CHAPTER 14

Environmental Hazards and Risks of Nanomaterials

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14.1 INTRODUCTION

Owing to their small size and composition, nanomaterials (NMs) display unique properties that have diverse applications in various fields such as biomedical, electronics, cosmetics, agriculture, environmental and engineering industries. The range of applications of nanotechnology is vast and expanding from the last decade. Although nanotechnology is emerging as a multi-disciplinary science for the development of new products using engineered NMs and many benefits are expected from the on-going research in nano-technology, serious concerns are being expressed about the potential hazards that nanoparticles (NPs) can poses on the environment, ecosystems and human health. Since significant physical and chemical property alters as the particle size reduced to the nano range (typically 1–100 nm), the biological property of engineered nanoparticle (ENPs) may also be altered from their bulk counterparts. Several analyses and assessment in the past few years on the hazardous risks of NMs has shown the adverse effects of many nano-products on the environment, aquatic organisms, human beings and few other flora and fauna. Thus, harmonized methods for structured assessment of fate of ENPs in the environment and their toxicological properties to ecologically relevant organisms are necessary to assess their risk.

The present chapter focuses on the current status of impact of nano-technology and ENPs on the environment and the various hazard and risk assessment methods employed to tackle these problems.

14.2 OVERVIEW ON RISK ASSESSMENT

Risk assessment is the task of characterizing a level of risk, typically in terms of a relative score or ranking. The goal of performing a risk assessment is to provide the important information that will be helpful to evaluate alternatives (Calow 1998). Usually the risk assessment is divided into the following four steps:

- Hazard assessment.
- Dose-response assessment.
- Environmental exposure assessment.
- Risk characterization.

Steps involved in risk assessment are to recognize and characterize the hazards, establish the link between dose and response for various endpoints, and then predict the probability of exposure (Figure 14.1).

14.2.1 Hazard Assessment

Hazard identification entails using the results of scientific research to describe the characteristics of a chemical or substance and its potential to contribute to adverse health effects of human, terrestrial or aquatic organisms. For a hazardous material to cause harm, it must be involved in processes by which the material contacts or enters the body and interacts with cells locally or systemically, leading to tissue-damaging process. Until recently the potential negative effects of NMs on human health and the environment were given very little attention. However, this has changed within the past few years, and a number of scientific studies have indicated that exposure to some NPs can lead to adverse effects in various organs of test animals (Lam et al. 2004; Oberdorster 2004; Poland et al. 2008). Various studies have been conducted to evaluate the potential toxicity and ecotoxicity of NPs (Baggs et al. 1997; Cheng 2004; Oberdorster 2004; Baker et al. 2005;

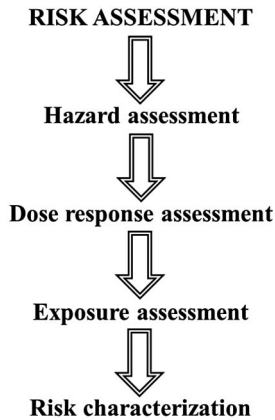

Figure 14.1. Overview of steps involved in risk assessment of NMs

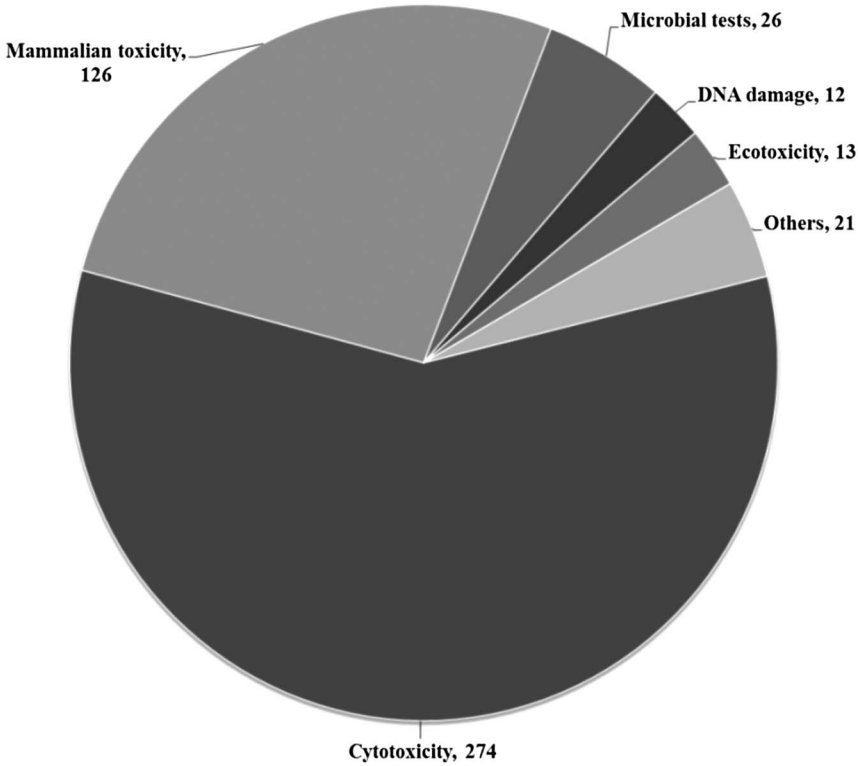

Figure 14.2. Distribution of toxicological studies on NPs (The numbers corresponds to the number of studies among the total 472 studies)
SOURCE: Data from Hansen et al. (2007)

Sayes et al. 2005; Hansen et al. 2007). The studied materials have mainly been water suspended and airborne NPs. Hansen et al. (2007) identified 428 studies reporting on toxicity of NPs. The studies were divided into cytotoxicity, DNA damage, ecotoxicity, mammalian toxicity and microbial test (Figure 14.2). In these studies, 965 tested NPs of various chemical compositions showed adverse health effects.

