [7]. DRA assumes that no-effect concentrations (NECs) are established and although a number of studies observed dose-response relationships, they do not explicitly state any NEC values. DRA is severely hindered by the fact that it is still unclear what the most suitable dose-descriptors for most ENPs are. EA is hampered by difficulties in monitoring nanomaterial exposure in the workplace and the environment, and by deep uncertaincies in regard to the environmental fate and the biological pathways of ENPs.

5. Overcoming the Limitations to the Risk Assessment of ENPs

5.1. Recommendations on Future Research

We argue that future research strategies must have a strong focus on the characterization of ENPs to enable the identification of clear causality between their inherent properties and the adverse effects they cause. For all types of ENPs, the most suitable dose-descriptors need to be determined (e.g., surface area, mass, morphology, chemical composition). Prior to achieving this, it would be relevant to report doses with respect to several characteristics, instead of choosing an irrelevant one. Given the large number of diverse ENPs, we recommend the use of a *tiered* testing approach, in which *in vitro* screening tests are designed to uncover particular properties that would then trigger more extensive evaluation. In addition, to facilitate the appropriate interpretation of testing results, standard reference materials, testing methods, and reporting formats must be elaborated. The development of reproducible and validated test standards and protocols would help to ensure that toxicological studies with ENPs generate comparable, standardized outputs, which would greatly aid their univocal HI.

EA of ENPs must build on a realistic environmental, occupational and consumer exposure scenarios (ESs). The ESs must be based on known or anticipated ENP production, trade/transport, use and disposal figures and coupled with a range of possible loss routes (e.g., accidents, leaks). With respect to EEA of ENPs it is necessary to establish the degree of environmental mobility of ENPs and their potential to bioaccumulate in order to identify whether ENPs can be taken up by living organisms and cause harm to them. In order to facilitate effective OEA of ENPs it is essential to obtain detailed data on the sources, dispersion mechanisms and concentrations of ENPs in the working settings and well study their occupational exposure pathways. To aqurately estimate ENP concentrations, adequate measurement and sampling methods and tools need to be developed. To characterize the consumer

exposures to ENPs, it is necessary to identify the ENP-containing products with a high priority for future exposure studies. Furthermore, it is essential to collect reliable data on how many consumers use these products, including information about which products they use, how often and for how long. The needs for further research with respect to environmental and health risk assessment of ENPs are summarized in the following table.

Table 4. Needs for further research with respect to the risk assessment of ENPs.

Field(s)	Research Needs
Metrology and Characterization of ENPs	(1) Develop sampling and measurement methods to detect and quantify ENPs in the environment, occupational settings and in consumer products (2) Establish standardized requirements for ENP characterization
HI and DRA	(1) Establish clear causality between ENP characteristics and the observed toxic effects. Identify the most suitable dose metrics (e.g., surface area, chemical composition, particle morphology) (2) Establish dose-response relationships for ENPs of different composition and particle size for well defined target organisms/cell types and endpoints (3) Study the toxicokinetics of ENPs, including translocation, excretion dynamics, acute vs. chronic toxicity, toxicity mechanisms, *etc.* (4) Develop *tiered* approaches for toxicological testing (5) Develop standard reference materials, testing methods, and reporting formats to facilitate the appropriate interpretation of testing results
EEA	(1) Study the mobility of ENPs in soils, sediments, water and air (2) Study the adsorption/desorption behavior in relation to organic, mineral and biological components of soil, sediments and water (3) Develop appropriate environmental exposure scenarios (ESs)
OEA	(1) Study the sources, dispersion mechanisms and concentrations of ENPs in the occupational settings (2) Study the behavior and fate of ENPs in occupational settings
CEA	(1) Develop of inventories of production volumes, use and waste streams (2) Study the distributions of different ENPs in consumer products (3) Study the behavior and fate of ENPs in products

Furthermore, it would be useful to compile results and establish open access databases, which can serve the international scientific society and reduce the duplication of research efforts.

5.2. On-Going Research Efforts

5.2.1. Hazard identification and dose-response assessment-related studies

In January 2008, the US National Institute of Standards and Technology (NIST) issued its first reference standards for ENPs (*i.e.*, 10, 30 and 60 nm gold spheres) [99]. These new reference materials are three of the five ENP samples, currently being used in an American Society for Testing and Materials (ASTM) interlaboratory performance-benchmarking study, intended to improve the consistency and quality of testing results across organisations. The comparative study began in January 2008, as NIST works in partnership with other national measurement institutes in several

countries, as well as with the European Commission's Joint Research Centre Institute for Reference Materials and Measurements (IRMM) [100].

