Chapter 11
Ecotoxicology of Engineered Nanoparticles

Karl Fent

11.1 Introduction

Nanotechnology opens new applications in many fields including medicine, material science, manufacturing and various technologies. A increasing variety of products on the market contain engineered nanoparticles (ENP) (Schmid and Riediker, 2008). Metal nanoparticles are among the most widely used, but Buckminster fullerenes, carbon nanotubes and also carbon black have widespread use. ENP are in use in many industrial sectors and products, including cosmetics, paints, fillers, catalysts, semiconductors, microelectronics and drug carriers in medicine to name a few. Considerable quantities of silver, aluminium oxide, iron oxide, silica oxide, titanium dioxide (TiO_2) and zinc oxide (ZnO) ENP are applied. The specific design and synthesis of ENP that create particular physico-chemical properties has influence on their biological and environmental behaviour.

Nanomaterials enter the environment in the production process and via applications and weathering of materials, but presently, the amount of ENP in nanotechnology-based materials and their concentrations are unknown. ENP may have particular properties that may be associated with higher health and environmental risks than natural NP formed by geochemical and combustion processes and found in the atmosphere and aquatic systems. Human health risks may arise from inhalation of ENP with associated inflammation, dispersion in the human body and exposure of vulnerable organs (e.g. heart, brain) and tissues with associated toxicity. Adverse effects on the immune system, damage of cells as well as cancerogenicity may represent health risks. In the environment, ENP are dispersed, and organisms may be exposed to these nanoparticles in the aquatic environment or they may get inhaled via air. Exposure of organisms in the environment may result in adverse

K. Fent (✉)
School of Life Sciences, University of Applied Sciences Northwestern Switzerland, CH-4132 Muttenz, Switzerland; Department of Environmental Sciences, Swiss Federal Institute of Technology (ETH Zürich), CH-8092 Zürich, Switzerland
e-mail: karl.fent@bluewin.ch

F.H. Frimmel, R. Nießner (eds.), *Nanoparticles in the Water Cycle*,
DOI 10.1007/978-3-642-10318-6_11,

effects in cells and organs, similarly as in the human body. In addition ENP may be accumulated by organisms and they may even be accumulated in the food chain.

Due to their unique physical and chemical properties nanomaterials are extensively studied. Natural nanoparticles (NP) were investigated for their physico-chemical behaviour in the environment (Buffle and Leppard, 1995; Buffle, 2006; Novak and Bucheli, 2007), but less for human health and environmental consequences with the exception of small-sized dust particles. ENP may pose environmental risks not associated with NP due to their specific properties. Whereas nanotechnology is gathering increasing attention, some concerns have arisen with respect to potential health effects on humans and the environment, particularly in the public opinion and media. It is important to consider them as early warnings of nanotechnology for its acceptance (Hansen et al., 2008). At present there is a need to evaluate the health and environmental risks associated with ENP as an exponentially increasing number of nanoparticle-based products are developed and brought to the market.

To date, little is known about the occurrence, fate and potential effects of ENP in the environment. The potential for effects on ecosystems will depend not only on the amount of nanoparticles emitted but also on their physico-chemical characteristics (size, surface/volume ratio, shape, chemical composition), which can be influenced by environmental parameters. Exposure of organisms in the environment to ENP will occur not only via wastewater from manufacturers, but also via municipal wastewater and direct inputs from weathering and ageing of products containing nanomaterials. The exposure of organisms will largely depend not only on the concentration, size and surface characteristics of dissolved NP but also on adsorption and aggregation phenomena. Knowledge on the chemistry of natural NP and colloids in the aquatic system (Buffle and Leppard, 1995) may in part be transferred to ENP. Adsorption to environmental media such as sediments, biofilms and microsurface layers of open waters may influence the bioavailability and thus exposure. To date, studies devoted to evaluate the bioavailability in a systematic fashion are lacking. As extrapolated from natural colloids, the ionic strength is of influence for the aggregation tendency; the high ionic strength in seawater will cause aggregation of ENP (Novak and Bucheli, 2007).

11.2 Bioavailability, Uptake and Distribution in Organisms

Methods of nanoparticle preparation for experiments and the behaviour of ENP during exposure influence the bioavailability and therefore the activity and toxicity. Surface chemistry, shape and size of ENP are important factors for the uptake, toxicokinetics and toxicity. ENP exist as emulsions or suspensions in the aqueous environment, they are not in true dissolution as are chemicals. They may form precipitate and agglomerates that differ in toxicity.

Aquatic organisms come in contact with ENP mainly through their surfaces. Uptake routes include direct ingestion and entry across epithelial surfaces such as gills or, to some extent, through body surfaces. Uptake via epithelia in gills and gut is most prominent (as with chemicals), although ENP also cover surface epithelia

(e.g. cornea, skin) and the mucus layer of fish, which may result in uptake to some extent. Carbon nanotubes (CNT), for instance, were found to readily associate with gill mucus in trout (Smith et al., 2007). The same kind of nanoparticles gets taken up by various cell types through diverse pathways (Nel et al., 2006).

As aquatic organisms are exposed to ENP both via water and ingestion, gills (oxygen uptake) and gut (ingestion) are the primary and prominent tissues and organs exposed to ENP, where toxicity may occur. As in case of environmental pollutants, the bioavailability of NP is an important factor for uptake and toxicity. Absorption to surfaces, aggregation behaviour of the particles and their environmental chemistry play a dominant role. The bioavailability will be influenced by the presence of organic matter, changes of pH and ions in environmental media. Carbon black acts as an effective sorbent for organic contaminants. It was shown to reduce toxicity of pesticide to algae (Knauer et al., 2007). Based on colloid chemistry, it seems that increases in salinity influence aggregation. The high ionic strength of not only seawater but also Ca^{2+} will cause ENP to aggregate (Handy et al., 2008b). Although probably to a lesser extent, aggregates will also be bioavailable, as found with TiO_2 and C_{60} in *Daphnia* (Baun et al., 2008a). Uptake and depuration of radioactive-labelled carbon nanotubes have been studied in an oligochaete, showing that CNT are associated with sediments remaining in the guts of organisms and not adsorbed in cellular tissues, which is in contrast to the behaviour of chemical compounds. This indicates that CNT are not readily absorbed into tissues of oligochaetes (Petersen et al., 2008).

The uptake of ENP is concentration and size dependent. Passive diffusion seems most important, although in the gut, vesicular uptake (endocytosis) may be important (Moore, 2006). Transport of colloidal particles across the cell wall is not occurring in bacteria. This is possible in eukaryotes having cellular uptake processes such as endocytosis or phagocytosis. Active uptake via ATP-binding cassette (ABC)-transporters is not probable. ENP may cause inflammation of the gill and injury to the gut at milligram concentrations as found for TiO_2 (Federici et al., 2007) or CNT (Smith et al., 2007). This may facilitate the uptake, because severe inflammation or erosion harms epithelial layers. Larger moieties such as aggregates are less transferred than smaller particles. As with chemicals, uptake through the skin mucus in fish, or carapax in crustacean, and insects is not or less probable.

After crossing the cell membrane, ENP may not only be stored in vesicles, mitochondria and additional organelles within epithelial cells but also induce adverse effects. Indeed, epithelia are targeted by ENP. The small size and large surface area and the ability to generate reactive oxygen species play a major role in the toxicity of ENP; they may generate oxidative stress and cytotoxicity (Oberdörster, 2004). In human epidermal keratinocytes, quantum dots (QD) were shown to be internalized into early endosomes and then transferred to late endosomes or lysosomes (Zhang and Monteiro-Riviere, 2009). QD endocytic pathways were primarily regulated by the G protein-coupled receptor-associated pathway and low-density lipoprotein receptor/scavenger receptor. The surface coating, size and charge of QD nanoparticles are important parameters in determining how nanoparticle uptake occurs in mammalian cells. Silica ENP led to formation of protein aggregates in the cell nucleus, thus impairing nuclear function (Chen et al., 2004). Uptake of tungsten

carbide nanoparticles has been studied in fish gill cell line RTgill-W1 (Kühnel et al., 2009). In these cell cultures, the localization seemed to be restricted to the cytoplasm. The current literature suggests that the uptake of ENP depends on the characteristics of the ENP (size, surface characteristics) and the specific cell type.

After absorption ENP are incorporated and they cross the epithelial cells and are further transported to the blood or haemolymph (in invertebrates) or, alternatively, through tight junctions into the blood stream, where they get transported and distributed to tissues and organs of the body. ENP are probably transported via sorption to blood proteins to other organs and tissues. They may also form aggregates in the blood. Distribution may also occur directly via cellular transport; ENP may be transported directly into the brain via the olfactory nerve through axonal transport (Oberdörster et al., 2006).

