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1. Introduction

The most recent definition for nanomaterials (NMs) was recommended by European Commission in 2011 (*The European Commission 2011*). In this report, a nanomaterial is defined as a “natural, incidental or manufactured material containing particles, in an unbound state or as an aggregate or as an agglomerate and where, for 50 % or more of the particles in the number size distribution, one or more external dimensions is in the size range 1 nm-100 nm”. Furthermore, the engineered or manufactured nanomaterials (MNMs) are defined as NMs that have been specifically engineered for an application (*Colvin 2003*).

MNMs have important applications in the medical, cosmetic, pharmaceutical, energy, remediation, electronics, textile, plastics and food industries (*Benetti, F., et al., 2014, Nowack and Bucheli 2007*) boosting the nanotechnology industry to reach an estimated market value of \$ 3 trillion by 2015, accounting for the 15% of global manufacturing output (*Howard and Murashov 2009*). A recent report of Piccinno *et al.* (2012) provided new information on production amounts and product distribution of ten different MNMs (TiO₂, ZnO, FeO_x, AlO_x, SiO₂, CeO₂, Ag, quantum dots (QDs), carbon nanotubes (CNTs) and fullerenes). The most produced MNM is TiO₂ with up to 10,000 t of worldwide production. CeO₂, FeO_x, AlO_x, ZnO, and CNT are produced between 100 and 1000 t/year. The data for SiO₂ range from less than 10 to more than 10,000 t/year. Finally Ag, QDs and fullerenes are produced in Europe in amounts less than 10 t/year.

With MNMs' increasing production, increasing quantities of nanoparticles (NPs) are entering the environment during their synthesis and during the manufacture, usage, disposal, and recycling of products that contain them (*Köhler et al. 2008*). The hazards and risks of NPs are different qualitatively and quantitatively from larger particles (*Owen and Handy 2007*). Due to their small size, large surface to volume ratio and high reactivity and mobility, concerns have been raised regarding the potential hazards of MNMs on human health (*Botelho et al. 2014, Schug et al. 2014*) and their biogeochemical behaviour and toxicity in the environment (*Wang Z., et al. 2013, Thomas et al., 2011*). A very recent study by *Watson et al. (2014)* suggested that certain nanoparticles, incorporated in many every day products, can harm DNA. This study relied on a high speed screening technology to analyse DNA damage. This approach allows to study nanoparticles potential hazards at a much faster rate and larger scale than previously, provides a standardized method to assess the engineered nanomaterials that are being developed and probably could help to design safer forms of nanoparticles. Evaluating the existing literature data until 2010 on toxicity (L(E)C50 values) of MNMs in environmentally relevant species, Kahru and Dubourguier (2010) ranked Ag NPs, ZnO NPs, CuO NPs TiO₂ NPs, CNTs, and fullerenes according to their lowest median L(E)C50 value for the tested organisms. They classified Ag and ZnO NPs as “extremely toxic”, (L(E)C50 < 0.1 mg/l), fullerenes and CuO NPs as “very



toxic”, (L(E)C50 0.1–1 mg/l), CNTs as “toxic” (L(E)C50 1–10 mg/l) and TiO₂ NPs as “harmful”, (L(E)C50 10–100 mg/l). For this reason, one of the main recommendations in the early review of Handy *et al.* (2008), the interdisciplinary approach to the problem has been already realized with research foundations and centers exclusively dedicated to study the environmental implications of nanotechnology are being established in many countries. However, although there are few studies of the long-term consequences of NPs on human health, governmental agencies (e.g. the United States National Institute for Occupational Safety and Health, Japan's Ministry of Health) have recently raised the question of whether apparently harmless materials (e.g. carbon-based nanotubes) should be treated with the same concern as known carcinogens such as asbestos (Sharify *et al.* 2012). If one adds also the exponential increment of the number of articles and reviews about this topic over the last few years then there is no doubt that the phrase of Kahru and Dubourguier (2010) “the signature of the next decade will be the (eco)toxicogenomics and nano(eco)toxicology 2010s” was prophetic.

Predicting and assessing the environmental impact of MNMs is a challenging task complicated mainly by: a) Lack of information regarding their production and release volumes. b) The identification of hazardous MNM properties including properties such as chemical composition, size, shape, aspect ratio, surface charge, redox activity, dissolution, crystallinity, surface coatings, and the state of agglomeration or dispersal (Thomas *et al.*, 2011). c) The large and rapidly increasing number of different MNMs. d) The different behaviour of MNMs in different environments. Furthermore, the vast majority of research until now has taken place under highly controlled laboratory conditions, while very little is known about the fate, transport, and behaviour of MNMs under environmentally relevant conditions preventing meaningful and accurate assessment of MNMs risk (Musante and White 2012).

Fate and transport processes that can act on nanomaterials in products, and after their release, include photochemical transformation, oxidation and reduction, dissolution, precipitation, aggregation, adsorption, desorption, combustion, reactions with biomacromolecules and abrasion, among other biogeochemically driven processes (Westerhoff *et al.*, 2013, Nowack *et al.* 2012, Liu *et al.* 2012). In addition, MNMs are also affected by agglomeration or aggregation and settling (Chen *et al.* 2010). The expected nature of transformations will depend on the solution conditions and even slight variations between systems may lead to different behaviors. Many of the transformations are dynamic but not readily reversible and therefore the history of the MNM will affect its properties and state in the environment. MNMs will possibly undergo multiple transformations simultaneously (e.g. interaction with NOM and oxidation or reduction), or successive irreversible transformations (e.g. irreversible adsorption of different proteins upon successive exposures to plasma and intercellular materials). These transformations and others will alter the fate, transport, and toxicity of nanomaterials. The nature and extent of these transformations must be understood before significant



progress can be made toward understanding the environmental risks posed by these materials (*Lowry et al. 2012*).

The present report is aimed to summarize the current literature on the subject of environmental risk assessment with emphasis on the most recent results. Studies about the implications of MNMs on terrestrial regions, aqueous regions and the atmosphere will be seen separately, and the developed strategies for examining their ecotoxicity and potential environmental impact will be discussed. Methods of decreasing the observed toxic effects, developing safer MNMs and remove MNMs from the environment will also be mentioned. Finally, some suggestions for environmental risk assessments and the overall management of MNMs' exposure in the environment are given.