A team of materials scientists and toxicologists announced in 2008 the formation of the International Alliance for Nano Environmental, Health and Safety (EHS) Harmonization (IANH). IANH is a new international research network to establish protocols for reproducible *in vivo* and *in vitro* testing of ENPs [101]. The goals of the alliance are to:

1. Create standard ENP toxicological testing protocols to use in a round-robin study and obtain identical toxicological results for ENPs.
2. Implement a round-robin laboratory set of tests, based on the standard protocols.
3. Facilitate further development of protocols, which take into consideration the properties of ENPs and their relationships to toixicity.

The alliance activities will potentially validate existing toxicity assessment strategies and thereby improve reproducibility and overall confidence in the reported results.

In 2009, the Institute of Occupational Medicine (IOM) launched the ENPRA, a project under the European 7th Framework Program. One of the main objectives of the project is to identify the critical ENP characteristics, responsible for the toxicity, observed in *in vivo* and *in vitro* studies [102]. The € 3.7 million worth ENPRA project would combine the knowledge and capabilities of 15 European and six U.S. partners, including three U.S. Federal Agencies: EPA, the National Institute for Occupational Safety and Health (NIOSH) and the National Institute of Environmental Health Sciences (NIEHS) [102].

Two large-scale projects, which plan to develop and employ *tiered* approaches, using *in vitro* screening tests, are currently in progress (Table 5). The objective of the SUNANO project is to establish and validate toxicological test systems in order to perform an integrated hazard characterization of free nanoparticles of Ag, zirconium dioxide (ZrO_2), SiO_2 and TiO_2, using a new *tiered* approach [103]. The toxicity of the particles will be assessed as a function of size, shape and dose (*i.e.*, mass and surface area). The NIRT project studies the mechanisms of interaction between carbon-based ENPs and cells, identifying potential adverse effects. The study uses *in vitro* and *in vivo* (*rat*) models and a tiered testing approach.

Table 5. Studies, aimed to develop and employ *tiered* approaches for HI of ENPs (modified after [103]).

Study Title	Country	Funding Institution	Budget (€)
SUNANO—Risk Assessment of Free Nanoparticles	Denmark	The Danish Strategic Research Council, Programme Commission on Nanoscience, Biotechnology and IT (NABIIT)	1 064 000
NIRT: Understanding Robust Large Scale Manufacturing of Nanoparticles and Their Toxicology	USA	National Science Foundation (NSF)	771 767

5.2.2. Environmental exposure assessment-related studies

A number of studies, focused on the environmental fate of ENPs are currently in progress (Table 6). The outputs of these studies may deliver important knowledge of the environmental pathways of ENPs, their biological and chemical transformation potentials as well as of their tendency to bioaccumulate.

Table 6. Ongoing environmental fate studies with ENPs (modified after [68]).

Study Title	**Country**	**Funding Institution**	**Budget (€)**
CRAEMS: Fundamental Studies of Nanoparticle Formation in Air Pollution	USA	NSF	680 786
NIRT: Nanoscale Processes in the Environment: Atmospheric Nanoparticles	USA	NSF	925 037
Aggregation and Deposition Behaviour of Carbon Nanotubes in Aquatic Environments	USA	NSF	221 661
Photochemical Fate of Manufactured Carbon ENPs in the Aquatic Environment	USA	EPA	110 775
Pharmaceutical and Cosmetic Silica Nanoparticles: towards an Understanding of their Structure, Fate and Behaviour in Aquatic Systems	UK	Natural Environment Research Council (NERC)	71162
Colloid Interfacial Reactions in Open Microchannel, Representing Unsaturated Soil Capillaries	USA	United States Department of Agriculture (USDA)	53189
Solubilisation of Carbon Nanotubes and Fullerenes in Natural Waters under Environmental Conditions	Switzerland	Swiss National Science Foundation (SNSF)	76076
NIRT: Nanoparticle-Environment Interfaces: Interactions in Natural Systems	USA	NSF	830935
Assessing the Environmental Impacts of Nanotechnology on Organisms and Ecosystems	USA	EPA	207768
Agglomeration, Retention, and Transport Behaviour of Manufactured Nanoparticles in Variably-Saturated Porous Media	USA	EPA	221085
Carbon Nanotubes: Environmental Dispersion States, Transport, Fate, and Bioavailability	USA	EPA	206043
Carbon Nanoparticles in Combustion: A Multiscale Perspective	USA	NSF	132 997
Quantitative Risk Assessment of Nanoparticles in the Environment: Exposure Modelling and Ecotoxicological Considerations	Switzerland	EMPA	163 762

