14

Ecotoxicology: Nanoparticle Reactivity and Living Organisms

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Nanotechnology is a major source of innovation with important economic consequences. However, the potential risks for health and the environment have raised questions on national, European, and international levels. Past experience of sanitary, technological, and environmental risks has shown that it is not a good policy to attempt to deal with them after the fact. It is thus crucial to assess the risks as early on as possible. A particular problem is the potential dissmination of mass produced man-made nanoparticles into the environment [1, 2]. Nanomaterials represent a particular hazard for humans due to their ability to penetrate and subsequently damage living organisms [3]. Indeed, the data available at the present time shows that some nanomaterials, especially insoluble particles, can cross biological barriers and distribute themselves within living organisms.

The surge of interest in nanoparticles is a result of their unique properties, or nano-effects, often radically different to those of the same macroscopic materials (see Chap. II). The main cause underlying the change in properties is the very high surface to volume ratio. A nanoparticle of diameter 6 nm will have 35% of its atoms at the surface and hence an exceptionally high interfacial reactivity. These novel properties on the nanoscale lie at the heart of current scientific work on drug delivery, tumour targeting, the replacement of silicon in microelectronics by carbon nanoparticles, the synthesis of tougher materials, and many other projects. Considering the huge range of applications, it seems reasonable to expect their dissemination in the environment at each step in their life cycle, from design through production to use and disposal of finished products. As a consequence, it is important to study the risks for the biological components of the various repository media, and in particular concentrating media, such as the aquatic compartment.

By definition, a toxic product is a chemical compound which can harm the environment by affecting the biological organisms that occupy it, including human beings. Owing to their novel properties, the ecotoxicological impact of nanoparticles cannot be studied in the same way as other xenobiotics in the environment, e.g., pesticides, medicines, etc. Nanoparticles have mass, charge,

P. Houdy et al. (eds.), *Nanoethics and Nanotoxicology*,
DOI 10.1007/978-3-642-20177-6_14,

and above all surface area. They are subject to the phenomena of classical and quantum physics. Their reactivity means that their surface atoms are labile, easily change their redox state, and highly reactive with respect to compounds in the aqueous phase.

It was because nanoparticles were seen as conventional pollutants that the first nanotoxicological investigations often led to contradictory results [4, 5], and consequent controversy between research groups. These differences arose because the properties of nanoparticles, and the conditions of exposure of organisms, were poorly controlled. Most of the physicochemical properties of nanoparticles have a potential impact on their interaction with living beings. Among the most significant are their chemical nature, crystal structure, specific surface area, size, and morphology (e.g., spherical, acicular, fibre), surface charge, surface functionalisation (presence of chemical functions), and state of aggregation. A poor understanding of the physicochemical behaviour of nanoparticles is likely to lead to an erroneous interpretation of ecotoxicological data. For example, the differences observed over the last few years in the biological effects of carbon nanoparticles (C_{60}) can be imputed at least in part to the presence of residues from the synthesis and from the organic solvent used to disperse them [6].

It is thus difficult to understand the results of studies about the ecotoxicity of nanoparticles on different organisms such as bacteria (see Sect. 14.2), aquatic organisms (see Sect. 14.3), or plants (see Sect. 14.4), without first considering their physicochemical properties (see Sect. 14.1). To illustrate the interactions between nanoparticles and organisms, this chapter will mainly discuss metal nanoparticles (e.g., Fe, Ag), metal oxide nanoparticles (TiO_2, CeO_2, Fe_3O_4, γ-Fe_2O_3, ZnO), and carbon nanoparticles (C_{60} and carbon nanotubes) which are stimulating a great deal of interest today in terms of development and applications.

14.1 Physicochemical Properties and Ecotoxicity of Nanoparticles

In most cases, the nanoparticles studied are poorly characterised, or not characterised at all, from the physicochemical point of view. However, in order to assess the potential risks due to the presence of nanoparticles in the environment, a systematic characterisation is essential. One of the main problems in interpreting published work stems from the poor understanding and/or excessive diversity of the samples. Nanoparticles can have widely different morphologies, crystal structures, and surface properties. Several different methods must therefore be combined in the research effort, including ecotoxicology, physicochemistry, and crystal chemistry. In this section, we shall show that the ecotoxic response can be very different depending on the state of aggregation of the nanoparticles (see Sect. 14.1.1), their chemical stability (see Sect. 14.1.2), or modifications to their surface (see Sect. 14.1.3).