Uptake of ENP has experimentally been investigated in several organisms. Nanosized ZnO was internalized by bacteria (Brayner et al., 2006). It was also shown that multi-walled carbon nanotubes were taken up by protozoans and localized in mitochondria (Zhu et al., 2006a). Uptake of NP into zooplankton and fish has also been studied. CNT, nC$_{60}$, fullerene (C$_{60}$), single-walled carbon nanotubes (SWCNT) and TiO_2 are taken up by *Daphnia* and incorporated (Oberdörster, 2004; Oberdörster et al., 2006). Uptake of fluorescent carboxylated ENP by *Daphnia* was found to be followed by translocation from the gut to fat droplets (Fernandes et al., 2007). C$_{60}$ nanoparticles in the digestive tract were also excreted. Translocation of polystyrene nanoparticles (20 nm) from the digestive tract to other parts of the body was observed (Baun et al., 2008b). After 48 h exposure of *Daphnia magna* to a 0.4 mg/L solution of dispersed nanotubes, the CNT comprised of 6% of the residual organism dry mass. *Daphnia* were unable to excrete the nanotubes unless they feed on algae. Residual accumulated CNT remained in the gut and were not absorbed into cellular tissues (Petersen et al., 2009). SWCNT were shown to be internalized by the ciliata *Tetrahymena thermophila*, which also causes these protozoa to aggregate, which in turn impedes their ability to ingest and digest their prey bacteria (Ghafari et al., 2008).

Water-suspended dispersed non-ionized fluorescent latex ENP (polystyrene microspheres) were used to investigate uptake, distribution and effects of nanoparticles in the eggs and bodies of medaka (Kashiwada, 2006). Different sizes of NP were adsorbed to the egg shell (chorion) of medaka eggs and accumulated in oil droplets. Interestingly, 474 nm particles and not smaller ENP (39 nm particles) showed highest uptake into eggs. These nanoparticles accumulated in the yolk and gall bladder during embryonic development. However, other ENP such as SWCNT (Cheng et al., 2007) and fluorescent silica ENP did not penetrate the chorion of fish eggs (Fig. 11.1). In contrast, in vivo imaging of transport of silver nanoparticles (5–46 nm) shows that they are transported into and out of zebrafish embryos through chorion pore canals and exhibit Brownian diffusion (Lee et al., 2007). Individual Ag nanoparticles were observed inside embryos at each developmental stage. Dose-dependent abnormalities (deformities) and mortality was a consequence.

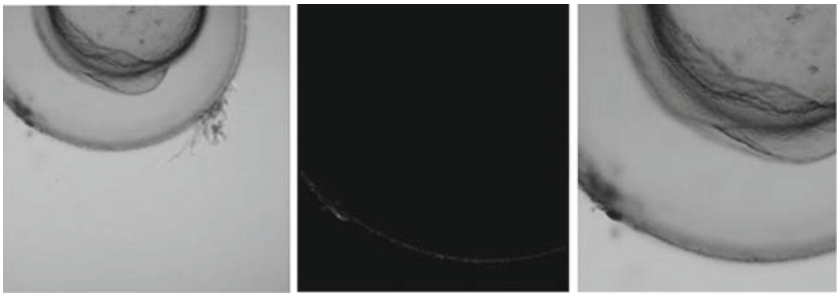

Fig. 11.1 Engineered fluorescent 200-nm silica nanoparticles (SNP) do not penetrate the egg chorion of fish and adhere to the surface. *Left*, SNP on the chorion surface, *middle* and *right*, fluorescent SNP at the surface shown in the confocal and transmission microscope (K. Fent, C. Weisbrod, A. Heller-Wirth, unpublished)

Adult fish accumulated the 39-nm sized ENP mainly in the gills and intestine when exposed to 10 mg/L ENP. They were also distributed into blood and detected in testis, liver and brain. Obviously, these ENP may penetrate the blood–brain barrier. In summary, the data from several organisms indicate that ENP may be bioavailable and taken up by organisms mainly via gill and intestine. They get distributed without metabolism into the body similar to chemical compounds. Therefore, the concept used for studying kinetics and adverse effects of chemicals applies also to ENP.

11.3 Determining Exposure and Effect Concentration

Thus far, toxicity experiments with ENP were performed using nominal concentrations; effective concentrations have not or very rarely been determined. This is not satisfactory, as similar to ecotoxicological experiments with chemicals, adsorption and aggregation processes may lower the actual exposure concentrations. Aggregation occurs when nanomaterials are introduced into media in exposure tanks and containers; hence, exposure concentrations may differ from nominal concentrations. It seems not completely clear, whether effects should be based on the total mass of ENP, its number or surface area in environmental media. Cell culture experiments indicate that the effects are dependent on the total mass, number of particles and their surface area, as well as on their concentration (Lison et al., 2008).

More emphasis is placed in recent studies to determine actual exposure concentrations (and forms) of ENP. Electron microscopy and various spectrophotometric methods applied such as MS, LC-MS and ICP-MS were used to determine organic and inorganic constituents of nanoparticles, but the latter destroy the particles. In exposure vessels ENP settle and concentrate on the bottom. Therefore, exposure concentrations fall relatively fast in the exposure containers. For instance, fullerenes form dispersions of water-soluble aggregates when agitated in fresh or deionized

water for prolonged periods of time. Increasing the ionic strength of an aqueous solution (such as NaCl) containing nC$_{60}$ results in the formation of large-sized aggregates (Brant et al., 2005b; Brant et al., 2006).

Characterization of experimental test material is important, since without characterization, it is difficult or impossible to compare studies and recognize parameters that influence toxicity. An important challenge in experimental investigations is the dissolution of NP in exposure vessels, and its characterization. Solubilizing ENP is critical, as aggregates have a different bioavailability, uptake and toxicity than single ENP. Sonication methods may be the best for solublizing SWCNT, but much easier are fullerenes (C$_{60}$) to test. Solubilization can be achieved by either using an organic solvent such as tetrahydrofuran (THF) or stirring for a long period of time (often 2 months), which is environmentally more accurate and relevant (Oberdörster et al., 2006). During solubilization ENP form aggregates or clusters. The solubilization method has influence on the toxicity. Tetrahydrofuran-nC$_{60}$ were more toxic than stirred nC$_{60}$ in Daphnia and fish (Oberdörster et al., 2006), likely due to presence of solvent residues (Brant et al., 2005a). Hence, it remains unclear whether effects are related to this solvent rather than to the particles itself (Oberdörster et al., 2006). The same is true for cell culture experiments, where formation of aggregates in cell culture media may occur. In conclusion, solubilization is a critical issue that must carefully be controlled in ecotoxicological experiments (Klaine et al., 2008).

In case of metallic ENP, toxicity may be based rather on metal residues than the nanoparticle feature itself. It was demonstrated that the toxicity of bulk metals such as ZnO is similar than that of ZnO nanoparticles. Both nano-ZnO and ZnO aqueous suspensions delayed zebrafish embryo and larva development, decreased survival and hatching and caused tissue damage at similar concentrations (Zhu et al., 2007).

11.4 Ecotoxicity

Nanomaterials have been studied in toxicology, mainly for their acute effects in rodents. The LD50 values are in the high mg/kg to g/kg range, which means that they are moderately acutely toxic. Target organs are members of the reticuloendothelial system, including liver and spleen (Stern and McNeil, 2008). Also the kidneys are targeted as they are the primary clearance route for many nanoparticles. Of course the major target of ultrafine air particles is the lung. In contrast to acute effects, the long-term effects are only little known, and the risks of ENP associated with chronic toxicity are unknown.

In ecotoxicology, ENP have been studied for uptake and toxicity only in a small number of organisms belonging to bacteria, algae, protozoa, zooplankton (*Daphnia*), oligochaetes, mussels, amphibians (*Xenopus laevis*) and fish (for review Baun et al., 2008a; Klaine et al., 2008; Navarro et al., 2008a). They belong to the classical species used in ecotoxicological testing, but whether they are the most important ecological species remains open. In general, species differences in sensitivity will also occur in the sensitivity against ENP. Furthermore, exaggerated

concentrations of ENP are studied, but the potential effects at environmental realistic concentrations remain elusive.

In general, toxicity may be dependent on particle concentration, particle size and shape and surface properties (surface chemistry). A toxicological review of quantum dots (QD) suggests that the toxicity depends on physico-chemical and environmental factors (Hardman, 2006). QD size charge, concentration, outer coating bioactivity have been implicated as determining factors in toxicity. Most data on the ecotoxicology of NP were generated in classical ecotoxicity tests using established test systems similar to the regulatory framework of testing of chemicals. Thus far, mainly acute toxicity was studied in a few species, particularly *D. magna*, but chronic toxicity data are largely lacking. The acute toxicity data indicate that the acute systemic toxicity of many ENP in aquatic and terrestrial organisms appears to be rather low. This is in contrast to the pulmonary toxicity of certain ENP such as CNT that show significant toxicity in mammals and humans. However, it should be noted that the risks of ENP are related to chronic and long-term effects that are not yet adequately studied. It should also be noted that the behaviour of ENP and associated toxicity may be different at environmentally relevant concentrations as compared to the high levels used in acute toxicity studies (Gao et al., 2009). Furthermore, partial oxidation ("ageing") and surface modification in the environment may alter the toxicity of EHP (Phenrat et al., 2009).