5.2.3. Occupational exposure assessment-related studies

OEA of ENPs requires detailed information on the sources ENPs in occupational settings, their concentrations, the number of workers, exposed to the particles, and the pathways of exposure. A number of studies, currently in progress, are expected to deliver important knowledge of these aspects and thus contribute to the OEA of ENPs (Table 7).

Table 7. Ongoing studies on the sources, dispersion mechanisms and concentrations of ENPs in occupational settings (modified after [68]).

Study Title	Country	Funding Institution	Budget (€)
NANOPLAST: Nano-technological Materials and Products in the Plastics Industry: Exposure Assessment and Toxicological Properties	Denmark	Danish Working Environment Research Fund (DWERF)	602 629
NANOKEM: Nanoparticles in the Paint and Lacquer Industry. Exposure and Toxic Properties	Denmark	DWERF	1 192 378
Experimental and Numerical Simulation of the Fate of Airborne Nanoparticles from a Leak in a Manufacturing Process to Assess Worker Exposure	USA	NSF	221 663
An Ultrafine Particle Intervention Study in Automotive Production Plants	USA	NIOSH	N/A
The Measurement and Control of Workplace ENPs	USA	NIOSH	N/A
Bypass Leakage and Recirculation of Workplace Aerosols	USA	NIOSH	N/A
Assessment Methods for Nanoparticles in the Workplace	USA	NIOSH	222 063

5.2.4. Consumer exposure assessment-related studies

To identify consumer products with a high priority for future exposure studies, it is essential to know how many consumers use products, containing ENPs, which products they use, how often and for how long. Furthermore it is important to study the behavior of ENPs in consumer products and identidy potantial exposure pathways. The following table lists several ongoing studies, expected to fill some of the current data gaps.

Table 8. Ongoing studies in regard to CEA of ENPs (modified after [68]).

Study Title	Country	Funding Institution	Budget (€)
Analysis of ENPs Exposure on Humans in Switzerland: Identification of Frequent Situations for Exposure Situations with Today's and Possible Future Use of Consumer Products on the Basis of ENPs	Switzerland	BAG (Federal Office of Public Health)	107,885
Assessment of Current and Projected Applications of Nanotechnology for Food Contact Materials in Relation to Consumer Safety and Regulatory Implications.	UK	FSA	76,643
Characterisation and Toxicological Evaluation of Nanoparticles from Liquid-based Nanofilm Products	Denmark	Nanocover Scandinavia A/S	322,741

6. Managing Uncertainty

As it was identified in the previous sections, a decent number of limitations and severe gaps in knowledge exist in regard to each of the four elements of CRA, when it is applied to ENPs. Considering these flaws, one can conclude that the risk assessment of ENPs is hampered solely by the lack of scientific knowledge. There is another viewpoint, however, it might be that conventional CRA methodology is simply inadequate to apply to ENPs and it should be substituted for an alternative methodology.

It would be naive to state that the conventional CRA should be entirely substituted for an alternative methodology due to the multiple and profound limitations. It is, however, also difficult to accept that it will be able to overcome these limitations in near future and effectively serve its purpose. It has been suggested by several authors that the CRA framework can be applied effectively to nanoparticles if it is aided by certain tools to reduce the deep uncertainties, which currently pervade every step of the procedure [7, after 104,105]. Some approaches, which are considered relevant in this respect are the: (1) Multi Criteria Decision Analysis, (2) Weight-of-evidence and (3) Expert Elicitation.

The common purpose of Multi Criteria Decision Analysis (MCDA) methods is to evaluate and choose among different decision alternatives, based on multiple criteria, using systematic and structured analysis [7]. MCDA methods have evolved as a response to the inability to effectively analyze multiple streams of conflicting information [106]. There are different MCDA methods, based on different theoretical foundations, such as optimization, goal aspiration and outranking [106]. MCDA tools could help in deciding what criteria to use to judge ENPs, to determine the relative importance of each of the criteria, score it, and finally compare the scores to identify the best alternative [106]. MCDA can be used for toxicity and risk assessment of selected ENPs as well as for the development of regulatory criteria for them [107].