Several scientists, governments and non-government agencies have reviewed the environmental, health and safety issues of NMs. Current state of knowledge of the hazards of various ENPs based on some important scientific studies and findings with regard to hazard identification are discussed in the following sections.

Carbon Nanotubes (CNTs). CNTs have attracted a great deal of attention due to their unique structural, physical and chemical properties and show promise for a wide array of applications in various fields, such as electronics and medicine. However, concerns have been raised over the safety of CNTs. In particular, CNTs have come under scrutiny due to their thin fibre-like structure and presumed

insolubility in the lungs, both attributes of harmful asbestos fibres (Donaldson and Poland 2009). Lam et al. (2004) 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). Cui et al. (2005) observed a dose and time dependent inhibition of cell proliferation, and a decrease in cell adhesive ability in human embryo kidney 293 cells after exposure to SWCNTs in concentrations between 0.78–200 g mL^{-1} for up to 5 days. Studies indicate CNTs may promote allergic immune responses (Nygaard et al. 2009) and exacerbate airway inflammation (Inoue et al. 2009) based on research conducted in mice using intranasal or intratracheal administration respectively. Several studies indicated that SWCNTs induce the frequent DNA damage in a dose-dependent manner in Chinese hamster lung fibroblast V79 cells (Kisin et al. 2007), mouse embryo fibroblast cells (Yang and Watts 2005) and human epithelial BEAS 2B cells (Lindberg et al. 2009). DNA damage in mouse embryonic stem cells exposed to multi-walled carbon nanotubes (MWCNTs) has also been reported, but this study is of limited value due to single dose tested and lack of positive controls (Zhu et al. 2007).

C_{60} Fullerenes. Fullerenes have attracted great attention in electronic, biological and medical applications due to their fascinating properties such as substituent modifications, endohedrality and superconductivity. Nevertheless, the safety of these materials is of great concern, and strong attention has been paid to the potential risk of C_{60} NPs (nano particles) to human health and environmental impact. Although C_{60} is poorly soluble in water, several methods have been developed to prepare dispersible colloidal aggregates of C_{60} (nC_{60}) in aqueous solutions (Brant et al. 2005, 2006). These nC_{60} particles are stable for months to years. This implicates that nC_{60} could be chronically exposed to the biological and environmental systems.

Previous toxicity tests of aqueous fullerene C_{60} demonstrated both positive and negative results. Yamawaki and Iwai (2006) observed dose dependent cytotoxicity of $C_{60}(OH)_{24}$ (1–100 μg mL^{-1}), resulting in decreased cell density and lactate dehydrogenase (LDH) release in human umbilical vein endothelial cells cavity. A dose-dependent decrease in the viability of human epidermal keratinocytes after exposure to C_{60}-phenylalanine was also observed by Rouse et al. (2006). Several toxicological studies suggest that C_{60} tend to induce oxidative stress in living organisms (Lai et al. 2000; Oberdorster 2004; Zhu et al. 2006; Hristozov and Malsch 2009). Lai et al. 2000 observed a significant increase in lipid peroxidation (LP) products after intravenous administration of 1 mg kg^{-1} $C_{60}(OH)_{18}$ in male mongrel dogs. Elevated LP was also observed by Zhu et al. (2006) in the cephalic ganglion and gills of *Daphnia magna* after exposure to hydroxylated C_{60} fullerenes ($C_{60}(OH)_{24}$) and tetrahydrofuran (THF)-dissolved C_{60}. Recently, Song et al. (2012) reported a size-dependent inhibition of DNA polymerase and reduced-size enhanced cytotoxicity in human lung adenocarcinoma cell line A549 by C_{60}. These size dependent effects were observed at the high exposure doses (4–6 mg L^{-1}). There are further reports which showed negative effects of nC_{60} toxicity (Jia et al. 2005; Fiorito et al. 2006; Bobylev et al. 2012).

On the other hand, Jia et al. (2005) incubated alveolar macrophage (from adult pathogen-free healthy guinea pigs) with nC_{60} (up to 226 μg cm^{-2}) and found no significant cytotoxicity. Bobylev et al. (2012) reported that complexes of C_{60} fullerene with polyvinyl pyrrolidone, C_{60}-NO_2-proline and C_{60}-alanine had no toxic effect on human laryngeal carcinoma cells, HEp-2.

Quantum Dots (QDs). Some NMs are made of more complicated structures than just one or two elemental species in their molecular formula. QDs are one of such NPs which typically contain between 2 and 5 different elements in a core/shell structure. An example is a core of cadmium selenide (CdSe), surrounded by a thin shell of zinc sulfide (ZnS). The toxicity of quantum dots (QDs) was found to be influenced by several factors such as constituting metals, size, metal ratio, surface charge and coating of the QDs. As mentioned in Table 14.1, several *in vitro* and *in vivo* studies in different animal models suggest that QDs are generally considered as toxic to the organisms. Furthermore, QDs are almost always made with toxic heavy metals, including the known human carcinogens such as cadmium or selenium. Thus, humans may also become exposed as QDs degrade. Due to the known toxic components in most QDs, the possibility of degradation in the environment and the extent to which this might happen should be thoroughly investigated. Nevertheless, such critical studies have yet to be performed.

Nano Metals (Metal and Metal Oxide NPs). Due to tremendous advances for the utility of metal based NPs, there is a great amount of data that has been published on NP properties and toxicity. The toxicity of metal NPs is being addressed by a number of standardized approaches with *in-vitro*, *in vivo* as well as detailed genomic or biodistribution studies (Schrand et al. 2010). Ag and Cu NPs have demonstrated a greater potential to travel through the organ systems compared to larger materials and may not be detected by normal phagocytic defenses, allowing them to gain access to the blood or cross the blood-brain barrier into the nervous system (Chen et al. 2006, 2007).