11.4.1 Toxicity Mechanisms and Target Organs

The mechanistic basis of NP toxicity is not well understood, both in toxicology and in ecotoxicology. Toxicity studies in mammals demonstrated various types of cellular effects, mainly on the respiratory tract (Oberdörster, 2004; Oberdörster et al., 2006). Ultrafine particles and nanomaterials have experimentally quite well been studied in mammals due to their occurrence in air dust. Exposure analysis of humans via air has mainly been performed in epidemiological studies. At present air pollution is by far the most important factor for human exposure occurring via natural and traffic-derived NP. Pulmonary effects of ultrafine particles are known for long, yet the effects of ENP have been investigated only in the last few years (Oberdörster et al., 2006). Hence, respiratory toxicity and inflammation reactions to NP are known. Epidemiological studies clearly support an association between particulate air pollutants and pulmonary, cardiovascular and CNS disease (Stern and McNeil, 2008).

In short, the effects of nanoparticles in mammals (and humans) can be summarized as follows: inflammation, cytokine production, cytoskeletal changes, altered vesicular trafficking, oxidative stress, apoptosis, as well as changes in gene expression and cell signalling in response to different types of ultrafine particles. This was found in a number of mammalian cells in vitro and rodents in vivo (Oberdörster, 2004; Oberdörster et al., 2006). The targets within cells include the lysosomes. Change in lysosomal permeability and the subsequent release of lysosomal enzymes is one of the mechanisms involved in the induction of alveolar macrophages by silica

microparticles (Thibodeau et al., 2004). Recently, it was shown in mice that inhaled multi-walled CNT led to suppression of systemic immune function (Mitchell et al., 2007), and this is based on the activation of cyclooxygenase enzymes in the spleen in response to a signal from the lungs (Mitchell et al., 2009).

ENP induce cytotoxicity in vitro in cell lines at high concentrations (Herzog et al., 2007). As an example, single-walled carbon nanotubes (SWCNT) were found to have a very low acute toxicity (Davoren et al., 2007). Silver ENP (15 and 30 nm) lead to more than 10-fold increase of reactive oxygen species (ROS) levels in cells exposed to 50 mg/L, suggesting that the cytotoxicity is likely to be mediated through oxidative stress. In addition, a significant inflammatory response was observed by the release of TNF-alpha, MIP-2 and IL-1beta to cell culture media (Carlson et al., 2008). In vitro cytotoxicity of many ENP has well been documented (Brunner et al., 2006). Different ENP were investigated for cytotoxicity and ability to cause DNA damage and oxidative stress in human lung epithelial cell line A549 (Karlsson et al., 2008). A high variation among different ENP was observed. CuO nanoparticles were most potent regarding cytotoxicity and DNA damage. The effects were not due to soluble metal impurities.

Mechanisms of actions known in mammals may similarly apply for other organisms. Silver ENP are well-known antibacterial agents causing toxicity via multiple mechanisms (Morones et al., 2005). They may alter the membrane properties, affecting the permeability and respiration of bacterial cells. They can also penetrate into bacteria and cause DNA damage and release toxic Ag^+ ions. In addition to direct effects, indirect effects by ENP are important. ENP may lead to physical blockage of the gills of aquatic organisms and/or digestive tract. For instance masses of black CNT were observed in the intestine of amphibian larvae, which lead to acute toxicity at high concentrations of 10–500 mg/L in addition to blockage of gill respiration (Mouchet et al., 2008).

Prime targets of ENP in aquatic organisms including fish (Handy et al., 2008a) are uptake organs such as gills and intestine. Their epithelia are exposed directly to the particles via water and food, respectively. Adsorption to and interaction with epithelial cells may harm cell membranes and cellular structures. In fact, it has been demonstrated that fish gills are sensitive to ENP such as TiO_2 (Federici et al., 2007) or single-walled carbon nanotubes (SWCNT) (Smith et al., 2007). Adsorption and attachment to the cell surface compromise its integrity and function. ENP may also indirectly cause membrane damage through the generation of ROS, leading to lipid peroxidation and damage of proteins and nucleic acids. Therefore, cell membrane damage is an important mechanism by which ENP act on the cellular level. The cytotoxic response of cells is dependent on the degree of functionalization in case of SWNT (Sayes et al., 2006). Exposure of cells in vitro increased ROS levels and reduced glutathione levels (Lin et al., 2006). The dose-dependent cytotoxicity was closely correlated to increased oxidative stress.

Additional modes of actions of ENP have been determined in mammals. Oxidative stress is induced by iron nanoparticles in human bronchial epithelial cells, leading to cytotoxicity and cell damage (Keenan et al., 2009). The response is equivalent to the response observed when cells are exposed to the same

concentration of dissolved Fe(II), however. Fullerenes (C_{60}) may act as antioxidants; however, a strong oxidative potential through photoactivation has also been shown. ENP may interact with proteins directly or by producing ROS or other damaging radicals. Some ENP (e.g. fullerenes) are photoreactive. Induction of oxidative stress seems to be an important mechanism also in aquatic and terrestrial species. Fullerenes induced potentially harmful lipid peroxidation in the brain of fish (largemouth bass) (Oberdörster, 2004). In contrast, no such effects have been found in *Fundulus heteroclitus*, however (Blickley and McClellan-Green, 2008).

ENP also induce pulmonary inflammation, fibrosis, granulomatosis and emphysema in mammals. CNT have been compared to asbestos, due to their needle-like shape, raising concerns that they may lead to mesothelioma and lung cancer in mammals (Stern and McNeil, 2008). Indeed multi-walled CNT resulted in asbestos-like pathology in mice such as inflammation and formation of lesions known as granulomas (Poland et al., 2008). The asbestos-like hazard of such CNT is a cause of concern important for the production and application of such ENP, in particular at the working place. This is because CNT may have unusual toxicity properties, such as a special ability to stimulate mesenchymal cell growth and to cause granuloma formation and fibrogenesis (Donaldson et al., 2006). CNT may exhibit some of their effects through oxidative stress and inflammation.

Less is known so far in aquatic and terrestrial toxicology. Similar to mammals, possible toxicity mechanisms of ENP may include among others the following:

1. Disruption of membranes or membrane potential
2. Formation of reactive oxygen species and oxidative stress
3. Induction of apoptosis and necrosis; induction of stress-related genes
4. Oxidation and denaturation of proteins and other biomolecules

Recently it was demonstrated that fullerenes C_{60} elicit oxidative stress response in embryonic zebrafish (Usenko et al., 2008). Gene expression analysis with zebrafish using microarrays on the effects of C_{60} demonstrated alterations in several key stress response genes including glutathione-*S*-transferase, glutamate cysteine ligase, ferritin, alpha-tocopherol transport protein and heat-shock protein 70 (Usenko et al., 2008). These alterations in gene expression patterns clearly indicate induction of oxidative stress in zebrafish. Furthermore, there was an upregulation of development, cell cycle (including induction of apoptosis) and signal transduction genes.

Genotoxicity has been investigated with TiO_2 in fish cells in vitro (Vevers and Jha, 2008). Increased levels of DNA strand breaks were observed only in combination with UVA light. Obviously, genotoxicity was photo-induced. Fullerenes were found to be nonmutagenic in the Ames test and non-genotoxic in a Chinese hamster lung cell chromosomal aberration assay up to 5 mg/mL (Mori et al., 2006).

Silver ENP led to cellular and DNA damage as well as to carcinogenic and axidative stresses in medaka, as suggested by analysis of gene expression alterations (Chae et al., 2009). Silver resulted in a lower overall transcriptional stress response in the liver than silver ENP.

Recently, molecular and histological effects following exposure to 0.1 mg/L nanocopper and 1 mg/L nanosilver and nano-TiO_2 (particle sizes between 45 and 688 nm) have been studied in gills after exposure of zebrafish for 24 and 48 h (Griffitt et al., 2009). Nanocopper (100 μg/L) increased mean gill filament width. Gene expression analysis demonstrated that the exposure to each nanometal or soluble metal produced a distinct gene expression profile, suggesting that each exposure is producing a biological response by a different mechanism. The effect of each nanoparticle does not appear to be based solely on the release of soluble metal ions into the water, but by the particulate feature as well. Although nano-TiO_2 did not show toxicity, different genes are altered, demonstrating a transcriptomal effect. Whereas 1 mg/L nano-TiO_2 altered a number of genes involved in ribosome structure and activity, nanocopper upregulated over 100 genes involved in the cellular processes such as apoptosis, mitogenesis and cell proliferation, as well as in cancer progression. Interestingly, no genes involved in oxidative stress were altered. Therefore, the extent to which oxidative stress can generally be assumed as a key molecular process by which ENP act on the cellular level remains elusive.