Another tool, widely used in risk assessment applications and considered applicable to the risk assessment of ENPs, is the Weight-of-evidence approach [105,107]. Using the Weight-of-evidence methodology, assessors weigh various lines of evidence and apply professional judgment and/or calculations to decide where the weight of evidence lies—or, whether the various lines of evidence point to a potential risk or not [106]. Weight-of-evidence evaluations may be either qualitative or quantitative, as the quantitative approach is often preferred because its results are considered more consistent and less subjective [108].

Expert Elicitation (EE) is the synthesis of opinions of experts on a subject where high uncertainty is present due to insufficient or conflicting data. EE is essentially a methodology, based on scientific consensus, used to quantify uncertainty [109,110]. The subjective judgment of experts is usually represented as a "subjective" probability density function (PDF), as effort is made to minimize subjective judgment and the errors related to it in the elicited outcomes [109,110].

7. Conclusions and Recommendations

This chapter presents the main conclusions drawn from this study. In addition, suggestions and recommendations on further research are given and focal points are discussed.

Objective 1: Investigate the Current state of Knowledge of the Risks of ENPs for the Environment and Human Health

It is possible that ENPs can cause novel environmental problems; impose risks to human health or do both. It is impossible, however, at this point of time and stage of knowledge to make any collective judgment about the potential risks of exposure to nanomaterials. ENPs are expected to affect living organisms in different ways than their bulk alternatives and considering their significant diversity, it is anticipated that ENPs would also differ a lot from each other in terms of toxicity.

Most of the reviewed toxicity studies with ENPs demonstrate some degree of hazardous effects on the tested organisms. Some *in vivo* toxicological studies with CNTs suggest that these materials tend to cause interstitial inflammation and lesions in mammals [13,14]. Shorter MWCNTs were shown to cause less severe inflammation than longer MWCNTs and dissolved MWCNTs caused almost no adverse response, which suggests that MWCNT toxicity is influenced rather by particle morphology than by chemical composition [17]. This notion was supported by Kang *et al.* [40], who compared the cytotoxicity of commercially obtained MWCNTs in bacterial systems before and after physicochemical modification and concluded that uncapped, debundled, short, and dispersed in solution MWCNTs exhibit highest cytotoxicity. Most *in vivo* studies with C_{60} fullerenes suggest that these materials tend to induce oxidative stress in living organisms [18-21]. The toxicity of QDs was found to be influenced by their composition, size, surface charge and coating, as well as by the exposure to light and on temperature [46-49]. Smaller QDs were shown to be more toxic than larger QDs [51], while the exposure to UV light and higher temperatures also tend to increase the tocicity of QDs. Exposure to Zn ENPs causes pulmonary (lung) inflammatory response in mammals [22,26], as the smaller the particle size is, the greater the adverse effect is [25]. Exposure to FeO and TO_2 ENPs decreases the viability of human monocyte macrophages and human lymphoblastic cells, respectively [52,54]. It was also shown that nano-Ag acts as an effective bactericide [55,56]. Despite that most (eco)toxicity studies with ENPs observed some degree of toxicity, it is still unclear which physical and/or chemical characteristics of ENPs are responsible for it. The uncertainty in this respect is mainly due to the fact that most ENPs, used in toxicity tests, are seldom well characterized.

Since a very limited number of studies are made in the field of environmental fate of ENPs, their behavior in the environment is still largely unexplored. When addressing the environmental fate of ENPs, most of the literature uses imprecise general considerations and comparison with data, obtained for larger particles. It is very important to study the environmental fate of ENPs in order to understand their pathways of environmental and human exposure. Occupational exposures to ENPs are most likely to occur via inhalation and/or dermal contact [7,88]. In most cases exposures are more likely to occur after the manufacturing process is complete (*i.e.*, while handling and bagging the materials and also during cleaning operations) [85,88]. Hansen *et al.* [96] estimated that the expected consumer exposure is highest for appliances, health, fitness, home and garden products. They noted that for most products, no information on the used materials is available, which is alarming since some of these materials might be potentially hazardous for their users.