Furthermore, nano-sized metal oxides demonstrated toxicity in the form of reactive oxygen species (ROS) generation and irritation, during cell culture experiments and during inhalation studies. Ag, Cu, and Al NPs may induce oxidative stress and generate free radicals that could disrupt the endothelial cell membrane. This disturbance may cause blood-brain barrier dysfunction resulting in the entry of NPs into the central nervous system (Sharma and Sharma 2007). Given the wide use of metal oxide NPs for sunscreen, a focused recent research has shown that NPs such as TiO_2 and ZnO can penetrate skins and be retained within the human stratum corneum and into some hair follicles (Schrand et al. 2010). Li et al. (2009) recently demonstrated that *in utero* exposure to NPs contained in diesel exhaust affects testicular function by suppressing the production of testosterone. A study by Yang and Watts (2005) on the effect of Al-NPs on the relative root growth (RRG) in *Zea mays* (corn), *Glycine max* (soybean), *Brassica oleracea* (cabbage), and *Daucus carota* (carrot) showed a significant inhibition in the growth of the plants after administration of 2 mg mL^{-1} for 24 h. Table 14.2 further summarizes some of the toxicological effects of metal NPs on various organisms.

Table 14.1. Different recent investigations on quantum dot toxicity

Effect	*Cell line/animal model*	*Quantumdot type*	*Response*	*Refs.*
Effect on embryo/larva development	Zebrafish embryo	CdSe/ZnS	Surface property dependent toxicity	King-Heiden et al. (2009)
	Zebrafish embryo	$CdSe_{core}/ZnS_{shell}$ with oxidative weathering	Increased cadmium body burden in the case of exposed larvae	Wiecinski et al. (2013)
	Danio rerio Embryos	CdSe/CdS/ZnS/S, S-dihydrolipoic acid/ polyacrylic acid	Defective swimming bladder, localized edemas, tail curvature	Zolotarev et al. (2012)
Effect on cell lines	Human breast cancer cells	CdSe/CdS	Uptake was controlled by surface property	Chang et al. (2006a)
	Mouse lymphocytes	CdSe/ZnS	Cytotoxicity	Hoshino et al. (2004)
	Cancer cells	CdSe/CdS	Cytotoxicity	Chang et al. (2006b)
Effect on animal models	Many different cell lines	Quantum dots of different surface properties	Surface property and size dependent cytotoxicity.	Pelley et al. (2009)
	Sprague–Dawley rats	CdSe/ZnS quantum dots	Did not result in any appreciable toxicity	Hauck et al. (2010)
	Mice	Quantum dots with various surface chemistries	Model animals were normal after the test	Hardman (2006)

SOURCE: Data from Wise et al. (2013)

Table 14.2. Studies on metal oxide NM toxicity

Effect	*Organism/cell line*	*Metal Oxide*	*Response*	*Reference*
Effect on different cell lines	Human dermal fibroblast (HDF)	TiO_2	Cell death	Sayes et al. (2006)
	Human lymphoblastoid	TiO_2	Decrease in cell viability	Wang et al. (2007)
	Human monocyte macrophages	Fe_3O_4 ENPs coated with dextran	Decrease in cell viability	Muller et al. (2007)
	Mouse fibroblast	TiO_2	Cell death, oxidative stress as well as decrease in cell viability & function	Jin et al. (2008)
	S. epidermidis	Ag	Bactericidal	Alt et al. (2004)
	E. coli	Zn, Cu, Al, La, Fe, Sn and Ti	Irrespective of identity, higher-charged particles were more toxic	Hu et al. (2009)
Effect on animal models	Mice	TiO_2 aerosols	Inflammatory response	Grassian et al. (2007)
	Human, pig and rat skin	TiO_2 and ZnO	No penetration in healthy skin	Nohynek et al. (2007)
	Rat	MnO, Fe_2O_3	Translocation of MnO NPs into olfactory bulb of brain	Elder et al. (2006), and Petri-Fink and Hofmann (2007)

SOURCE: Data from Wise et al. (2013)

14.2.2 Dose-Response Assessment (DRA)

According to the European Commission Technical Guidance Document (European Commission JRC 2003), 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." It is the process of characterizing the relationship between the dose of an agent, administered to or received, and the consequent adverse health effects on an individual (Hansen 2009; Hristozov and Malsch 2009). In toxicological studies a dose is the quantity of anything that may be received by or administered to an organism. Normally, dose refers to 'dose by mass' (i.e., μg, mg, g). However, based on the experiences gained in DRA, it has been suggested that biological activity of NPs might not be mass-dependent, but is dependent on physical and chemical properties not routinely considered in toxicity studies (Oberdorster et al. 2005; Hansen 2009). For instance, Oberdorster et al. (2007) and Stoeger et al. (2006, 2007) found that the toxicity of low-soluble NPs was better described by their surface area than by their total mass. Whereas Warheit et al. (2007a, b, 2008) found that toxicity was related to the number of functional groups in the surface of NPs. Nevertheless, understanding about the physical and chemical properties of substances and materials is fundamental for their risk assessment. Studying the standard properties like composition, structure, molecular weight, melting point, boiling point, vapor pressure, water solubility, reactivity and stability is sufficient for the characterization of most chemical compounds (Hansen 2009; Hristozov and Malsch 2009). However, for NPs much more elaborated investigation is needed. Apart from the above mentioned properties, other properties, such as particle size distribution, surface area to volume ratio, shape, electronic properties, surface characteristics, state of dispersion/agglomeration and conductivity need to be studied.

Most of the current research on the properties of NPs is focused on the identification of metrics and associated methods for the measurement of NPs 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 NPs. Only few comprehensive studies on the development of standard, well-characterized reference NMs 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 (Hristozov and Malsch 2009).