11.4.2 Effects on Aquatic Organisms: Acute Toxicity

There are a few major classes of nanomaterials: metal and metal oxide nanoparticles, carbon-based nanoparticles and nanotubes, fullerenes and macromolecules. At present knowledge about the toxicity of these ENP is clearly not sufficient. Acute toxicity of ENP on a variety of different organisms has been studied. Most studies were performed with filter-feeding *Daphnia*, where general toxicity signs such as immobilization were usually assessed. Here, some of the most relevant findings are summarized in the following and in Table 11.1, other literature reviews are given elsewhere (Baun et al., 2008a; Klaine et al., 2008; Novak and Bucheli, 2007). So far, almost all studies deal with the acute toxicity of very high concentrations of ENP. In general, they found acute toxicities of various ENP in the range of around 1 to several mg/L (Table 11.1), and therefore it is not very high. Only a few studies show toxicity below 0.1–0.5 mg/L. Furthermore, toxicity seems to be dependent on the physico-chemical characteristics of the nanoparticles, therefore a range of non-toxic to toxic nanomaterials can be found.

High concentrations of ENP inhibit the growth of algae with EC_{50} in tens of mg/L (Hund-Rinke and Simon, 2006). Aggregates may be formed at the surface including cell walls leading to indirect effects on growth and photosynthesis. Mortality and toxicity in *Daphnia* was induced by single-walled nanotubes (SWNT) at 10 mg/L (Templeton et al., 2006), whereas the lowest observed effect concentration of filtrated fullerenes was 0.26 mg/L. Acute toxicity was also observed with inorganic ENP with decreasing toxicity for ZnO, SiO_2, TiO_2 (Adams et al., 2006).

Exposure of aquatic crustaceans (*D. magna, Artemia salina* and Gammarids) to several ENP (TiO_2, fluorescent polystyrene particles, carbon black) indicates that particles are readily ingested and accumulated in the gastrointestinal tract and distributed into body lipid droplets (Fernandes et al., 2007; Roberts et al., 2007) and

Table 11.1 Acute and chronic toxicity of engineered nanoparticles to aquatic organisms (selected examples)

Engineered nanoparticle	Size (nm)	Species	Effect	EC50/LC50/LOEC (Exposure time)	References
Ag	10–200 (mostly 25)	Algae *Chlamydomonas reinhardtii*	Inhibition of photosynthesis	829 nM (5 h)	Navarro et al. (2008b)
		Daphnia pulex	Mortality	40 μg/L (48 h)	Griffitt et al. (2008)
	90–2,000	*Ceriodaphnia dubia*	Mortality	2-46 μg/L (LC50, 48 h)	Gao et al. (2009)
	45–216	Zebrafish (*Danio rerio*)	Alteration of gene expression	1 mg/L (24 h, 48 h)	Griffitt et al. (2009)
TiO_2	25, 100	Algae *D. subspicatus*	Growth inhibition	14 mg/L	Hund-Rinke and Simon (2006) Lovern and Klaper (2006)
	10–20	*Daphnia magna*	Mortality	5.5 mg/L (48 h)	Hund-Rinke and Simon (2006)
	25	*D. magna*	Mortality	40% at 20 mg/L	Lovern et al. (2007)
	30	*D. magna*	Behavioural and physiological alterations Alterations in gills, liver, intestine	12.5 mg/L (48 h)	Federici et al. (2007)
		Rainbow trout (*O. mykiss*)	Alteration of gene expression	0.1–1 mg/L (LOEL) (14 d)	Griffitt et al. (2009)
	221–688	Zebrafish (*D. rerio*)		1 mg/L	
ZnO	73–408	Algae *Pseudokirchneriella subcapitata*	Growth inhibition	60 μg Zn/L (72 h)	Franklin et al. (2007)
	50–70	*D. magna*	Mortality	3.2 mg/L (24 h)	Heinlaan et al. (2008)
		Zebrafish embryos	Development, hatching, mortality	1.79 mg/L (LC50, 96 h)	Zhu et al. (2008a)

Table 11.1 (continued)

Engineered nanoparticle	Size (nm)	Species	Effect	EC50/LC50/LOEC (Exposure time)	References
CuO		*D. pulex*	Mortality	60 μg/L (48 h)	Griffitt et al. (2008)
	30	*D. magna*	Mortality	3.2 mg/L (24 h)	Heinlaan et al. (2008)
	100–8,000	*Ceriodaphnia dubia*	Mortality	7–48 μg/L (LC50, 48 h)	Gao et al. (2009)
	95–447	Zebrafish (*D. rerio*)	Alteration of gene expression	100 μg/L (24 h, 48 h)	Griffitt et al. (2009)
CeO_2	14, 20, 29 (aggregates 275–552)	Algae *Pseudokirchneriella subcapitata*	Growth inhibition	5.6 mg/L (LOEC)	Van Hoecke et al. (2009)
		D. magna	Mortality	(72 h) 56–100 mg/L (LC50);	
			Reproduction	18–32 mg/L (21 d)	
SiO2	14, 60, 930	*D. magna*	Mortality	70% at 10 mg/L (24 h??)	Adams et al. (2006)
Fullerene (C_{60})	720	*D. magna*	Mortality	0.46 mg/L (48 h) (THF), 7.9 mg/L (sonicated)	Lovern and Klaper (2006)
	10–20	*D. magna*	Heart rate, behavioural changes	0.26 mg/L (1 h)	Lovern et al. (2007)
	10–200 (aggregates)	*D. magna*	Delay in moulting	2.5 and 5 mg/L (21 d)	Oberdörster et al. (2006)
	10–200 (aggregates)	Fathead minnows	Reproduction effect	1 mg/L (THF-nC_{60}), no mortality in water-stirred	Oberdörster et al. (2006)
	100	Largemouth bass	Mortality		
	not reported	Zebrafish embryos	Lipid peroxidation in brain		
			Mortality, delayed development, edema, heartbeat, hatching	1.5 mg/L	Oberdörster (2004)
		Zebrafish embryos (dechorionated)		0.5, 1 mg/L	Zhu et al. (2007)
				1.5 mg/L (96)	Usenko et al. (2008)
			Mortality, edema	0.2 mg/L (5 d)	
			Gene expression (oxidative stress)		

Table 11.1 (continued)

Engineered nanoparticle	Size (nm)	Species	Effect	EC50/LC50/LOEC (Exposure time)	References
Single-walled carbon nanotubes (SWCNT)	1.2	*D. magna*	Mortality	100% at 20 mg/L (48 h) 36% at 10 mg/L	Roberts et al. (2007)
	1	*Amphiascus tenuiramis*	Life cycle mortality Ventilation rate	0.1 mg/L (3 d)	Templeton et al. (2006) Smith et al. (2007)
	1.1	Rainbow trout *(O. mykiss)*	Gill alterations Behavioural change	0.25 mg/L (10 d)	
Multi-walled carbon nanotubes (MWCNT)	1–3	*Xenopus laevis* larvae	Mortality	85% at 500 mg/L (12 d)	Mouchet et al. (2008)
	11	Zebrafish embryos	Delayed hatching	120 mg/L	Cheng et al. (2007)
Quantum dots (QD)		*Pseudokirchneriella subcapitata*	Growth inhibition	37.1 μg/L (EC_{50}, 96 h)	Bouldin et al. (2008)
		*Ceriodaphnia dubi*a	Mortality Lipid peroxidation	1.6 mg/L	Bouldin et al. (2008)
	CdTe QD	Mussel *Elliptio complanata*	Immune system	> 0.11 mg/L (48 h)	Gagne et al. (2008)
	CdSecore/ZnSshell-PLL, 382	Zebrafish embryo	Mortality	7 μM (Cd equiv.)	King-Heiden et al. (2009)

other parts of the body (Baun et al., 2008a). The ENP also adhered to the surfaces of organisms and may induce effects such as increased moulting of the carapace by neonates (Fernandes et al., 2007). *Daphnia* can also biomodify single-walled CNT by stripping the lipid coating, which is used as a nutrient (Roberts et al., 2007).

Thus far, only a few experiments have been conducted with saltwater species. The toxicity of fullerenes has been investigated in adult and larval fish *F. heteroclitus* (Blickley and McClellan-Green, 2008). Very little effects were noted and no median lethal concentrations could be calculated up to 10 mg/L. Aggregates of fullerenes adhered to the chorion of eggs and did not affect embryonic development or hatching. Exposed larvae exhibited a dose-dependent increase in total glutathione, which was accompanied by a decreasing trend in lipid peroxidation. Increases in glutathione were also noted in liver but not gills of adult fish.

Current knowledge of different categories of ENP on various organisms indicates the following:

Metallic ENP. High concentrations of various metallic and inorganic NP have been shown to inhibit the growth of bacteria (Morones et al., 2005) and algae (Hund-Rinke and Simon, 2006), and cause mortality to invertebrates and fish. Due to their use as bactericides, nano-sized silver particles are quite well studied in bacteria. The cells of bacteria are damaged and this is dependent on the concentration, shape and size of the particles (Morones et al., 2005; Pal et al., 2007). Nanosilver appears to be more toxic than silver anions (Lok et al., 2006). Silver nanoparticles can be toxic because of their size and shape, but on the other hand, they could be toxic because they release silver ions, which are well known for their antibacterial activity and toxicity to algae. Exposure of algae to silver nanoparticles showed that in the first hour silver ions inhibit the photosynthesis about 18 times more than nanosilver did (Navarro et al., 2008b). But thereafter, the nanoparticles continued to be even more toxic than the ions alone. Obviously, the nanoparticles contributed to the toxicity as a source of silver, which is formed in the presence of algae.