2. Aquatic environment

In aquatic systems, where many contaminants ultimately reside, nanoparticles may be directly and/or indirectly toxic. Direct effects arising from the high density of reactive groups at the nanoparticle surface while indirect effects result from the production of reactive oxygen species (ROS) and dissolution of toxic ions or impurities into solution (*Li et al. 2013*). Natural waters and groundwater may be depleted of oxygen and result in MNM reduction (*Lowry et al. 2012*).

a. Metal oxide NPs

Because of their significant commercial relevance and potential toxicity, the environmental fate of CuO, ZnO, and TiO₂ NPs is of growing interest (*Bondarenko et al. 2013*). Once released into aquatic environments, the potential ecotoxicological and public health risks associated with metal oxide NPs will be influenced by their fate and transport within these systems. Those MNMs from consumer products may, pose threat to the 'non-target' organisms, such as natural microbes and aquatic organisms. Recent review summarizes the current research on (eco)toxicity of Ag, CuO and ZnO NPs on the toxicity towards algae, crustaceans, fish, bacteria, yeast, nematodes, protozoa and mammalian celllines (*Bondarenko et al. 2013*). The toxic range of all the three metalcontaining NPs to target- and non-target organisms overlaps, indicating that the leaching of biocidal NPs from consumer products should be addressed. The most toxic out of the three studied NPs was nanosilver and crustaceans, algae and fish proved most sensitive to the studied NPs.

Environmental research about CuO NPs' toxicity has definitely proved that they were highly toxic to aquatic organisms (*Pseudokirchneriella subcapitata* algae, *Aruoja et al. 2009*; *Danio Rerio* zebrafish, *Griffitt et al. 2007*; *Mytilus galloprovincialis* mussel, *Gomes et al. 2012*; *Landoltia punctata* duckweed, *Shi et al. 2011*; fish and bacteria *Bondarenko et al. 2013*; *Escherichia coli* bacteria, *Bondarenko et al. 2012*; *Allogamus ligonifer* shredder, *Pradhan et al. 2012*) even in low concentrations. Due to their soluble form, CuO NPs are easy to intake and aggregate in vital parts of



the organisms. Then they are dissolute to the high toxic Cu^{2+} triggering ROS and resulting even in DNA damage in the case of bacteria (*Chang et al. 2012*).

Bioaccumulation profiles and toxicological effects of ZnO and CuO NPs were examined by dietary and waterborne exposure of goldfish (*Ates et al. 2014*). The results mainly suggest a) that NPs accumulated mainly in the intestine, gills, and liver. b) The heart, brain, and muscle tissue did not exhibit any significant NP accumulation under any exposure condition. c) Uptake of NPs was lower in dietary exposure in comparison to waterborne exposure. d) the ingestion of contaminated food is a pathway for the transfer of the NPs from lower trophic levels to higher organisms in the food chain. e) that toxic effects of NPs could vary with route of exposure and chemical properties of NPs.

The suspensions of the ZnO NPs were not acutely toxic to goldfish. The suspensions of the CuO NPs, on the other hand, were more toxic in both dietary and waterborne exposure. This study shed light onto the short-term effects of exposure to ZnO and CuO NPs.

Earlier reports on ZnO NPs toxicity showed that they inhibited alga *P. subcapitata* growth (*Franklin et al. 2007*). Another study on endobenthic species indicated that clam burrowing behaviours and feeding rates were significantly impaired after exposure to ZnO NPs (*Buffet et al. 2012*). *Xiong et al. (2012)* proved that ZnO NPs acute toxicity to zebrafish was dose-dependent, and zebrafish death occurred once the concentration reached 2mg/L.

In freshwater, the short-term in vivo exposure to TiO_2 NPs was demonstrated to induce several changes on functional parameters of *Dreissena polymorpha* hemocytes (*Couleau et al. 2012*). Despite the absence of any adverse effect on hemocyte viability, a non-linear response in which phagocytosis was inhibited at the lower concentrations of NPs (0.1–1 mg/L) could be reported. Also the skin, the gill and especially the liver of *Cyprinus carpio* were affected when exposed in freshwater containing TiO_2 NPs (*Lee et al. 2012b*). Furthermore, *Fan et al. (2012)* showed that, apart from its own toxicity, TiO_2 NPs when they are present assist Cu to be sorped more easily and thus inducing higher levels of oxidative stress and physiological damage in *Daphnia magna*.

Recently the importance of UV-only effects on the ultimate phototoxicity of TiO_2 NPs to aquatic organisms has been demonstrated (*Li et al. 2014*). Furthermore, it was verified the importance of species sensitivity and exposure scenarios in future test guidelines of nanophototoxicity.

b. Metal NPs

Because of their antibacterial effects, chemical stability and relatively low cost of production, Ag NPs are among the fastest growing and most commercialised groups of engineered nanomaterials (*Fabrega et al. 2011*). In sea water, studies of the behaviour of Ag NPs are rather limited. Generally



the effects of metallic NPs on algae are predicted to be a function of both the rate of metal dissolution and the toxicity of the resulting ions.

Ag NPs are destabilized in seawater condition (Zhong 2014). An increased aggregation tendency of Ag NPs in seawater is attributed to its relatively high cation concentration that could neutralize the negatively charges adsorbed on the surface of Ag NPs and reduce the electrostatic repulsion forces between nanoparticles. These results may be helpful in understanding the environmental risk of discharged silver NPs in seawater conditions.