Objective 2: Estimate Whether Current Knowledge is Sufficient to Facilitate Comprehensive and Effective Risk Assessment of ENPs

The above analysis identified a number of severe limitations and flaws in relation to each of the four steps of the risk assessment procedure, when it is applied to ENPs. Toxicity has been reported for many ENPs, but for most of them further investigation and confirmation are needed before hazard can be identified [12]. Currently, most laboratories use non-standardized tests, generating non-reproducible results, which make the univocal hazard identification of ENPs very difficult. The DRA of ENPs is restricted by the enormous deficit of characterization data [59], which makes it impossible to determine which properties account for the inherent hazards of ENPs and identify appropriate dose metrics. Furthermore, despite that dose-response estimation assumes that no effect thresholds can be established, most studies, reporting dose-responce relationships, do not state any NECs [7]. EEA is hindered by the fact that the biological and environmental pathways of ENPs are still largely unexplored. OEA of ENPs is hindered by the lack of information about: (1) the number of workers, exposed to ENPs, (2) the type of ENPs workers are exposed to, (3) the occupational exposure pathways, and (4) the concentrations of ENPs in the working settings [85]. The deficiency of data in this regard is partly explained by the lack of adequate occupational exposure measurement methods and tools. CEA of ENPs requires complex modeling, addressing aspects such as ENP production volumes, number of nano-products, their market distribution, ENP releases from the products throughout their life-cycle *etc.* [96]. There is insufficient knowledge with respect to these parameters, which is partly attributable to the lack of studies and partly to a lack of access to industry-derived data.

As it was shown, in most cases, the available information about the risks of ENPs for the environment and human health is insufficient to facilitate comprehensive and effective risk assessment. Each step of the procedure is hindered by serious data flaws, as RC, being at the end of the line, sums all the limitations. In order to facilitate sound risk assessment of ENPs, more research is needed.

Objective 3: Provide Recommendations on Future Research in the Field of Risk Assessment of ENPs

Based on the information presented in the previous chapters, it becomes obvious that there are many data deficits in regard to the EHS of ENPs, which require ungent research activities. In order to facilitate accurate risk assessment of ENPs, it is essential to start with adequate property characterization to understand the causality between the properties of ENPs and their inherent toxicity and identify suitable dose metrics for them. Furthermore, given the great diversity of ENP types, it would be useful to use *tiered* testing approaches, in which *in vitro* screening tests are used to detect any potential hazards and indicate whether more extensive *in-vivo* evaluation is necessary. The credibility and the broad acceptance of the test results will depend greatly on the establishment and utilization of standard reference materials, testing methods, and reporting formats. Standard testing would generate reproducible results and greatly aid the univocal HI of ENPs.

The most urgent research need in regard to the environmental exposure of ENPs is to establish the degree of their environmental mobility and bioavailability. Understanding the environmental fate of ENPs would greatly help us assess their exposure of ecosystems and of *humans* via the environment. Furthermore, for accurate exposure estimation it is necessary to obtain reliable environmental

concentration data and therefore adequate sampling and monitoring technologies need to be developed. To facilitate effective OEA of ENPs it is essential to identify the exposure sources and pathways of ENPs in the working settings as well as to study the mechanisms behind their dispersion and measure their concentrations. For the latter, the development of adequate measurement techniques is a must. In order to facilitate proper CEA of ENPs future research should address multiple aspects (e.g., ENP global production volumes, number of products entailing ENPs, current and future market penetrations of these products, ENP releases throughout their life-cycles). Furthermore, a comprehensive inventory of consumer exposure data needs to be elaborated and made easily accessible to scientists and risk managers.

The deep uncertaincies, which currently pervade every step of the risk assessment of ENPs, make the procedure uncapable of properly serving its purpose. Data gaps with respect to EHS of ENPs are gradually filled by new research, but this process advances at low speed, while risk assessment results are urgently needed to triger adequate regulatory response. We recommend that the current risk assessment approach is adapted to reflect the challenges, discussed above, as it is aided by appropriate tools to manage the present uncertainties. Some approaches, which are considered useful in this respect are the Multi Criteria Decision Analysis, the Weight-of-evidence and the Expert Elicitation [106-110]. Implementing some of these non-conventional tools in the risk assessment framework holds promise to reduce uncertainties and deliver accurate risk characterization results very soon. This would enable current regulation to adequately reflect the risks of ENPs and protect the environment and the community.

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