Other inorganic ENP such as TiO_2, SiO_2 and ZnO show toxicity to bacteria and other organisms. Nano-sized filtered TiO_2 induced acute toxicity to *D. magna* at 2 mg/L and higher (Lovern and Klaper, 2006). The effect was greater for these and C_{60} particles when prepared in THF than with sonication, and C_{60} was more toxic than TiO_2. Toxicity of nano-sized and bulk ZnO, CuO and TiO_2 was studied in bacteria (Microtox assay), *D. magna* and the crustacean *Thamnocephalus platyurus*. Metal-specific recombinant biosensors were used to differentiate the effects induced by the metal oxides or nanoparticles (Heinlaan et al., 2008). Suspensions of nano and bulk TiO_2 were not toxic even at 20 g/L, whereas ZnO and CuO of bulk and nano-compounds showed acute toxicity. It was shown that the toxicity was largely due to soluble Zn and Cu ions, respectively.

A comparative toxicity study of bulk and nano-sized ZnO, TiO_2 and aluminium oxide (Al_2O_3) to zebrafish early life stages was undertaken to understand potential effects of such nanoparticles (Zhu et al., 2008a). Both bulk and nano-ZnO aqueous suspensions delayed zebrafish embryo and larval development, decreased hatching rate and survival and caused tissue damage. The 96-h LC_{50} of nano-ZnO and ZnO was 1.79 and 1.55 mg/L, respectively. No effects were found for the other two ENP

at the concentrations tested. This study showing no different toxicities between bulk and nano-ZnO indicates that the toxicity is mainly based on the metal oxide concentration rather than the nature of nanoparticles.

Similarly, a series of metallic ENP was tested in an algae, daphnids and zebrafish and compared to metal ions (Griffitt et al., 2008). Nanosilver and nanocopper caused toxicity in all organisms with a 48-h median lethal concentration as low as 40 and 60 μg/L, respectively, in *Daphnia pulex*, whereas TiO_2 did not cause toxicity. Susceptibility differed among species, with filter-feeding invertebrates being more susceptible compared with zebrafish. This study also found that soluble metals were more toxic than the nano-particulate forms.

Effects of a range of metallic ENP were assessed on rainbow trout (Federici et al., 2007) and zebrafish (Griffitt et al., 2007; Griffitt et al., 2008). Exposure of rainbow trout to dispersed nanoTiO_2 (0.1–1 mg/L) for 14 d in a static-renewal experiment caused some effects on gills such as oedema and thickening of lamellae (Federici et al., 2007). Decrease in Na^+K^+-ATPase activity in gills and intestine and a trend of decreased activity in the brain were also observed. An increase in total glutathione levels the gills is a sign of anti-oxidative defence and depletion in the liver. Liver cells showed minor fatty change and lipidosis, and some liver cells showed condensed nuclear bodies. Fish probably ingested TiO_2 during exposure leading to erosion on the intestinal epithelia. Obviously, ENP are not major iono-regulatory toxicants, although they lead to oxidative stress, organ pathologies and induction of anti-oxidative defences in form of glutathione level increase.

Silica nanoparticles show low acute toxicity. No cytotoxicity occurred in cell cultures up to 0.1 mg/mL silica nanoparticles (Jin et al., 2007). The growth of algae is only little affected by 20 and 28.8 mg/L (Van Hoecke et al., 2008). The 12.5 and 27 nm-sized ENP were found to adhere to the outer cell surface of algae. No signs of uptake were found. This is also the case with 50 nm and 200 nm fluorescent nanoparticles in fish eggs (Fig. 11.1).

Fullerenes (C_{60}) have been studied to a larger extent than other non-inorganic ENP. Increasing the salinity or ionic strength of aqueous media causes aggregations of fullerenes to be formed (Fortner et al., 2005). However, an increase in humic acids stabilizes the suspension allowing them to remain in solution. Hence, organisms may be exposed to C_{60} dissolved in water (Chen and Elimelech, 2007). The dispersion of fullerenes and their toxicity in natural waters vary significantly with water chemistry and the reactivity, as shown in two toxicity tests with *Ceriodaphnia* and a bioassay; C_{60} did not exhibit toxicity even at concentrations of >3 mg/L (Gao et al., 2009).

Acute toxicity to bacteria (Lyon et al., 2008), *D. magna* (Lovern and Klaper, 2006; Oberdörster, 2004) and fish (Oberdörster, 2004; Oberdörster et al., 2006; Zhu et al., 2006b) has been studied. The exposure of juvenile largemouth bass to 0.5 and 1 mg/L nC_{60} resulted in signs of lipid peroxidation in the brain (Oberdörster, 2004). These were assumed to reach the brain via the olfactory nerve. Cytochrome P450 enzymes were not affected by these ENP. THF-pre-treated C_{60} induced mortality in fathead minnows, whereas stirring of ENP in water did not affect survival. However, lipid peroxidation was observed in the gill (Zhu et al., 2006b). This shows that the

method of ENP preparation is important for their biological activity. The formation of aggregates usually reduces the bioavailability and toxicity. The use of solvents may also alter the toxicity as it may contribute to the effect. In case of metal ENP, the dissolution of metal ions (in addition to the nanoparticles) may contribute to the toxicity. Buckminster fullerene aggregates suspended in water (nC_{60}) affected zebrafish embryo survival, hatching rate, heartbeat and caused pericardial oedema at 1.5 mg/L and higher (Zhu et al., 2007). Moreover, embryo and larval development was delayed. Subsequent treatment with antioxidant glutathione mitigated toxicity, suggesting that oxidative stress was the cause for observed toxicity. Fullerol (a hydroxylated C_{60} derivative, $C_{60}(OH)_{16–18}$ had no such effect even at 50 mg/L (Zhu et al., 2007). An oxidative stress response was also observed in embryonic zebrafish (Usenko et al., 2008). Exposure of fish *F. heteroclitus* embryos larvae and adults to nC_{60} resulted in very little mortality up to 7 mg/L (Blickley and McClellan-Green, 2008). Fullerenes adsorbed to the chorion of eggs but did not affect development of the embryos or their hatching success. In larvae and adults, increase in total glutathione and a decreasing trend in lipid peroxidation were the only signs of fullerene exposure.

Baun et al. (2008b) studied the effect of combinations of ENP and chemical contaminants as a mixture. In *D. magna* and an algal species it was observed that C_{60} may serve as a contaminant carrier (Baun et al., 2008b). Suspensions of C_{60} were made by a 2-month stirring in medium and subsequently mixed with different contaminants. Depending on the contaminant, different toxicities occurred in *D. magna* and the algae *Pseudokirchneriella subcapitata*. The possibility of ENP serving as contaminant carriers has environmental relevance and should further be explored.

CNT induced dose-dependent growth inhibition in a protozoan (Zhu et al., 2006a). When CNT were coated with lipids they were readily taken up by *Daphnia*. They modified the solubility of the CNT through digestion of the lipids coating (Roberts et al., 2007). In fish, single-walled CNT (SWCNT) exposure caused a dose-dependent rise in ventilation rate, alteration in gills (oedema, altered mucocytes, hyperplasia), which points to a respiratory toxicant (Smith et al., 2007). In addition, increases in glutathione levels in gills and liver and condensed nuclear bodies in the liver were observed. SWCNT were ingested by fish resulting in precipitates in the gut and intestine. The brain showed swelling in parts of the cerebellum. Aggressive behaviour and gill irritation/damage led to the conclusion that SWCNT are respiratory toxicants in trout.

Effects of CNT have also been investigated in developing zebrafish (Cheng et al., 2007). Delay in hatching was observed at high concentrations for both single- and multi-walled CNT, but the toxicity may have been in part due to possible contamination of CNT. At very high concentrations of 120 mg/L hatching was delayed, whereas carbon black had no effect (Cheng et al., 2007). SWCNT did not influence embryonic development and survival. It was found that the chorion of zebrafish embryos acts as a protective barrier to prevent the passage of large nanotube aggregates through the pores. Delayed hatching was regarded as an indirect effect of hypoxia, because the nanotube aggregates hindered oxygen uptake. In contrast, 40 nm fluorescent latex nanoparticles are taken up by embryos and larvae of medaka

and accumulate in the yolk and gallbladder (Kashiwada, 2006). Double-walled CNT induced acute toxicity at 10–200 mg/L in larvae of the frog *X. laevis* due to physical blockage of the gills and/or digestive tract. This material has been taken up into the intestine (Mouchet et al., 2008).