Ag NPs are predicted to be among the more toxic NPs to algae in both fresh water and marine environments. This is because Ag^+ is one of the most phytotoxic metal ions (Hiriart-Baer et al. 2006) and, in the presence of chloride, aqueous toxicity may be augmented by the formation of the moderately hydrophobic, neutral chloro complex (Reinfelder and Chang 1999). However, according to Turner et al. (2012) concentrations of Ag NPs high enough to elicit a phytotoxic response are unlikely to be encountered in the marine environment. These findings were supported by another recent sea water study related with the accumulation of Ag NPs by the gastropod, *L. littorina*, where was little measurable Ag accumulation by (or association with) *L. littorina* (Li et al. 2013). In a recent study about the toxic effect of silver NPs in *Artemia nauplii* (Chinnasami et al. 2014) hatched in sea water showed that that the nanomolar concentrations of AgNPs have toxic effect on both *Artemia nauplii* and cysts. As the concentration of AgNPs increased, the mortality rate, aggregation in gut region, apoptotic cells, and DNA damage increased in nauplii, whereas the percentage of hatching in *Artemia* cysts decreased.

In another study, the skin, the gill and more severely the liver of *Cyprinus carpio* were affected when they were exposed in freshwater containing a critical dose of dispersed citrate-capped Ag NPs (Lee et al. 2012a).

A literature survey on the existing data on the dissolution of Ag NPs revealed that only qualitative trends can be documented as the nature of the NPs and of the immersion medium are practically not comparable (Losa et al. 2014). The dissolution effects were confirmed by cell culture experiments (human mesenchymal stem cells and neutrophil granulocytes) where Ag NPs that were stored under argon had a clearly lower cytotoxicity than those stored under air. They also led to a less formation of reactive oxygen species (ROS). The result highlight that Ag ions are the toxic species.

The studies on the effects of water hardness and humic acids on the bioaccumulation and toxicity of AgNPs coated with polyvinyl pyrrolidone (PVP) to the freshwater snail *Lymnaea stagnalis* after dietary exposures indicated that bioaccumulation and toxicity of Ag from PVP-AgNPs ingested with food are not affected by water hardness and by humic acids, although both could affect interactions with the biological membrane and trigger NP's transformations. Therefore results



suggest that correcting regulations for water quality could be irrelevant and ineffective where dietary exposure is important (López-Serrano Oliver *et al.* 2014).

Although Au NPs are generally considered non toxic like bulk gold, following marine bivalve *Scrobicularia plana* exposure to Au NPs during 16 days of very high dose ($100 \mu\text{g Au L}^{-1}$) treatment, the clams accumulated Au in their soft tissues and their burrowing behaviour was impaired (Pan *et al.* 2012).

Taking into consideration that metals are already a part of the body's metabolic system and the evidences regarding the use of nanometals from ancient times the concept of using metallic NPs for therapeutic applications could be the best choice if these NPs are safely designed to over-come the toxicity profile and hazardous effects (Sengupta *et al.* 2014).

c. Carbon-based MNMs

Carbon nanotubes (CNTs) are hydrophobic in nature and thus tend to accumulate in sediments if released into aquatic environments. Mwangi *et al.* (2012) evaluated the toxicity of different types of CNTs in 14-d water-only exposures to an amphipod (*Hyaella azteca*), a midge (*Chironomus dilutus*), an oligochaete (*Lumbriculus variegatus*), and a mussel (*Villosa iris*) and found that 1.00 g/L of commercial sources of CNTs significantly reduced the survival or growth of the invertebrates. Toxicity was influenced by the type and source of the CNTs, by whether the materials were precleaned by acid, by whether sonication was used to disperse the materials, and by species of the test organisms. Their results suggest that releases of as-produced CNTs will cause toxic effects on the benthic invertebrates. This will not only reduce the population of the invertebrates but also likely interfere with ecological balances and has the potential to disrupt the food chain in the impacted area.

In vivo study of the biological response to carbon nanotubes network probed by the zebrafish model it was concluded that tubes agglomeration at an average bulk size of 500 nm before their exposure to cells probably diminishes their chemical and/or oxi-reduction reactivity, suppressing their cyto- and genotoxic effects (Filho, 2014). Findings are important and shall encourage studies into environment safety and remediation based on the use of CNTs. Raman spectroscopy and microscopy conducted in the intestine of the zebrafish revealed complete clearance of the CNT network as well as minimal disturbances, such as aneurysms, hyperemia, and reversible inflammatory focus in the zebrafish gills.



3. Terrestrial environment

a. Soil

MNMS are applied deliberately for soil remediation and are also released unintentionally through various other pathways to soil. The pathways by which MNMs enter soils could be very different depending on the life-cycle of the product. For example, personal care products (such as cosmetics, coatings, cleaning agents and dietary ingredients) usually contain well-dispersed MNMs and these enter the environment mostly *via* sewage treatment plants. Some MNMs in plastics, glass and ceramics are released in the solid disposal area. The main pathway for MNMs abraded and released from textiles (mostly Ag NPs) is through the sewage treatment plant. Alternatively, MNMs in capacitors, filters, plastics and electronic equipment could be released during incineration (*Pan and Xing 2012*).

Culture-dependent and -independent methods on fungal and bacterial soil microbial communities showed that the bacterial soil community is transiently affected by the presence of SWNTs with the major impact of SWNTs on bacterial community observed after 3 days of exposure and complete recovery was observed after 14 days (*Rodrigues et al. 2013*). On contrary, the fungal community did not recover during the experiment. In another study, Zhou *et al.* (2013) indicated that CNTs either in the form of SWCNTs or MWCNTs have more significant impacts on the environmental fate of the common hydrophobic organic pollutant 2,4-Dichlorophenol (2,4-DCP), when they are entering soil together via strong sorption of CNTs than 2,4-DCP already present in soil. The effects of CNTs on the degradation of ^{14}C -2,4-DCP in the soil by endogenous microorganisms depended also on the concentration and type of CNTs with SWCNTs having a stronger effect than MWCNTs on the fate of ^{14}C -2,4-DCP in soil (lower mineralization rate of ^{14}C -2,4-DCP). Furthermore, Chung *et al.* (2011) demonstrated that MWCNTs significantly inhibited the activities of 1,4- β -glucosidase, cellobiohydrolase, xylosidase, 1,4- β -N-acetylglucosaminidase, phosphatase and microbial biomass-C and -N in soils. In contrast, the influence of C_{60} in soil microorganisms is insignificant in the short term, as their mobility is drastically reduced due to the adsorption by the soil organic matter (SOM) (*Tong et al. 2007*).