14.2.3 Exposure Assessment (EA)

Exposure is an important aspect in risk assessment of NMs as it is a precondition for the potential toxicological and ecotoxicological effects to take place. EA is defined as an evaluation of the concentrations/doses to which human populations come across via the environment or environmental compartments. An EA seeks to decide the concentrations and bioavailable forms of a contaminant in the environment, with a concern of fate and exposure period, effects on target

organisms. It will be useful because measurements disclose the concentrations, chemical and physical properties of the compound in the field that are truly responsible for exposure. For NMs, it has been demonstrated that size and surface charge are critical parameters. Nanoparticle net surface charge was observed as an important measure of the extent to which their dispersion is stabilized by electrostatic repulsive forces. EA explains the sources, pathways, routes, and the uncertainties in the assessment.

After the release of NMs into the environment, they might behave differently from their larger counterparts of the same chemical composition and/or operate differently from the intended use. The tendency of NPs to undergo agglomeration, aggregation, adhesion, diffusion, dissociation, degradation, adsorption of different species, and bioaccumulation in organisms as well as biomagnifications in trophic pyramids depends not only on their size/shape, but also on the local environmental and cellular conditions (OCED 2012). Therefore, the evaluation of the effect of physical (size, surface area, shape, agglomeration state), chemical (charge, chemical composition, chemical reactivity), biological (route of administration, metabolism, excretion, adduction to biological molecules) and environmental (temperature, pH, presence of microbes, salinity, acidity, viscosity) factors on NPs EA is mainly needed (Majestic et al. 2010; Scown et al. 2010).

The potential for exposure to NMs starts with the production (as is the case for chemical compounds). Therefore, information on quantitative aspects linked to production, purification, functionalization, conditioning, packaging and transport is essential. Calculation of industrial release must be based upon knowledge on the day-to-day operations, including the events that are likely to be the most important for emission rates, e.g. those relating to elevated temperatures and high pressures, high material flows and all waste streams. When considering environmental exposure it is also noteworthy to consider the frequency and magnitude of incidents that may lead to release to air, water, and soil (Robichaud et al. 2007). For environmental exposure it is necessary to have empirical data or procedures to calculate the persistence and mobility in air, soil and water. Adsorption capacity, degree of aggregation, photolytic degradation, dispersibility, interactions with soil particles are example of factor that may be needed to make predictions on the environmental fate (Robichaud et al. 2007).

EA can be classified into three sub-areas: (1) Environmental exposure assessment (EEA) (including indirect human exposure from the environment); (2) Occupational exposure assessment (OEA); and (3) Consumer exposure assessment (CEA). Details about each of these three sub-areas are described below.

Environmental Exposure Assessment (EEA). The environment may be exposed to NPs during all stages of their life-cycles: raw material production, transport and storage, industrial exercise, consumer use, waste disposal. The destiny of NPs, released in the environment is determined by their mobility in the different settings (soil, water, air), as well as by their potential to biodegrade or undergo chemical transformation. To facilitate the determination of the extent of environmental exposure to NPs, it is necessary to understand their behavior in the environment. Up to now, only a limited number of environmental fate studies

with NPs have been reported, and the fundamental mechanisms behind their distribution are still not clearly understood. The fate of NPs in the air is determined by three main factors: the period of time particles remain in airborne, their interaction with different particles or molecules in the atmosphere, and the distance they are capable to travel in the air (Aitken et al. 2009).

The processes important to be aware of the dynamics of NPs in the atmosphere are diffusion, agglomeration, deposition and gravitational settling (Aitken et al. 2009). The rate of diffusion and gravitational settling is inverse and directly proportional to the particle diameter, respectively (Aitken et al. 2004). It is usually considered that particles in the nanoscale ($d < 100$ nm) have shorter residence time in the air, compared to medium-sized particles ($100\text{ nm} < d < 2{,}000$ nm), because they rapidly agglomerate into larger particles and settle on the ground (Dennekamp et al. 2002). NPs with anti-agglomerate coatings create an exemption, and their residence time cannot be predicted (Dennekamp et al. 2002). It is considered that once NPs are deposited, generally they are not likely to be re-suspended or re-aerosolized in the atmosphere (Colvin 2003; Aitken et al. 2004). Many nano-sized particles are photoactive (Colvin 2003), but it is not clear whether they are susceptible to photodegradation in the atmosphere. NPs also show high absorption coefficients (Wiesner et al. 2006), and a lot of them can act as catalysts.

The fate of NPs in water is decided by several factors like aqueous solubility, reactivity of the NPs with the chemical environment and their relations with certain biological processes. Because of their lesser mass, NPs usually settle more slowly to the bottom than larger particles of the similar material (Hristozov and Malsch 2009). However, due to their high surface-area-to-mass ratios, NPs readily absorb to soil and sediment particles and as a result are more liable to removal from the water column (Oberdorster et al. 2005). Some NPs might be subject to biotic and abiotic degradation, which can eliminate them from the water column as well. Abiotic degradation methods that may happen include hydrolysis and photo catalysis (Colvin 2003) near to the surface of NPs that are exposed to sunlight. It is likely that light-induced photoreactions can explain for the removal of certain NPs and for varying the chemical properties of others (Colvin 2003).

In contrast to the removal procedures mentioned above, a number of insoluble NPs can be stabilized in aquatic environments. Hoon et al. (2007) studied the aqueous stability of multi-walled carbon nanotubes (MWCNTs) in the occurrence of natural organic matter (NOM). MWCNTs were easily dispersed as an aqueous suspension and remained stable for over 1 month. They also observer 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. The C_{60} fullerenes were observed to spontaneously form insoluble, dense aqueous colloids of nanocrystalline aggregates and remain in the aqueous phase for long periods (U.S. EPA 2007). Another known relation 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 affix NPs to their surfaces and transport them over long distances (Moore 2006).