Quantum dots (QD) are semiconductor nanocrystals which contain a cadmium crystalline core (Cd/Se and CD/Te) coated with a shell of ZnS and an outer organic polymer coating. QD are incorporated by primary rat hepatocytes, which may result in toxicity (Derfus, 2004). Aqueous toxicity and food chain transfer of QD were studied in freshwater algae and *Ceriodaphnia dubia* (Bouldin et al., 2008). No lethality was observed at 48-h exposure to *C. dubia* up to 110 μg/L; however, the alga *P. subcapitata* was affected with a 96 h EC_{50} of 37.1 μg/L. It was further shown that QD were transferred from dosed algae to *C. dubia*, indicating that these nanoparticles are transferred via food chain. It has to be further investigated to what extent bioaccumulation or even biomagnification in the aquatic food chain will occur (Holbrook et al., 2008). Effects of QD (cadmium telluride) were also studied in freshwater mussel *Elliptio complanata*, a filter feeder (Gagne et al., 2008). Exposures led to oxidative stress in gills and to DNA damage. Increased oxidative stress has also been indicated by increased carbon black concentrations in *D. magna* (Zhu et al., 2006b). Toxicity was influenced in zebrafish embryos by the QD coating, which also contributed to the QD suspension stability (King-Heiden et al., 2009). At sublethal concentrations, many QD produced not only signs of cadmium toxicity but also distinctly different toxicity that could not be explained by cadmium release.

11.4.3 Effects on Aquatic Organisms: Chronic Toxicity

In constrast to studies on the acute mortality in various organisms including *Daphnia* (Lovern and Klaper, 2006; Lovern et al., 2007; Oberdörster, 2004; Oberdörster et al., 2006; Zhu et al., 2006b), an estuarine meiobenthic copepod (Templeton et al., 2006) and fish (Kashiwada, 2006; Oberdörster, 2004), the long-term toxicity (sublethal alterations or subtle chronic effects) of nanomaterial has been studied only marginally. Potential impacts of environmentally realistic exposures (low concentrations) or chronic toxicity of ENP are not or inadequately known. Currently, there is a lack of knowledge about the long-term risks and potential mechanisms of toxicity of ENP, and therefore, there is a clear need to address these questions instead of focusing on acute toxicity only.

Similar to the situation with chemical compounds such as pharmaceuticals (Fent et al. 2006), chronic toxicity data of ENP are very sparse or lacking. There are only very few studies so far. Some data are given in Table 11.1. *D. magna* were exposed for 21 d to 2.5 and 5 mg/L fullerenes, resulting in a delay of moulting and reduced offspring production, a clear sign of chronic toxicity (Oberdörster et al., 2006). The full life cycle effects of SWNT on an estuarine copepod (*A. tenuiremis*) were studied over 28 d exposure. No significant mortality, development and reproduction effects

were observed with purified SWNT, but effects occurred at the highest concentration of 10 mg/L of more complex SWNT mixtures (Templeton et al., 2006).

Growth reduction and signs of oxidative stress, as indicated by a decrease in glutathione and an increase in antioxidant enzymes superoxide dismutase and catalase in gills and liver were observed in juvenile carp that were exposed to 0.04–1 mg/L fullerene aggregates for 32 days (Zhu et al., 2008b). In addition, lipid peroxidation was altered in liver and gills. Single-walled carbon nanotubes induced effects on gills (increase of Na^+K^+-ATPase activity) in rainbow trout when exposed up to 10 days. Increase of glutathione was observed in gills, liver and intestinal tract. Furthermore, aggressive behaviour was observed (Smith et al., 2007).

11.4.4 Terrestrial Plants and Soil Organisms

Less attention has been paid so far on implication of ENP to the terrestrial environment. Agglomerates of C_{60} applied to soil (up to 50 mg/kg dry soil) had little effects (Johansen et al., 2008). Respiration and microbial biomass were unaffected, whereas the number of fast-growing bacteria was decreased after ENP application. Also protozoans reacted not very sensitive to fullerenes.

Phytotoxicity was studied in ryegrass. In the presence of ZnO ENP, ryegrass biomass significantly reduced, root tips shrank and root epidermal and cortical cells were destroyed (Lin and Xing 2008). ZnO ENP greatly adhered on to the root surface. Individual ZnO ENP were observed present in apoplast and protoplast of the root endodermis and stele, but little ZnO ENP could translocate up in the ryegrass.

Reproduction of earthworm *Eisenia veneta* was affected by double-walled nanotubes (DWNT) administered through food at concentrations above 37 mg DWNT/kg food. The most sensitive toxicological parameter was reproduction (cocoon production), with no effect on hatchability, survival or mortality up to 495 mg DWNT/kg and 1000 mg C_{60}/kg. The toxicity of ENP and bulk ZnO, Al_2O_3 and TiO_2 has been evaluated in the nematode *Caenorhabditis elegans*. Both ENP and their bulk counterparts inhibited growth and the reproductive capability at mg/L concentrations (Wang et al., 2009). The toxicity of Al_2O_3 and TiO_2 nanoparticles was higher than that of their bulk counterparts. Silver nanoparticles decreased reproduction potential in *C. elegans*. Increased expression of the superoxide dismutase 3 and other genes occurred with 0.1 and 0.5 mg/L exposures concurrently with decreases in reproduction (Roh et al., 2009). Oxidative stress was indicated to be an important mechanism of silver ENP toxicity.

11.5 Conclusions

Recent studies suggest a risk of nanotubes that may act similar as asbestos fibres with a potential cancerogenic activity (Poland et al., 2008). Moreover adverse effects on the immune system have been indicated (Mitchell et al., 2009). This

suggests a toxic potential for human exposure, previously known with respect to inhalation of nanoparticles. Much less is known, however, on the potential risks to the environment. Thus far, the acute toxicity of a variety of ENP with different physico-chemical properties and sizes has been studied in various organisms at high concentrations. However, the effects at low and environmentally relevant concentrations remain largely unknown. Furthermore, it is almost unknown whether or not, and which chronic effects occur at realistic environmental concentrations. In addition, basic questions such as the bioavailability from environmental media, uptake and distribution in the body of organisms remain to be studied in more detail. The current knowledge is yet too restricted to draw conclusions about potential environmental hazards and risks of ENP. Further investigations are needed on the mode of actions and the toxic mechanisms of different ENP, particularly at low and environmentally realistic concentrations. Emphasis should be placed particularly on long-term effects, chronic toxicity and risks for bioaccumulation and transfer in the food chain. All measures should be taken to avoid or at least to reduce the introduction of ENP into the environment. Precaution is needed, because environmental consequences are largely unknown, in particular with respect to long-term effects.

References

Adams LK, Lyon DY, McIntosh A, Alvarez PJ (2006) Comparative toxicity of nano-scale TiO_2, SiO_2 and ZnO water suspensions. Water Sci Technol 54(11–12): 327–334.

Baun A, Hartmann NB, Grieger K, Kusk KO (2008a) Ecotoxicity of engineered nanoparticles to aquatic invertebrates: a brief review and recommendations for future toxicity testing. Ecotoxicology 17: 387–395.

Baun A, Sorensen SN, Rasmussen RF, Hartmann NB, Koch CB (2008b) Toxicity and bioaccumulation of xenobiotic organic compounds in the presence of aqueous suspensions of aggregates of nano-C_{60}. Aquat Toxicol 86: 379–387.

Blickley TM, McClellan-Green P (2008) Toxicity of aqueous fullerene in adult and larval *Fundulus heteroclitus*. Environ Toxicol Chem 27: 1964–1971.

Bouldin JL, Ingle TM, Sengupta A, Alexander R, Hannigan RE, Buchanan RA (2008) Aqueous toxicity and food chain transfer of quantum dots in freshwater algae and *Ceriodaphnia dubia*. Environ Toxicol Chem 27: 1958–1963.

Brant JA, Lecoanet H, Hotze M, Wiesner MR (2005a) Comparison of electrokinetic properties of colloidal fullerenes (n-C_{60}) formed using two procedures. Environ Sci Technol 39: 6343–6351.

Brant JA, Lecoanet H, Wiesner MR (2005b) Aggregation and deposition characteristics of fullerene nanoparticles in aqueous systems. J Nano Res 7: 545–553.

Brant JA, Labille J, Bottero JY, Wiesner MR (2006) Characterizing the impact of preparation method on fullerene cluster structure and chemistry. Langmuir 22: 3878–3885.

Brayner R, Ferrari-Illiou R, Brivois N, Djediat S, Benedetti MF, Fievet F (2006) Toxicological impact studies based on *Escherichia coli* bacteria in ultrafine ZnO nanoparticles colloidal medium. Nano Lett 6: 866–870.

Brunner TJ, Wick P, Manser P, Spohn P, Grass RN, Limpach LK, Bruinink A, Stark WJ (2006) In vitro cytotoxicity of oxide nanoparticles: comparison to asbestos, silica, and the effect of particle solubility. Environ Sci Technol 40: 4374–4381.

Buffle J, Leppard GG (1995) Characterisation of aquatic colloids and macromolecules. 1. Structure and behavior of colloidal material. Environ Sci Technol 29: 2169–2175.

Buffle J (2006) The key role of environmental colloids/nanoparticles for the sustainability of life. Environ Chem 3: 155–158.