Concerning metal and metal oxide NPs, recent data have shown that they affect soil microorganisms (*Peyrot et al. 2014, Dinesh et al. 2012*). At the low Ag concentrations, AgNPs caused greater toxic effects to the soil enzymes, while for the larger concentrations of total soil Ag, causes of the negative effects on enzyme activities are less obvious but colloidal forms of Ag play a role (*Peyrot et al. 2014*).

Hänsch and Emmerling (2010) showed that although Ag NPs did not influence microbial biomass-N, enzyme activities, soil pH and organic C the microbial biomass was significantly decreased while basal respiration and metabolic quotient (basal respiration per unit of microbial biomass) was increased



with increasing NP application rate. Furthermore, TiO₂ and ZnO NPs reduced both microbial biomass and bacterial diversity and composition (*Ge et al. 2011*) and they significantly inhibited soil protease, catalase, and peroxidase activities (*Du et al. 2011*). Moreover, Zn and ZnO NPs inhibited the activities of dehydrogenase, β -glucosidase and acid phosphatase in soils (*Kim et al. 2011*). Another toxicity study showed that terrestrial isopods *Porcellio scaber* exposed to ZnO on NPs died following bioaccumulation (*Pipan-Tkalec et al. 2010*). There are suggestions that bioaccumulated Zn is primarily from Zn²⁺ dissolved from the ZnO NPs, not from the particles themselves NPs', and thus dissolubility of NPs plays significant role on the toxicity (*Chang et al. 2012*).

The study on the influence of soil type and environmental conditions on ZnO, TiO₂ and Ni nanoparticles phytotoxicity has shown that in a majority of cases, aging and increase of temperature caused a reduction of toxicity of NPs (ZnO, TiO₂ and Ni), while light conditions increased the toxic effect of NPs (*Josko et al. 2013*). Additionally, the influence of five metal nanoparticles (Ag, Cu, TiO₂, ZnO) present in biosolids on soil microbial community has been studied as a function of time (*Shah et al. 2014*). Results indicate that ZnO and Zero Valent Cu NPs were not toxic to soil bacterial community. Biosolids mixed with Ag NPs and TiO₂ in contrast changed the bacterial richness and composition in wavering pattern as a function of time. Finally, it has been suggested caution when interpreting the toxicity of NPs based on single time point study.

Concerning the implications of QDs, *Hsu et al. (2012)* investigated the potential toxicity of CdSe/ZnS mercaptosuccinic acid (MSA)-capped quantum dots (QDs-MSA) and the sublethal effects on reproduction of nematode *Caenorhabditis elegans*. They found that QDs-MSA might have disrupted the motor neurons during the reproduction process and that QDs-MSA affected *Caenorhabditis elegans* by a different mechanism than the well-known toxic Cd metal.

b. Plants

The interactions between MNMs and vascular plants are of great importance, as plants closely interact with soil, water, and the atmosphere, and constitute one of the main routes of exposure for higher species, i.e. accumulation through the food chain. MNMs affect plants in a different way than bulk materials. For example, *Musante and White (2012)* found that Ag NPs were more phytotoxic (as measured by plant growth and transpiration) than bulk Ag powder.

Current literature shows contradictory evidence on the phytotoxicity of MNMs (*Mirales et al. 2012*) probably because the phytotoxicity and tissue-penetration ability of an MNM depend strongly on its physical and chemical properties. Cellular penetration is the most accepted mode of action by which MNMs interact with plants. To assess the potential hazard of MNMs in the terrestrial environment, the toxicity in several types of plants, specifically the effects on seed germination and root growth have to be studied.



One of the most used MNMs for soil remediation is zero-valent iron (ZVI). For ZVI NPs, El-Temsah and Joner (2012) suggested that at low concentrations they can be used without detrimental effects on plants as complete inhibition of germination of ryegrass, barley, and flax was observed only at 1000–2000 mg L⁻¹. In addition, the presence of soil had a modest influence on toxicity, and inhibitory effects were observed at 300 mg ZVI NPs L⁻¹ water in soil. Complete inhibition was observed at 750 and 1500 mg L⁻¹ in sandy soil for flax and ryegrass, respectively, while for barley 13% germination still occurred at 1500 mg L⁻¹. In clay soil, inhibition was less pronounced.

Regarding metal oxide MNMs, at a concentration of 2000 mg/L ZnO, inhibition of germination was seen for corn (*Zea mays*) but not for the other various plant species studied. In examining root growth, a variety of adverse outcomes were observed at different concentrations of ZnO in soybean (*Glycine max*), mesquite (*Prosopis juliflora-velutina*), palo verde (*Parkinsonia florida*), tumbleweed (*Salsola tragus*), and corn (*Zea mays*) (Thomas et al., 2011). The effect of a 7 day exposure to 100 mg/L ZnO, fullerene soot or TiO₂ NPs on gene expression in *Arabidopsis thaliana* roots was studied using microarrays (Landa et al. 2012). The three types of MNMs resulted in qualitatively and quantitatively distinct gene expression responses. It is evident that ZnO NPs deserve particular attention from the perspective of potential environmental hazards. It elicited the highest stress response, in terms of both gene expression and phenotype. Fullerene soot also caused significant gene expression response, resulting in the upregulation of mainly stress-related genes. Fullerene soot should be considered as potentially phytotoxic material. In contrast, TiO₂ showed only weak impact on *A. thaliana* gene expression, indicating potentially minimal toxicity and concern with regard to the overall risk to plants. Other data suggest that only the smallest TiO₂ NPs may be accumulated in wheat plants, although in limited amounts and that their impact is moderate (Larue et al. 2012).

Although several studies have shown that CeO₂ nanoparticles are accumulated in plants without modification, recent investigations revealed that CeO₂ induced a type of aggregation NPs within the components of the roots and that changes the nutritional properties of cilantro (Morales et al. 2013). On the other hand, atmospheric NPs can be taken up and distributed within plant tissues, which could represent a threat for environmental and human health (Hong, 2014).