The performance of NPs in soil media can greatly vary, depending on the physical and chemical characteristics of the material. A number of NPs can strongly sorb to the soil particles and turn into completely inert and immobile (U.S. EPA 2007). In contrast, if NPs do not sorb to the soil matrix, they might demonstrate even greater mobility than bigger particles, because their small size might permit them to travel easily through the pore spaces between the soil particles. The chance to sorb to soil and the respective sorption strength of NPs is influenced by their size, chemical composition and surface characteristics (U.S. EPA 2007). Studies by Zhang (2003), Lecoanet and Wiesner (2004) and Lecoanet et al. (2004) showed substantial differences in mobility of some insoluble NPs in porous media. The properties of the soil, such as porosity and grain size, influence the mobility of the particles. Just like the mineral colloids, the mobility of NPs, agglomerated in colloid-like structures might be strongly affected by electrical charge variations in soils and sediments (Zhang 2003). Surface photoreactions might provoke photochemical transformations on the soil surface (Colvin 2003).

Occupational Exposure Assessment (OEA). While manufacturing NP-based materials, formulating them into products, transporting, handling in the storage facilities, workers may be exposed to NMs. Because higher concentrations of NMs and higher rate of exposure to them are more likely to happen in workplace surroundings, occupational exposures need special consideration. The primary route of exposure for workers, engaged in manufacturing NPs is considered to be through inhalation and/or dermal contact after the manufacturing process is complete (Hansen 2009). Exposure is less likely to take place during the manufacturing process itself, since most ENP manufacturing processes are performed in closed reaction chambers (Hansen 2009). Contamination and exposure of workers are more likely to occur while handling and bagging the NMs and also during cleaning operations (Luther and Malanowski 2004).

In the production phase, an occupational exposure mainly occurs while unloading the materials from shipping containers and cleaning the process equipment and vessels. During product manufacturing, exposures to NPs are highly process-specific. On the contrary, particles, bound in nanocomposites are not likely to release and handling of composites would result in lower occupational exposure levels. High exposures take place during product machining (i.e., cutting, drilling and grinding), repair, destruction and recycling (NIOSH 2006; 2009).

A study by Aitken et al. (2004), aimed to identify exposure scenarios, related to the manufacture and use of NPs, examined the production methods of fullerenes, CNTs, metals and metal oxides. They confirmed four main groups of ENP production processes: vapor deposition, gas-phase, colloidal and attrition processes. All production processes can potentially result in occupational exposure through inhalation, dermal or ingestion routes (Aitken et al. 2004). Maynard et al. (2004) performed exposure capacity of unprocessed airborne SWCNTs in production at four facilities that were using either the HiPCO (High-Pressure CO Conversion) or laser ablation production methods to evaluate the propensity for

aerosol particles to be released during agitation and to determine the size of particles released into the air while SWCNT material was removed from production vessels and handled before processing. The study concluded that occupational exposures of SWCNTs are most likely to occur during handling and bagging of the materials and there is high risk of dermal uptake (Maynard et al. 2004). Han et al. (2008) calculated occupational exposures in the production cycle of MWCNTs. Air samples were taken and the MWCNTs in the samples were counted by a transmission electron microscope (TEM). The outcomes yielded that most of the MWCNT exposure levels (0.43 mg m^{-3}) were lower than the current threshold limit value (TLV) for carbon black (3 mg m^{-3}). Yeganeh et al. (2008) studied the concentrations of airborne NPs, released during manufacturing of carbonaceous NMs, such as carbon nanotubes (CNTs) and fullerenes, 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 sites: inside the fume hood where NMs were produced, just external the fume hood, and in the background. Average mass concentrations and particle number concentrations were not considerably different inside the facility versus outdoors. On the other hand, large, some degree of increases in PM 2.5 and particle concentrations were associated with the physical handling of NMs. In many cases, an augment in the number of sub-100 nm particles accounted for the majority of the increase in total number concentrations. Photoionization results inferred that the particles suspended during handling, within the fume hood, were carbonaceous and so likely to include NPs, whereas those suspended by other activities, going on outside the fume hood, were not. Based on the outcomes of the study, the engineering controls at the facility were efficient at limiting exposure to NPs (Yeganeh et al. 2008). Fujitani et al. (2008) compared the particle size distributions and morphology of aggregated/agglomerated fullerenes at Frontier Carbon Corporation in Japan, for the duration of work and non-work periods as well as an agitation process, and compared it to near outdoor air. They observed that the particle number concentration of particles with a diameter <50 nm was not larger during the removal of fullerenes from a storage tank for bagging and/or weighing than in the non-work period. On the other hand, the concentration of particles with a diameter >1000 nm was observed to be larger during the non-work period. They also found that the use of a vacuum cleaner reversed these observations.

A significant concern is related to the processing including drilling and cutting of NM-hybrid composites. Bello et al. (2008) investigated the airborne exposures generated in a research lab during the dry and wet cutting of nanocomposites, consisting of fibers and polymer matrices, containing CNTs. No major difference in air concentrations during wet cutting, which is the usual procedure for such composites, was identified. Dry cutting, on the other hand, generated statistically considerable quantities of nanoscale and fine particles; in any case of the composite type. Using a variety of measuring instruments simultaneously Bello et al. (2008) evaluated the potential exposure to MWCNTs during chemical vapor deposition (CVD) growth in a university research lab, and

during subsequent handling as the CNTs are removed from the furnace and detached from the growth substrate. In contrast to Maynard et al. (2004) and Han et al. (2008), Bello et al. (2008) found no augment in the total particle number concentration and any particle size range during furnace operations compared to background. According to Biswas and Wu (2007), active operations in production will direct to high spikes of ultrafine particle number concentration. Once these operations stop, a steady decay will be observed due to primarily coagulation, evaporation, dilution, and/or deposition. The effects of spatial and temporal alteration are important as well in order to evaluate exposure precisely. Whereas the fraction of the total ultrafine particle number concentrations usually reduces, fine particle number concentrations raises with time and distance from the point of emission. Biswas and Wu (2007) observed that there is linear dependence between the active operations in production and the concentrations of NPs in the working settings, while many other authors suggested that the influences of background concentration as well as the potential special and temporal variations of exposure are very significant and have to be taken into consideration (Mazzuckelli et al. 2007; Mohlmann 2005; Schneider 2007). Major restrictions to the occupational exposure assessment are that official data on the number of workers exposed to NPs are not available. The concentrations of NPs in the working settings are rarely appropriately measured and the occupational exposure pathways are still not well studied. (Brun et al. 2008; Hansen 2009).