Carlson C, Hussain SM, Schrand AM, Braydich-Stolle L, Hess KL,, Jones RL, Schlager JJ (2008) Unique cellular interaction of silver nanoparticles: size-dependent generation of reactive oxygen species. J Phys Chem B 112: 13608–13619.

Chae YJ, Pham CH, Lee J, Bae E, Yi J, Gu MB (2009) Evaluation of the toxic impact of silver nanoparticles on *Japanese* medaka *(Oryzias latipes)*. Aquat Toxicol 94: 320–327.

Chen KL, Elimelech M (2007) Influence of humic acid on the aggregation kinetics of fullerene (C_{60}) nanoparticles. Langmuir 22: 10994–11001.

Chen Y, Chen J, Dong J, Jin Y (2004) Comparing study of the effect of nanosized silicon dioxide and microsized silicon dioxide on fibrogenesis in rats. Toxicol Ind Health 20: 21–27.

Cheng J, Flahaut E, Cheng SH (2007) Effect of carbon nanotubes on development zebrafish (*Danio rerio*) embryos. Environ Toxicol Chem 26: 708–716.

Davoren M, Herzog E, Casey A, Cottineau B, Chambers G, Byrne HJ, Lyng FM (2007) In vitro toxicity evaluation of single walled carbon nanotubes on human A549 lung cells. Toxicol in Vitro 21: 438–448.

Derfus A (2004) Probing the cytotoxicity of semiconductor quantum dots. Nano Lett 4: 11–18.

Donaldson K, Aiken R, Tran L, Stone V, Duffin R, Forrest G, Alexander A (2006) Carbon nanotubes: A review of their properties in relation to pulmonary toxicology and workplace safety. Toxicol Sci 92: 5–22.

Federici G, Shaw BJ, Handy RH (2007) Toxicity of titanium dioxide to rainbow trout (*Oncorhynchus mykiss*): Gill injury, oxidative stress, and other physiological effects. Aquat Toxicol 84: 415–430.

Fent K, Weston AA, Caminada D (2006) Ecotoxicology of human pharmaceuticals. Aquat Toxicol 76: 122–159.

Fernandes TF, Christofi N, Stone V (2007) The environmental implications of nanomaterials. In: Monteiro-Riviere N, Lang Tran C (eds.) Nanotoxicology: Characterization, Dosing and Health Effects. CRC Press, Boca Raton, FL, pp. 405–420.

Fortner JD, Lyon DY, Sayes CM et al. (2005) C_{60} in water: nanocrystal formation and microbial responses. Environ Sci Technol 39: 4307–4316.

Franklin NM, Rogers NJ, Apte SC, Batley GE, Gadd GE, Casey PS (2007) Comparative toxicity of nanoparticulate ZnO, bulk ZnO, and $ZnCl_2$ to a freshwater microalga *(Pseudokirchneriella subcapitata)*: The importance of particle solubility. Environ Sci Technol 41: 8484–8490.

Gagne F, Auclair J, Turcotte P, Fournier M, Gagnon C, Sauve S, Blaise C (2008) Ecotoxicity of CdTe quantum dots to freshwater mussels: impacts on immune system, oxidative stress and genotoxicity. Aquat Toxicol 86: 333–340.

Gao J, Youn S, Hovsepyan A, Llaneza VL, Wang Y, Bitton G, Bonongo JCJ (2009) Dispersion and toxicity of selected manufactured nanomaterials in natural river water samples: effects of water chemical composition. Environ Sci Technol 43: 3322–3328.

Ghafari P, St-Denis CH, Power ME, Xu J, Tsou V, Mandal HS, Bols NC, Xiaowu T (2008) Impact of carbon nanotubes on the ingestion and digestion of bacteria by ciliated protozoa. Nature Nanotech 3: 347–351.

Griffitt RJ, Hyndman K, Denslow ND, Barber DS (2009) Comparison of molecular and histological changes in zebrafish gills exposed to metallic nanoparticles. Toxicol Sci 107: 404–415.

Griffitt RJ, Weil R, Hyndman KA, Denslow ND, Powers K, Taylor D, Barber DS (2007) Exposure to copper nanoparticles causes gill injury and acute lethality in zebrafish (*Danio rerio*). Environ Sci Technol 41: 8178–8186.

Griffitt RJ, Luo J, Gao J, Bonzongo JC, Barber DS (2008) Effects of particle composition and species on toxicity of metallic nanomaterials in aquatic organisms. Environ Toxicol Chem 27: 1972–1978.

Handy RD, Henry TB, Scown TM, Johnston BD, Tyler CR (2008a) Manufactured nanoparticles: their uptake and effects on fish—a mechanistic analysis. Ecotoxicology 17: 369–409.

Handy RH, Owen R, Valsami-Jones E (2008b) The ecotoxicology of nanoparticles and nanomaterials: current status, knowledge gaps, challenges, and future needs. Ecotoxicology 17: 315–325.

Hansen SF, Maynard A, Baun A, Tickner JA (2008) Late lessons from early warnings for nanotechnology. Nat Nanotechnol 3: 444–447.

Hardman R (2006) A toxicologic review of quantum dots: toxicity depends on physicochemical and environmental factors. Environ Health Perspect 114: 165–172.

Heinlaan M, Ivask A, Blinova I, Dubourguier HC, Kahru A (2008) Toxicity of nanosized and bulk ZnO, CuO and TiO_2 to bacteria *Vibrio fischeri* and crustaceans *Daphnia magna* and *Thamnocephalus platyurus*. Chemosphere 71: 1308–1316.

Herzog E, Casey A, Lyng FM, Chambers G, Byrne HJ, Davoren M (2007) A new approach to the toxicity testing of carbon-based nanomaterials-The clonogenic assay. Toxicol Lett 174: 49–60.

Holbrook RD, Morphy KE, Morrow JB, Cole KD (2008) Trophic transfer of nanoparticles in a simplified invertebrate food web. Nat Nanotechnol 3: 352–355.

Hund-Rinke K, Simon M (2006) Ecotoxic effect of photocatalytic active nanoparticles (TiO_2) on algae and daphnids. Environ Sci Poll Res 13: 225–232.

Jin Y, Kannan S, Wu M, Zhao JX (2007) Toxicity of luminescent silica nanoparticles to living cells. Chem Res Toxicol 20: 1126–1133.

Johansen A, Pedersen AL, Jensen KA, Karlson U, Hansen BM, Scott-Fordsmand JJ, Winding A (2008) Effects of C_{60} fullerene nanoparticles on soil bacteria and protozoans. Environ Toxicol Chem 27: 1895–1903.

Karlsson HL, Cronholm P, Gustafsson J, Müller L (2008) Copper oxide nanoparticles are highly toxic: a comparison between metal oxide nanoparticles and carbon nanotubes. Chem Res Toxicol 21: 1726–1732.

Kashiwada S (2006) Distribution of nanoparticles in see-trough medaka (*Oryzias latipes*). Environ Health Perspect 114: 1697–1702.

Keenan CR, Goldstein R, Lucas D, Sedlak DL (2009) Oxidative stress induced by zero-valent iron nanoparticles and Fe(II) in human bronchial epithelial cells. Environ Sci Technol 43: 4555–4560.

King-Heiden TC, Wiecinski PN, Mangham AN, Metz KM, Nesbit D, Pedersen JA, Hamers RJ, Heideman W, Peterson RE (2009) Quantum dot nanotoxicity assessment using the zebrafish embryo. Environ Sci Technol 43: 1605–1611.

Klaine SJ, Alvarez PJJ, Batley GE, Fernandes TF, Handy RH, Lyon DY, Mahendra S, McLaughlin MJ, Lead JR (2008) Nanomaterials in the environment: Behavior, fate, bioavailability and effects. Environ Toxicol Chem 27: 1825–1851.

Knauer K, Sobek A, Bucheli TD (2007) Reduced toxicity of diuron to the freshwater green alga *Pseudokirchneriella subcapitata* in the presence of black carbon. Aquat Toxicol 83: 143–148.

Kühnel D, Buscha W, Meissner T, Springer A, Potthoff A, Richter V, Gelinskyc M, Scholz S, Schirmer K (2009) Agglomeration of tungsten carbide nanoparticles in exposure medium does not prevent uptake and toxicity toward a rainbow trout gill cell line. Aquat Toxicol 93: 91–99.

Lee KL, Nallathamby PD, Browning LM, Osgood CJ, Xu XHN (2007) In vivo imaging of transport and biocompatibility of single silver nanoparticles in early development of zebrafish embryos. ACS Nano 1: 133–143.

Lin D, Xing B (2008) Root uptake and phytotoxicity of ZnO nanoparticles. Environ Sci Technol 42: 5580–5585.

Lin W, Huang YW, Zhou XD, Ma Y (2006) In vitro toxicity of silica nanoparticles in human lung cancer cells. Toxicol Appl Pharmacol 217: 252–259.

Lison D, Thomassen LC, Rabolli V et al. (2008) Nominal and effective dosimetry of silica nanoparticles in cytotoxicity assays. Toxicol Sci 104: 155–162.