The effects of graphene on root and shoot growth, biomass, shape, cell death, and ROS of cabbage, tomato, red spinach, and lettuce, were investigated using a concentration range from 500 to 2000 mg/L. The results indicated that after 20 days of exposure, graphene significantly inhibited plant growth and biomass. Concentration dependent increase in ROS and cell death as well as visible symptoms of necrotic lesions were observed on cabbage, tomato, and red spinach mediated by oxidative stress necrosis while little or no significant toxic effect was observed with lettuce seedlings under the same conditions (Begum et al. 2011).



Overall, the mechanisms by which engineered nanomaterials penetrate plants are not well understood, and further research on their interactions with plants is needed. Important questions needed to be resolved including a determination of actual or predicted concentrations of MNMs, assessment of the nutritional content modifications and/or accumulation of MNMs, component metal, and cocontaminants in edible plants and their implications on human diet, nutrition, and health, assessment of the consequences of MNM-induced changes in soil health, physiological process, and yield on agricultural production and food security, and transfer of MNMs in trophic levels. Given the significant implications of MNMs exposure and the rather large knowledge gaps that exist, it will be prudent to observe judicious and targeted use of MNMs so as to minimize environmental release until a comprehensive environmental fate and effects assessment can be undertaken (*Gardea-Torresdey, 2004*).

4. Atmosphere

Since most of the NPs that are present in aquatic and terrestrial environments are originally formed in the atmosphere, it is important to distinguish the various emission sources of airborne NPs. Natural NPs concentration in the atmosphere from different natural sources including volcanic eruptions, forest fires, hydrothermal vent systems, physical and chemical weathering of rocks, precipitation reactions, and biological processes is low compared to the levels caused by man-made activities like combustion processes, diesel and gasoline-fueled vehicles and stationary combustion sources. It has been assessed that the amount of incidental NPs in the atmosphere due to human activity is more than 36% of the total particulate concentrations, and the forecast for the years ahead is that there will be a strong increase on atmospheric NPs due to the activity in industries related to the use of MNMs. Nevertheless, at present there are no real environmental data (*Farré et al. 2011*). In their review Kumar *et al.* (2010), implied that in urban atmosphere the dominant source of NPs is road transport. However in special locations like airports (*Hu et al. 2009*), ports (*Saxe and Larsen 2004*) and demolition or building sites (*Hansen et al. 2008*), other NP sources may be dominant. The contribution to urban nanoparticles from non-vehicle exhaust sources (e.g. road–tyre interaction, construction and demolition, aircraft, ships, municipal waste incineration, power plants, domestic biomass burning, forest fires, cigarette smoking, cooking, and secondary formation.) may become more pronounced in future (Kumar *et al.* 2014)

While in other media (i.e. liquid solutions, soil, and living organisms) MNMs are classified mainly to organic and inorganic, atmospheric NPs are classified according to their size in three modes. The airborne NPs in the range of 1-30 nm are called nucleation (or nuclei) mode particles and they are predominantly a mixture of two or more mutually exclusive aerosol populations (Lingard *et*



al., 2006). These are not present in primary exhaust emissions, but are thought to be formed through nucleation (gas-to-particle conversion) in the atmosphere after rapid cooling and dilution of emissions when the saturation ratio of gaseous compounds of low volatility reaches a maximum. Nucleation mode particles are found in high number concentrations near sources. Collisions with each other and with particles in the accumulation mode are largely responsible for their relatively short atmospheric life time. Accumulation mode particles or soot mode NPs are carbonaceous (soot and/or ash) agglomerates in the range of 30-300 nm. They derive mainly from the combustion of engine fuel and lubricant oil by diesel-fuelled or direct injection petrol-fuelled vehicles (*Wehner et al. 2009*), as well as from the coagulation of nucleation mode particles. Due to their relatively long atmospheric life times (typically days to weeks) they can travel over very long distances in the atmosphere. Furthermore, the size of accumulation mode particles is comparable with the wavelengths of visible lights causing visibility impairment problem in many urban areas. Finally, The Aitken mode is an overlapping fraction, in the range of 20-100 nm, of the nucleation and accumulation mode particles (*Seinfeld and Pandis 2006*). Particles in this mode arise from the growth or coagulation of nucleation mode particles as well as by production in high numbers by primary combustion sources such as vehicles (*Kulmala et al. 2004*).

Because atmospheric NPs sooner or later are deposited on the ground or water, concerns expressed over their direct impact are limited only in urban areas where their concentration is much higher than in rural areas. The impacts of atmospheric NPs through direct inhalation, ingestion, or skin exposure are subjected to epidemiological or occupational safety studies associated exclusively with human health. Short-term inhalation studies with aerosols generated from 13 nanomaterials provided information on the biokinetics and pulmonary and extra-pulmonary effects of the test materials (*Landsiedel et al. 2014*). The reader can find more information about this topic in SIINN deliverables 1.4 and 1.5.

5. Measurements and modelling of MNMs in the environment

a. Identification and characterization of MNMs

To date, no single property, or even the combination of several properties, has been established to describe the behaviour of MNMs. Therefore, various characterization techniques are generally combined to describe MNMs from different perspectives (*Pan and Xing 2012*). Before they can be characterized and quantified, effective separation of the different types of MNMs is required. Different types of nanoparticles, both naturally-occurring and man-made particles, are present in the



same system. To understand or evaluate the environmental behaviour and risks of certain types of MNMs, it is important to identify, separate and characterize the species within the complex system.

Separation methods include differential size filtration, micro-filtration, nano-filtration cross-flow filtration, ultracentrifugation, field-flow fractionation (FFF), size exclusion chromatography, liquid chromatography and capillary electrophoresis (*Ferreira da Silva et al. 2011; Simonet and Valcarcel 2009*). However, MNMs do not have exact molecular weights, homogeneous particle size or even surface charge. Distinguishing MNMs qualitatively in natural environments is a major issue that hinders their monitoring and risk assessment.