Consumer Exposure Assessment (CEA). Widespread consumer exposure via direct contact with ENP-containing products such as food and cosmetics are already taking place. It is also expected that the nature of consumer exposure will be disparate too, as the spectra of the nano-products is very diverse. Hansen et al. (2008) classified ENP-containing products into several categories (appliances, foodstuff and beverages, health and fitness, home and garden and goods for children). They noticed that the expected consumer exposure is higher for products in the categories of appliances, health and fitness, home and garden. These products represent a sunscreen lotion, facial lotion, a fluid product for outdoor surface treatment, and a spray product for indoor surface treatment. The next outline compares between the probability of exposure and the types of NPs used in the manufacturing of the products. The lack of information about the NPs, used in these products, is alarming since some of these materials might be potentially hazardous for their users. The evaluation of the consumer exposure to NPs is considerably limited by the lack of access to information about which commercially available products contain NPs, the exact nanomaterial content of these products and the consumer behavior towards them (Hansen 2009). For a lot of products, the number of users is also unidentified (Wijnhoven et al. 2009).

14.2.4 Risk Characterization

Risk characterization (RC) is the concluding step of the risk assessment procedure. RC is defined as evaluation of the incidence and severity of the adverse effects likely to happen in a human population or environmental compartment due to

real or predicted exposure to a substance, and may include risk estimation (ECJRC 2003; Leeuwen and Vermeire 2007). In this phase, all information, collected during the first three steps of risk assessment is taken together, weighted and the risk is quantified. RC is the ultimate step in the risk assessment method, in which the information from the hazard identification, dose-response and exposure steps are considered together to conclude and relate the actual likelihood of risk to exposed populations.

The quantitative RC evaluates the predicted environmental concentration (PEC) of a chemical agent with its predicted no-effect concentration (PNEC). The PNEC is the concentration, lower than which the exposure to the substance is not predicted to cause any adverse 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 below than 1, it is considered that no further testing or risk reduction measures are needed (ECJRC 2003). If it is greater than 1, further testing can be started to reduce the PEC/PNEC ratio (Nielsen et al. 2007). If that is not achievable, risk reduction measures should be implemented (ECJRC 2003). Important issues in this final step is an evaluation of the overall quality of data, the postulations and uncertainties associated with each step, and the level of confidence in the resultant estimates.

In 2008, Muller and Nowack studied the first fully quantitative environmental risk assessment of NPs. They used nano-particulate Ag at threshold concentrations of 20 mg L^{-1} and 40 mg^{-1} L and exposed *B. subtilis* and *E. coli* bacteria to it. The outcomes revealed that, at the above concentrations, Ag NPs did not affect the integrity of the microorganisms (both concentrations were equivalent to NOEC). In addition, Muller and Nowack (2008) calculated the PNEC values of nano-particulate Ag, TiO_2 and CNTs in water, which were 0.04 mg^{-1} L, <0.001 mg^{-1} L and <0.0001 mg^{-1} L, respectively. Combining these PNEC-values with the estimated exposure, they measured the environmental concentrations of the above NPs in Switzerland, stemming from diverse industries (textiles, cosmetics, coatings, plastics, sports gear and electronics). Predicting worst-case exposure scenarios levels, they observed that the RQs for Ag NPs and CNTs were below than 0.001, and concluded that there was little or no risk that these materials would do harm to aquatic organisms. However, exposure to TiO_2, might possibly cause risks, since its RQs were ranging from 0.7 to 16. Park et al. (2008) studied the risk of cerium oxide (CeO_2) to cause lung inflammation and concluded that it was highly unlikely that exposure to CeO_2 at the monitored and modeled environmental levels would elicit pulmonary inflammation.

14.3 QUANTITATIVE NANOSTRUCTURE TOXICITY RELATIONSHIPS (QNTR)

It is of critical importance for nanotechnology to evaluate the biological effects originated by Manufactured Nano Particles (MNPs). Experimental studies, particularly toxicological, are time-consuming, costly, and most of the time

impractical, calling for the development of proficient computational approaches capable of predicting biological effects of MNPs. Therefore, scientists have investigated the cheminformatics methods such as Quantitative Nanostructure–Activity/toxicity Relationship (QSAR/QNTR) modeling to establish statistically important relationships among measured biological activity profiles of MNPs, physical, chemical, and geometrical properties of manufactured NPs, either measured experimentally or computed from the structure of manufactured NPs.

Experimental outcomes of the toxicities of manufactured NPs have so far revealed that acute or repeated exposure to certain manufactured NPs may cause systemic, cellular, and/or genomic toxicities. Since the effects on health of a nanoparticle made from a specific chemical may be altered with changes in its size, shape, specific surface area, surface reactivity, and surface coatings, understanding the cause of these NP-related toxicities is complex. Further, air-borne or biological molecules may adsorb on the surfaces of pristine or coated NPs and vary their properties; and these surface-adsorbed molecules may exchange as NPs move into different compartments of the body. Therefore, determining and understanding nanoparticle toxicities by experiment is not easy. Computational approaches are perfect for rapidly exploring the effects of a large number of variables in complex scenarios.

One of the most hopeful approaches for predicting the biological properties of NPs uses a method that has proven very useful in the pharmaceutical industry over several decades. Quantitative Nanostructure Toxicity Relationships (QNTR) methods use statistical or machine learning methods such as neural networks, decision trees, and support vector machines to model the relations between physical and chemical properties of NMs and their biological effects. These are well-validated and tested methods that have been improved to a large extent over the past decade by incorporation of recent developments in mathematics and statistics. These methods are also in use by regulatory agencies, to predict toxicities of industrial chemicals and environmental pollutants. The methods are robust and intrinsically applicable to modeling a wide range of properties including materials properties and biological effects of chemicals (Fourches et al. 2010).