Lok CN, Ho CM, Chen R, He QY, Yu WY, Sun HZ, Tam PKH, Chiu JF, Che CM (2006) Proteomic analysis of the mode of antibacterial action of silver nanoparticles. J Proteome Res 5: 916–924.

Lovern SB, Klaper R (2006) *Daphnia magna* mortality when exposed to titanium dioxide and fullerene (C-60) nanoparticles. Environ Toxicol Chem 25: 1132–1137.

Lovern SB, Strickler JR, Klaper R (2007) Behavioral and physiological changes in *Daphnia magna* when exposed to nanoparticle suspensions (titanium dioxide, nano-C_{60}, and $C_{60}HxC_{70}Hx$). Environ Sci Technol 41: 4465–4470.

Lyon DY, Brunet L, Hinkal GW, Wiesner MR, Alvarez PJJ (2008) Antibacterial activity of fullerene water suspensions (nC_{60}) is not due to ROS-mediated damage. Nano Lett 6: 1261–1268.

Mitchell LA, Gao J, Vander Wal R, Gigliotti A, Burchiel SW, McDonald JD (2007) Pulmonary and systemic immune response to inhaled multiwalled carbon nanotubes. Toxicol Sci 100: 203–214.

Mitchell LA, Lauer FT, Burchiel SW, McDonald JD (2009) Mechanisms for how inhaled multiwalled carbon nanotubes suppress systemic immune function in mice. Nat Nanotechnol 4: 409–410.

Moore MN (2006) Do nanoparticles present ecotoxicological risks for the health of the aquatic environment? Environ Int 32: 967–976.

Mori T, Takada H, Ito S, Matsubayashi K, Miwa N, Sawaguchi T (2006) Preclinical studies on safety of fullerene upon acute oral administration and evaluation for no mutagenesis. Toxicology 225: 48–54.

Morones JR, Elechiguerra JL, Camacho A, Holt K, Kouri JB, Ramirez JT, Yacaman MJ (2005) The bactericidal effect of silver nanoparticles. Nanotechnology 16: 2346–2353.

Mouchet F, Landois P, Sarremejean E, Bernard G, Puech P, Pinelli E, Flahaut E, Gauthier L (2008) Characterisation and in vivo ecotoxicity evaluation of double-wall carbon nanotubes in larvae of the amphibian *Xenopus laevis*. Aquat Toxicol 87: 127–137.

Navarro E, Baun A, Behra R, Hartmann NB, Filser J, Miao AJ, Quigg A, Santschi PH (2008a) Environmental behavior and ecotoxicity of engineered nanoparticles to algae, plants, and fungi. Ecotoxicology 17: 372–386.

Navarro E, Piccapietra F, Wagner B, Marconi F, Kaegi R, Odzak N, Sigg L, Behra R (2008b) Toxicity of silver nanoparticles to *Chlamydomonas reinhardtii*. Environ Sci Technol 42: 8959–8964.

Nel A, Xia T, Madler L, Li N (2006) Toxic potential of materials at the nanolevel. Science 311: 622–627.

Novak B, Bucheli T (2007) Occurrence, behavior and effects of nanoparticles in the environment. Environ Pollut 150: 7–22.

Oberdörster E (2004) Manufactured nanomaterials (fullerenes, C_{60}) induce oxidative stress in the brain of juvenile largemouth bass. Environ Health Perspect 112: 1058–1062.

Oberdörster E, Zhu SQ, Blickley TM, McClellan-Green P, Haasch ML (2006) Ecotoxicology of carbon-based engineered nanoparticles: effects of fullerene (C-60) on aquatic organisms. Carbon 44: 1112–1120.

Pal S, Tak YK, Song JM (2007) Does the antibacterial activity of silver nanoparticles depend on the shape of the nanoparticle? A study of the gram-negative bacterium *Escherichia coli*. Appl Eviron Microbiol 73: 1712–1720.

Petersen EJ, Huang Q, Weber WJ Jr. (2008) Ecological uptake and depuration of carbon nanotubes by *Lumbriculus variegatus*. Environ Health Perspect 116: 496–500.

Petersen EJ, Akkanen J, Kukkonen JVK, Weber WJJ (2009) Biological uptake and depuration of carbon nanotubes by *Dapnia magna*. Environ Sci Technol 43: 2969–2975.

Phenrat T, Long TC, Lowry GV, Veronesi B (2009) Partial oxidation ("aging") and surface modification decrease the toxicity of nanosized zerovalent iron. Environ Sci Technol 43: 195–200.

Poland CA, Duffin R, Kinloch I et al. (2008) Carbon nanotubes introduced into the abdominal cavity of mice show asbestos-like pathogenicity in a pilot study. Nat Nanotechnol 3: 423–428.

Roberts AP, Mount AS, Seda B, Souther J, Qiao R, Lin S, Ke PC, Rao AM, Klaine SJ (2007) In vivo biomodification of lipid-coated carbon nanotubes by *Daphnia magna*. Environ Sci Technol 41: 3025–3029.

Roh JY, Sim SJ, Yi J, Park K, Chung KH, Ryu DY, Choi J (2009) Ecotoxicity of silver nanoparticles on the soil nematode *Caenorhabditis elegans* using functional ecotoxicogneomics. Environ Sci Technol 3: 3933–3940.

Sayes CM, Liang F, Hudson JL et al. (2006) Functionalization density dependence of single-walled carbon nanotubes cytotoxicity in vitro. Toxicol Lett 161: 135–142.

Schmid K, Riediker M (2008) Use of nanoparticles in Swiss industry: a targeted survey. Environ Sci Technol 42: 2253–2256.

Smith CJ, Shaw BJ, Handy RD (2007) Toxicity of single walled carbon nanotubes to rainbow trout (*Oncorhynchus mykiss*): respiratory toxicity, organ pathologies, and other physiological effects. Aquat Toxicol 82: 94–109.

Stern ST, McNeil SE (2008) Nanotechnology safety concerns revisited. Toxicol Sci 101: 4–21.

Templeton RC, Ferguson PL, Washburn KM, Scrivens WA, Chandler GT (2006) Life-cycle effects of single-walled carbon nanotubes (SWNTs) on an estuarine meiobenthic copepod. Environ Sci Technol 40: 7387–7393.

Thibodeau MS, Giardina C, Knecht DA, Helble J, Hubbard AK (2004) Silica-induced apoptosis in mouse alveolar macrophages is initiated by lysosomal enzyme activity. Toxicol Sci 80: 34–48.

Usenko CY, Harper SL, Tanguay RL (2008) Fullerene C_{60} exposure elicits an oxidative stress response in embryonic zebrafish. Toxicol Appl Pharmacol 229: 44–55.

Van Hoecke K, De Schamphelaere KAC, Van der Meeren P, Lucas S, Janssen CR (2008) Ecotoxicity of silica nanoparticles to the green alga *Pseudokirchneriella subcapitata*: importance of surface area. Environ Toxicol Chem 27: 1948–1957.

Van Hoeke K, Quik JTK, et al. (2009) Fate and effects of CeO_2 nanoparticles in aquatic ecotoxicity tests. Environ Sci Technol 43: 4537–4546.

Vevers WF, Jha AN (2008) Genotoxic and cytotoxic potential of titanium dioxide (TiO_2) nanoparticles on fish cells in vitro. Ecotoxicology 17: 410–420.

Wang H, Wick RL, Xing B (2009) Toxicity of nanoparticulate and bulk ZnO, Al_2O_3 and TiO_2 to the nematode *Caenorhabditis elegans*. Environ Pollut 157: 1171–1177.

Zhang LW, Monteiro-Riviere NA (2009) Mechanisms of quantum dot nanoparticle cellular uptake. Toxicol Sci 110: 138–155.

Zhu L, Zhao Q, Li Y, Cai X, Li W (2006a) The interaction and toxicity of multi-walled carbon nanotubes with *Stylonychia mytilus*. J Nanosci Nanotechnol 6: 1357–1364.

Zhu S, Oberdörster E, Haasch ML (2006b) Toxicity of an engineered nanoparticle (fullerene, C_{60}) in two aquatic species, Daphnia and fathead minnow. Mar Environ Res 62(Suppl.): S5–S9.

Zhu X, Zhu L, Li Y, Duan Z, Chen W, Alvarez PJJ (2007) Developmental toxicity in zebrafish (*Danio rerio*) embryos after exposure to manufactured nanomaterials: buckminsterfullerene aggregates (nC_{60}) and fullerol. Environ Toxicol Chem 26: 976–979.

Zhu X, Zhu L, Duan Z, Qi R, Li Y, Lang Y (2008a) Comparative toxicity of several metal oxide nanoparticle aqueous suspensions to zebrafish (*Danio rerio*) early developmental stage. J Environ Sci Health A 43: 278–284.

Zhu X, Zhu L, Lang Y, Chen Y (2008b) Oxidative stress and growth inhibition in the freshwater fish *Carassius auratus* induced by chronic exposure to sublethal fullerene aggregates. Environ Toxicol Chem 27: 1979–1985.