Standard techniques for the assessment of chemical composition, agglomeration, crystallinity, size, shape, and surface area to interpret structure-activity relationships include: energy dispersive X-ray spectroscopy (EDXS), X-ray diffraction (XRD), atomic force microscopy (AFM), Fourier transform infrared spectroscopy (FTIRS), electron energy loss spectroscopy (EELS), transmission electron microscopy (TEM), scanning electron microscopy (SEM), Brunauer-Emmett-Teller (BET) methods, dynamic light scattering (DLS), and low-frequency Raman spectroscopy (LFRS), condensation particle counters (CPCs), aerosol particle charger (*McMurry, P. H., 2000, Bandyopadhyay et al. 2012*). While these methods are valuable, it is also necessary to characterize the nanoparticles under different exposure conditions. For example the aggregation status of MNMs strongly depends on the surrounding environment.

In soil, usual methods for surface area measurement are based on N₂ or CO₂ sorption experiments, because microscopy techniques as SEM or TEM usually require a dry sample. However, the dispersed MNMs may be re-aggregated during the drying process (*Ferreira da Silva et al. 2011*). In moist or aqueous environments, N₂ or CO₂ measured surface areas provide limited information on MNM surface properties, as water parameters, including pH, ionic strength and ionic composition, dictate the behaviour of MNMs because they affect the stability of surface charge and particle interactions. Particle suspension and stabilization in aqueous media must include consideration of the effects of ionic strength, mono- and divalent cations, pH and the presence of buffers, proteins, natural organic matter (NOM), *etc.* Because dispersions of nanomaterials agglomerate when introduced into the aqueous or terrestrial environment and tend to settle rapidly, additional MNM characterization methods are required for assessment of MNM size distribution, state of dispersion, suspension stability, change in the zeta potential, and assessment of dissolution characteristics and metal ion shedding (*Thomas et al. 2011*).

For atmospheric NPs quantitative measurements on the total number and the size distribution of NPs is essential. The most used instruments for such measurements are: Scanning mobility particle sizer (SMPS), Electrical low pressure impactor (ELPI), Aerodynamic particle sizer (APS), Differ-



ential mobility spectrometer (DMS), Fast mobility particle sizer (FMPS), Ultrafine particle (UFP) monitor, Laser aerosol spectrometer (LAS), and GRIMM nanoparticle measuring systems (Keogh *et al.* 2010). Issues that need to be considered in using such instruments in any regulatory framework include their portability, time response, detection limits, robustness for unattended operation over long durations, cost, calibration and maintenance requirements. Although most instruments claim to overcome many of these issues, reproducibility of data still remains a major issue. Most instruments are not capable of detecting particles below 3 nm, a size range that is important for secondary particle formation. Further advances in performance are needed to address this deficiency and enable real-time determination of nanoparticle physico-chemical properties and related gas phase species involved in nucleation and growth (Kulmala *et al.* 2004). Development of robust and cost-effective instruments that have high sampling frequencies and cover a wide range of particle sizes is needed so that individual and population exposure to particulate pollution in urban environments may be characterised. These capabilities are not currently met by a single particle monitoring instrument and use of more than one instrument is required to obtain such information (Kumar *et al.* 2010).

b. Modeling of MNMs concentration and environmental implication

Currently a compartmental multimedia model was developed to enable evaluation of the dynamic environmental multimedia mass distribution and concentrations of engineered nanomaterials. Model simulations for various environmental scenarios indicated that MNMs accumulation in the sediment increased significantly with increased MNMs attachment to suspended solids in water. Atmospheric dry and wet depositions can be important pathways for MNMs input to the terrestrial environment in the absence of direct and distributed MNM release to soil (Liu *et al.* 2014). Other investigators have applied simple algorithms to predict the discharge of NMs into the environment. A comprehensive model incorporating factors for estimated worldwide production volume, allocation of the production volume to product categories, particle release from products and flow coefficients within the environmental compartments was proposed by Mueller and Nowack (2008). Gottschalk *et al.* (2009) applied a probabilistic method for analysis of MNM environmental exposure while Grieger *et al.* (2011) used worst-case-definition models for modeling the fate of nZVI and C₆₀. Given the current lack of substantiated data, modeling is very useful for predicting environmental concentrations of MNMs. For example Gottschalk *et al.* (2010) developed a probabilistic material flow analysis model based on a life-cycle perspective to predict environmental concentrations of TiO₂ NPs and CNTs. As input parameters they used the production volumes of the MNMs, the manufacturing and consumption quantities of products containing those materials, and the fate and pathways of MNMs in natural and technical environments.



Furthermore, as indicated by Holmes (2007), the modelling of airborne nanoparticle number concentrations has become very challenging with the inclusion of the complex dilution and transformation processes that occur after their release into the atmosphere. Currently, there are several particle dispersion models available that include particle dynamics e.g. MAT (Ketzel and Berkowicz 2005), MATCH (Gidhagen et al. 2005), and MONO32 (Pohjola et al. 2003). On contrary, particle dynamics were disregarded and particle number concentrations predicted by using a modified Boxmodel (Kumar et al. 2009), OSPM (Berkowicz 2000), and the CFD code FLUENT (Solazzo et al. 2008). Particle number concentrations predicted by all three models were within a factor of three of the measured values. However, a number of studies indicate that particle dynamics should be included in city scale models where they may affect the total number concentrations considerably (Gidhagen et al. 2005; Ketzel and Berkowicz 2004). For a more detailed description of the above models as also other models the reader is referred to Holmes and Morawska (2006).

In aqueous environments, chemical fate modeling, material flow analysis and classical knowledge of colloid science have been used for developing transport and fate models of MNMs. However, a number of particle-specific fate equations regarding the sedimentation, agglomeration, and dissolution of MNMs are needed to be included in order obtain “nano relevance” (Quik et al. 2011). Because all these processes are dynamic their kinetics has to be considered. For example, Arvidsson et al. (2011) have developed such a kinetic model for the aquatic environment based on colloid chemistry principles. A state-of-the-science review funded by the U.S. Environmental Protection Agency to identify fate and transport models and alternative modeling approaches that could be used to predict exposure to engineered nanomaterials released into aquatic systems (<http://nepis.epa.gov/Exe/ZyPDF.cgi?Dockey=P1008R2S.PDF>).