14.4 WORLD EFFORTS ON RISK ASSESSMENT OF NMs

14.4.1 The European Commission's Efforts

The European Commission (EC) has started research projects, technology platforms, working groups and other committees that deal with various aspects of public acceptance and risks linked to nanotechnology: The European Commission has, through the EC Action Plan for nanotechnology, implemented the objectives to investigate nanotechnology risks on health and the environment through funding of a number of research projects within the 6th and 7th Framework Programs. A number of EU projects are thus concluded or running, and offer EC Research DG and other EC offices with views on research priorities and up to date

input on state-of-the-art. Requirements for standardization of nanotechnology nomenclature, materials, tests systems etc. including aspects of testing NMs for safety and risks are being treated in these projects. The SCCP (European Commission's Scientific Committee on Consumer Products) are continuously evaluating consumer products and their main chemical components with respect to potential harm. For example, they have estimated the use of TiO_2 NPs in sun screens and judged that this is safe in contact with skin at concentrations below 25%, irrespective of particle size (SCCP 2007). ZnO NPs were not approved as a UV blocker, but allowed for use as a coloring agent (Hristozov and Malsch 2009).

EFSA. The European Commission has requested a scientific judgment from the European Food Safety Agency (EFSA) relating to the risks happening from nano science and nanotechnologies on food and feed safety and the environment. It was also asked to identify the nature of the possible hazards associated with actual and foreseen applications in the food and feed area and to supply general guidance on data needed for the risk assessment of such technologies and applications (Hristozov and Malsch 2009).

ETPIS. The European Technology Platform on Industrial Safety is focusing a great part of their efforts on assessment risks for human exposure to NPs in industrial working environments. The Scientific Committee on Emerging and Newly Identified Health Risks (SCENIHR) recently adopted an opinion on "the appropriateness of existing methodologies to assess the potential risks of nanotechnologies". Delivered at Commission's demand, the report concludes that existing risk assessment methodologies require some modification to deal with hazards associated with nanotech. According to the report, the existing toxicological and ecotoxicological methods may not be sufficient to address all of the issues arising with NPs. SCENIHR pointed out that very little is known about the physiological responses to NPs. Therefore, conventional toxicity and ecotoxicity tests should undergo modification regarding hazards evaluation and the detection of nanoparticle distribution in the human body and in the environment (Hristozov and Malsch 2009).

14.4.2 Norwegian Efforts

The Norwegian Research Council had published a national policy for research on nanosciences and nanotechnology (Norwegian Research Council 2006) where health and environmental risks are incorporated. The establishment of the research program Nanomat subsequently issued a call where risks were among the priority basis. The Norwegian Technology Board informs commercial, public and legislative bodies on several recent and potential future implications of nanotechnology, together with safety and risks. The Norwegian Scientific Committee for Food Safety is, through the European Food Safety Agency, taking part in risk assessment of NMs in contact with food and animal feed. The Norwegian Pollution Control Authority (STF) is currently taking part in working groups on environmental risks of nanotechnology within the Organization for economic co-operation and development (OECD) and the European

Commission, and in that way following international efforts closely on this area (Hristozov and Malsch 2009).

14.4.3 U.S. Regulation Efforts

The possibility of establishing regulations on nanosilver is extremely debated and evaluated, both within individual countries, supra-national federations like the EU (Scientific Committee on Emerging and Newly Identified Health Risks (SCENIHR), European Food Safety Authority (EFSA) and European Committee for Standardization (CEN), and in international organizations like OECD and ISO (International organization for standardization). In general, the applicability of present laws and the modification or establishment of new ones is limited by the lack of data on properties and use of NMs in consumer products (Franco et al. 2007). The most important U.S. agencies concerned with environmental risks are the Environmental Protection Agency (EPA) and the Occupational Safety and Health Administration (OSHA).

The purpose of EPA is to protect human health and the environment. One of EPA's major purposes is to make sure that all Americans are protected from significant risks to human health and the environment where they live, learn, and work. The assignment of OSHA is to ensure safe and healthful working situation for working men and women by setting and enforcing standards and by providing training, outreach, education and assistance. Both are so directly concerned with environmental implications of nanotechnology. Regulation of the production and use of NMs is most likely to occur under the Clean Air Act (CAA) and the Toxic Substances Control Act (TSCA) and both of them are concerned with environmental health impacts. Under the Clean Air Act no specific requirements or regulatory procedures presently exist for NPs. The Clean Air Act provides a list of 189 substances that have been decided to be hazardous air pollutants. The Act also recommends procedures for adding and removing substances from this list. If unfavorable health and environmental effects are encountered as a result of emissions from the use or manufacture of NMs, the EPA will be enforced to list such substances as hazardous air pollutants and require emission controls. Commercial applications of nanotechnology are regulated under TSCA, which authorizes the EPA to review and set up restrictions on the manufacture, processing, delivery, use and/or disposal of new materials that create an unreasonable risk of injury to human health or the environment. The EPA can enforce limits on production, including an outright ban and the EPA may revisit a chemical's status under TSCA and change the degree or type of regulation when new health/environmental data warrant (Bergeson 2004; Bergeson and Auerbach 2004; Toxic Substances Control Act 2008). If the incident with genetically engineered organisms is any sign, there will be a push for not only EPA but also OSHA to update regulations in the future to reveal changes, advances, and trends in nanotechnology. U.S. has taken initiatives to classify silver NPs as a pesticide and is taking steps towards a possible ban. A strong demands from various organizations, together with the Natural Resources Defense Council, the

U.S. EPA decided to place nanosilver under the authority of the FIFRA (Federal Insecticide, Fungicide and Rodenticide Act), as it was redefined as an antimicrobial agent (Henig 2007). After transferring this regulatory issue to FIFRA, the U.S. EPA determined to regulate only specific nanosilver products, namely those are having antimicrobial properties (Henig 2007). The U.S. EPA is proposing a voluntary reporting program, called the Nanoscale Materials Stewardship Program (NMSP). The NMSP proposal encourages companies to willingly report to EPA information on existing NMs and nanobased products and the data should comprise chemical name; physical and chemical properties such as density, melting point, and surface area; expected uses; life cycle; and various byproducts that are likely to be produced during manufacture and use of the materials (Chatterjee 2007).

14.5 CHALLENGES IN RISK ASSESSMENT OF NMs

Each of the tasks of risk assessment holds certain limitations and challenges. RC, being the final step of risk assessment, sums all of these limitations. Though toxicity has been reported for many ENPs, in many cases further investigation and confirmation are needed before hazard can be identified (Hansen 2009). Secondly, according to DRA no-effect concentrations (NECs) are one of the important parameters in assessing the toxicity of ENPs, however, several studies observed dose-response relationships but they do not explicitly state any NEC values. This severely hindered the DRA by the fact that it is still unclear what the most suitable dose-descriptors are for many ENPs. Thirdly, EA is hampered by difficulties in monitoring nanomaterial exposure in the workplace and the environment, and by deep uncertainties in regard to the environmental fate and the biological pathways of ENPs (Hristozov and Malsch 2009). There is also a concern regarding NMs in food and feed that depends on their particular intrinsic characteristics, the lack of *in vivo* toxicity data and *in vitro* validated tests and the limited practical experience on risk assessment in this area (ECJRC ENPRA 2003).

According to Chaundry et al. (2006) the potential gaps of regulation of NMs falls into two main categories. First, the key piece of regulation relating to a sector, application, product or substance fails to address an aspect of particular interest. For instance, if a piece of legislation is intended to address the human health impacts but fails to address possible environmental impacts of NMs or nano products. Second, a piece of legislation is intended to address a specific aspect of particular interest to a sector, application, product or substance but fails to address it due to exemptions (e.g. threshold, volume or tonnage related), lack of foresight, limitations in technical or scientific knowledge, etc. Thus, in summary it is currently difficult to determine the most relevant risk indicator(s) for all the NMs. Nevertheless, continuous update of various regulation/guidance for NMs assessment as scientific knowledge evolves would improve the situation.

14.6 OVERCOMING THE LIMITATIONS TO RISK ASSESSMENT OF ENPs

There is a wave of interest among various government and non-governmental organizations to ensure the use of nanotechnology in a safe and appropriate manner. However, only a few plan of action on safe use of nanotechnology is actually in place. Many elaborated reports on impact of nanotechnology have been written recently by various governmental organizations of United Kingdom, Australia, Canada, the European Union, US EPA and NIOSH. (Wise et al. 2013). Additionally, several non-government organizations have produced numerous reports on nanotechnology, including Project on emerging nanotechnologies (PEN) (an extensive collection of reports, data, inventory and analysis), Friends of the Earth (several reports on nanotechnology in food and agriculture), and Environmental Defense for the responsible development of new NMs (Wise et al. 2013). Though all these reports vary in terms of their specific recommendations, the main them is a call for more information on exposure and toxicity, and for some sort of information-gathering mechanism.

Traditional approaches for risk assessment of substances cannot always be applied to all NMs due to the missing data or uncertainties with existing information. One of the alternative approaches is the utilization of control banding, which is a simplified approach to evaluate the risks from activities and the substances they involve into bands according to the potential for exposure and the hazard (UKNSPG 2012). For each risk band, control measures are then suggested. Furthermore, the control banding approach of COSHH essentials can be applied to NMs (UKNSPG 2012). This is a tried and tested, robust approach for many chemical hazards, however, there are currently no COSHH Essential control measures for NMs, but development is on-going (UKNSPG 2012).

Another tool that has already been applied on NMs is Multi Criteria Decision Analysis (MCDA) (Linkov et al. 2007). The common purpose of Multi Criteria Decision Analysis methods is to evaluate and choose among different decision alternatives based on multiple criteria, using systematic and structured analysis. A number of different MCDA-methods exist following various optimization algorithms, varying in both the types of value information needed and in the extent they are dependent on computer software. Some techniques rank options whereas others identify a single optimal alternative and again others differentiate between acceptable and unacceptable alternatives. Key issues in relation to MCDA are, who defines what the initial criteria are, what alternatives are available to the decision maker and how the different criteria are translated into a numerical score in order to rank the different alternatives (Mayer and Stirling 1999; Hansen 2009).

However, 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. Furthermore, a compilation of results and establishment of open access databases can serve the international scientific society and reduce the duplication of research efforts.

14.7 CONCLUDING REMARKS

Nanotechnology has an immense impact on our daily life by providing various useful products and solutions to global problems. However, one has to remember that influence of nanotechnology on human health and natural environment is neither completely understood nor established. Therefore, it is essential to perceive physico-chemical properties of NMs to determine their behavior in environment. The present research data on various aspects of environmental issues and risk assessment related to ENMs clearly shows that there are still many gaps in available experimental data devoted to risk assessment of ENPs that are already available on the market. Thus, a comprehensive assessment of the impact of NMs at different stages of production, use, and disposal/recycling is an immediate necessity to tackle these problems. This includes understanding environmental pathways, fate and transport processes, and reasonably foreseeable exposures. Since, it is the interaction of toxicity with persistence, which leads to the greatest harm and must be most actively guarded against. While it is clearly important to identify localized environmental concerns, it is absolutely essential to identify potential global environmental impacts associated with materials that are both persistent and toxic. Further, more detailed risk assessments based on precise models of environmental exposure routes may be reserved for ENMs found to be both persistent and toxic, or for high exposure scenarios. Finally, finding answers to the present challenges and using new and upcoming technologies/systems/methods would help to elucidate the toxicities of various NMs and be beneficial to nanotechnology, paving the way for safer products and a better quality of life.

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