It is suggested that the development and introduction of a high-throughput screening technology to expedite the toxicological assessment of MNMs is required (Thomas et al. 2011). A first step is to build libraries of materials primary characterized by MNMs’ properties. Model organisms in various trophic levels will be used to examine the toxicity of MNMs. This information will be used to build the structure–activity relationships established using cell studies and confirmed *in vivo*. Furthermore, Eckelman et al. (2012) in their study utilized existing life cycle inventory data for three methods of CNT production and combined toxicological studies with the USEtox impact assessment model to determine appropriate bounds on the ecotoxicity of CNTs released to the environment. USEtox is an integrated multimedia fate, transport, and toxicity model covering large classes of organic and inorganic substances.

6. Suggestions and solutions

a. Methods of developing safer MNMs



Following toxicity assessment, it is important to use the knowledge to develop safer nanomaterials, including re-engineering in such a way as to reduce or to eliminate the observed toxic effects of the native particles. **Prior the synthesis of the re-engineered safe MNMs, first, the toxic mechanisms of the primary nanoparticles of interest and then the behaviour of the re-engineered MNMs in the environment have to be investigated.** (e.g iron doped ZnO NPs are less toxic than pure ZnO NPs due to slower dissolution of NPs in mammalian cells *Thomas, 2011 or* NPs with the same distribution of a primary size could have different toxicological profiles, *Lu et al. 2014*).

In order to maintain stable aqueous suspensions of nanoparticles (without particle aggregation and settling), many MNMs are modified with surface coatings such as polymers, polyelectrolytes, and surfactants. In addition to the desired changes in the resulting MNM surface chemistry, such modifications may also alter a chemical's environmental transport behavior and toxicity. **Capsular extracellular polymeric substances (EPS)** could provide **an additional barrier against UV/TiO₂ nanoparticles toxicity.** Capsular EPS may play dual roles in minimizing nanoparticle toxicity as it may provide a physical barrier between the cell and particle at the cell surface, but also provides an alternative oxidation and consumption site for ROS (*Hessler et al. 2012*). This beneficial property of EPS was demonstrated also by protecting *Escherichia coli* bacteria against Ag NPs toxicity. The results showed that exogenous addition of EPS to a control strain results in an increase in cell viability as TEM observations showed that EPS traps the nanoparticles outside the cells and reduces the exposed surface area of cells to incoming nanoparticles by inducing cell aggregation. Moreover, nanoparticle size characterization in the presence of EPS indicated a marked tendency towards aggregation (*Joshi et al. 2012*). Finally, concerning CNTs, it is suggested that acid cleaning of CNTs, which removes significant amounts of easily soluble toxic metals, would make them less toxic to aquatic invertebrates (*Mwangi et al. 2012*).

Speciation refers to chemical form. Different chemical species of the same element often have very different properties (e.g., solubility, volatility). This fact has specific implications to their toxicological examination and their risk assessment, especially in aquatic systems (<http://globe.setac.org/2014/june/basel-aquatic-nanotechnology.html>).

In metal-based nanomaterials, free ion can be a determinant in the toxicity and may be behind some apparently **“particle-specific effects.”** However, whether the metallic nanoparticle itself exerts a “particle-specific” toxicity remains an elusive question. A specific answer would help both to advance antimicrobial applications of AgNPs and to clarify their potential behavior and impact in the environment (*Xiu et al. 2012*).



b. Removal of MNMs from the environment

One of the main issues that should be considered is the identification and quantification of all nanoparticle sources and to establish the relative toxicity of nanosized particles from each one of them (Kumar *et al.* 2013).

Bare ZnO NPs and TiO₂ NPs upon release into the aquatic environment may experience limited mobility due to aggregate formation and thus, likely can be effectively removed by water filtration techniques such as slow sand and riverbank filtration. However, given that a majority of commercially and industrially employed NPs will be surface-functionalized, stabilized, or matrix embedded, various derivatives of these basic metal oxide NP types may exhibit far greater mobility upon release and be more difficult to remove using contemporary water treatment approaches (Petosa *et al.*, 2012).

Results have shown that, by increasing the concentration of electrolytes the aggregation rate of TiO₂ NPs (Shih *et al.* 2012) and ZnO NPs (Liu *et al.* 2012) in water was enhanced and in the presence of divalent anions, TiO₂ and ZnO NPs tend to aggregate more than in the presence of monovalent ones. Hence, a possible technique for removing TiO₂ and ZnO NPs is through rapid precipitation by multivalent electrolytes.

At expected production/use levels, the presence of nanomaterials in biomass appears unlikely to influence current biosolids treatment processes (*e.g.*, anaerobic digestion) or landfill biogas production. Additional research is needed to be able to monitor the transformation and **removal of nanomaterials** throughout wastewater treatment plants and biosolids treatment to assure they are not released into the environment where they may pose human or ecological risks (Westerhoff *et al.* 2013 b).

Wang *et al.* (2012) quantified the **removal efficiency** of Carboxy-terminated polymer coated Ag, TiO₂, and carbonaceous MNMs from simulated wastewater and into biosolids using lab-scale sequencing batch reactors. They indicated that biological wastewater treatment plants operated using suspended biomass (*e.g.*, activated sludge) have the potential to remove engineered nanomaterials from wastewaters. Both small, negatively charged MNMs (*e.g.*, fn-Ag) and larger aggregates of MNMs (*e.g.*, TiO₂) were removed by interaction with biomass in systems operated with total suspended solids similar to that of full-scale wastewater treatment plants. In particular, TiO₂ and aqueous fullerenes (C₆₀) were removed by 95%, hydroxylated fullerenes (fullerols) by greater than 90% and fn-Ag less effectively by 88%.

c. Suggestions, public policy and ethics

It is crucial **before a risk assessment study is launched to determine whether the as-produced MNM or the product-modified MNM should be taken into account**. This is because the fate of a product-modified NM, including the transformations and the partitioning into environmental compartments, may vary with the matrix the manufacturer uses to protect or modify its surface properties (*Zhong 2014, Westerhoff P. et al. 2013, Nowack et al. 2012*). Impurities or artifacts introduced during the manufacturing process may also influence the fate of the MNM or may enhance toxicity. It is also necessary to develop ecological risk assessment procedures that take into account the environmental transformation of MNMs over the short to long term. It is entirely possible that some of these environmental transformed MNMs pose a greater risk to the environment than the original manufactured material, or vice versa (*Li et al. 2014, Losa et al. 2014, Zhong 2014, Lowry, G.V. et al. 2012, Nowack et al. 2012*).

After MNMs release, **the environmental concentrations of MNMs** have to be determined properly by extensive measurements in different terrestrial and aquatic regions as well as in high polluted atmospheric conditions of industrialized zones and mass-crowded cities as it was suggested also in SIINN deliverable 1.5. Predicted concentrations by numerical models certainly cannot lead to an authoritative risk assessment. As Eckelman *et al. (2012)* showed production-side ecotoxicity impacts of current carbonaceous nanomaterials are of at least equivalent magnitude to the impacts of their release, thus, it is mandatory **to create mechanisms through international cooperations for tracking and assimilating the production-level records that exist for each type of MNM**. Estimations of the maximal amounts of MNMs produced, disposed and possibly reaching the environment for new technologies using or releasing MNMs have to be calculated (*Shatkin J.A. et al. 2014*). For example, regarding the emerging organic photovoltaic (OPV) technology, an estimation, in the worst case scenario, calculated that OVP's contribution of 87.5 tons ZnO NPs and 50.9 tons TiO₂ would contribute less than 0.1% for the former and about 1 and 0.001% for the later to the total worldwide produced amount (*Zimmermann 2012*).

Furthermore, **the strategy for risk assessment of MNMs should take life cycle concepts** into consideration and vice versa in order to mitigate the situation of having only limited knowledge on the risks inherent in MNMs (*Miseljic et al. 2014, Som et al. 2010*). Considering that the life cycle of products provides information in relation to the release and exposure scenarios, life cycle concepts provide a means for identification of priorities for risk assessment, and analysis of MNMs release and monitoring. In particular, **life cycle assessment (LCA)** may provide added benefits in that it considers environmental sustainability impacts such as material and energy consumption, hazardous byproducts of MNM production, among others, to provide a comprehensive input for evaluation of the environmental sustainability potential of a nanoproduct (*Miseljic et al. 2014, Meyer et al. 2009*). In a



current review on LCA (*Miseljic et al. 2014*) is pointed out that LCAs have been applied mainly to assess the accountable production-related emissions. Thus, that the **future aims should be to further map, develop and validate the application of LCA of MNMs and, importantly, collect inventory data and develop impact characterisation factors to further the assessment of potential environmental and toxicological impacts from released MNMs/NPs.** Also in a recent review on LCA, Gavankar et al. (2012) found that while there is plenty of literature available promoting LCA as a viable tool for MNMs, **there are only a handful of studies where at least some parts of life cycle were evaluated for MNMs and none of them assessed nano-specific fate, transport, and toxicity effects as part of their evaluation.**

More studies using aquatic organisms, plant life, and higher organisms will broaden current knowledge of the risks of MNMs in the environment. Using this information, **combinatorial libraries can be developed** to help gaining a better mechanistic understanding of the nano/bio interface (*Aklilani et al. 2013, Thomas et al. 2011*). Pre-requirement is the finding of suitable organisms in aquatic and terrestrial environments that can be used for biomonitoring models. Furthermore, a strategy integrating the multi tiered biomarker sets to reveal the effects of biological responses from the infra-individual through the individual levels, in order that we can take necessary environmental management actions is needed (*Pan et al. 2012*).

Pointing out the example of Ag NPs “concealed” presence in the environment the last 120 years, Nowack *et al.* (2011) claimed that applying the general prefix “nano” **does not in itself automatically render a material harmful**, and thus any concept of risk must ultimately derive from chemical and physical characteristics of a specific material. Consequently **it will be fruitful for specific materials to re-evaluate and employ previous knowledge expanding in this way the available database.**

Regulations and standards covering a wide range of nanomaterials at the early stage of manufacturing and emission are required to prevent contamination from MNMs and protect the environment (*Kumar et al. 2014, Chen et al. 2011*). However, the current review article by Kumar (2014) indicates **that not much information is available on the regulation of MNM-related exposure and the scope of different available regulations and guidelines is limited to organic compounds and toxic substances, but they do not specifically address MNM-related pollution.** It is recommended that all information on elements existing in the nanomaterials available on the market should be included in the **materials’ safety data sheets** (*Mwangi et al. 2012*). Moreover, there is urgent need for legislation requiring manufacturers to report the use of MNMs in their products, as recent reports on regular (not marked as nanopowders) cosmetic powders showed that they contain also agglomerations of NPs (*Nazarenko et al. 2012*).

As a final remark, a more targeted effort from scientists to impart information about the environmental risks of NMs should be made. In this respect, the role of reports (as SIINN’s) is vital



because not only it accumulates the existing knowledge on MNMs, identifies the characteristics of the specific problems and points out the possible solving routes, but also it distributes worldwide this information to the scientists and public.



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Abbreviations

AFM	Atomic force microscopy
APS	Aerodynamic particle sizer
BET	Brunauer-Emmett-Teller methods
CNT	Carbon nanotube
DLS	Dynamic light scattering
DMS	Differential mobility spectrometer
EDXS	Energy dispersive X-ray spectroscopy
EELS	Electron energy loss spectroscopy
ELPI	Electrical low pressure impactor
MNM	Manufactured nanomaterial
EPS	Extracellular polymeric substance
FFF	Field-flow fractionation
FMPS	Fast mobility particle sizer
FTIRS	Fourier transform infrared spectroscopy
LAS	Laser aerosol spectrometer
LCA	Life cycle assessment
LFRS	Low-frequency Raman spectroscopy
MWCNT	Multi-walled carbon nanototube
NM	Nanomaterial
NOM	Natural organic matter
NP	Nanoparticle
OPV	Organic photovoltaics
QD	Quantum dot
ROS	Reactive oxygen species
SMPS	Scanning mobility particle sizer
SEM	Scanning electron microscopy
SOM	Soil organic matter
SWCNT	Single-walled carbon nanotube
TEM	Transmission electron microscopy
UFP	Ultrafine particle
XRD	X-ray diffraction