14.1.1 Nanoparticle Aggregation

Nanoparticles are not thermodynamically stable systems. One can define an interfacial tension which gives this dispersed state a high free energy. Without stabilisation via electrostatic repulsion (surface charge) and/or steric repulsion (adsorbed molecules), nanoparticles will agglomerate and hence be eliminated from the suspension by precipitation or flocculation. Once stabilised, nanoparticle suspensions can remain as such for long periods, but that will depend on the physicochemical conditions in the solution. For example, an increase in the ionic strength, a change of pH, or the presence of extracellular proteins [7] can perturb the stability of nanoparticle suspensions. And this type of modification is very common in ecosystems, in particular due to biological activity.

In ecotoxicity studies, nutrient solutions in equilibrium with aquatic organisms, micro-organisms, or plants contain nutrients, organic salts, sources of carbon and energy (glucose), sources of nitrogen (amino acids), and growth factors (vitamins, fatty acids). The high surface reactivities of nanoparticles for molecules and ions in solution associated with the environmental pH close to the zero charge point of most nanoparticles [8] will significantly perturb their colloidal stability. Such is the case with maghemite (γ-Fe_2O_3) nanoparticles (see Fig. 14.1) characterised by a mean hydrodynamic diameter of 20 nm in water at pH 3, but which form 50–100 µm aggregates in cell nutrient solutions.

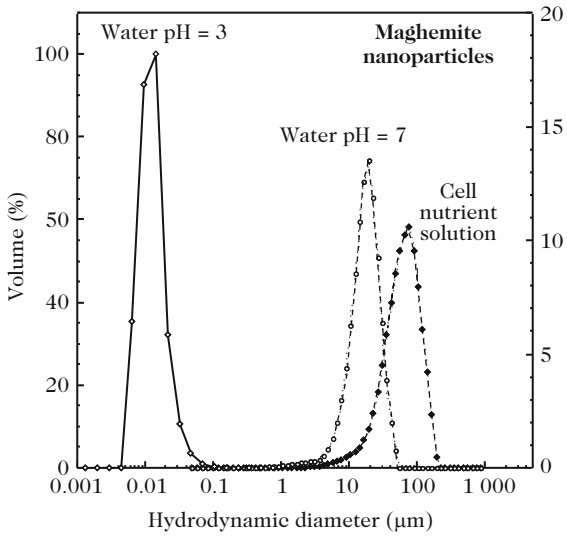

Fig. 14.1. Aggregation of nanoparticles in different aqueous media. Examples of maghemite (γ-Fe_2O_3) nanoparticles of diameter 6 nm in ultrapure water with acid pH, lower than the zero charge point (ZCP), neutral pH close to the ZCP, and in a cell nutrient solution

This aggregation of nanoparticle suspensions often contributes to the variability of the observed effects. For example, there are contradictions regarding size effects in the case of TiO_2 nanoparticles. According to Adams et al. (2006) and Verran et al. (2007), there is no effect, whereas Qi et al. (2006) find that the toxicity increases when the size of the nanoparticles is reduced [9–11]. These disagreements probably arise from the nanoparticle composition and the conditions under which the toxicity tests were carried out. In certain cases, nanoparticles may tend to aggregate, thereby reducing their contact with the given organism and hence also reducing their toxicity [10]. Sondi and Solopek-Sondi (2004) also observed that silver nanoparticles are toxic only when contact occurs on a solid medium, but not in a liquid medium where they note only slowed growth [12]. This can be explained by aggregation of the silver nanoparticles with intracellular components of dead cells. Once aggregated, their bactericidal effects are lessened and bacteria can develop normally. On the other hand, silver nanoparticle aggregation can be avoided by adding bovine serum albumin, and in this case, the bactericidal effect is maintained [13].

The destabilisation of nanoparticles in solution generally happens suddenly when physicochemical conditions are propitious. For the ionic strength, there is a critical coagulation concentration (CCC) beyond which contacts between nanoparticles cause them to stick together. The rate at which the solution is destabilised is then a question of kinetics. As a guide, one can use a simplified expression which gives the evolution of the concentration $N(t)$ of isolated particles in an initially stable suspension just after complete destabilisation, viz.,

$$\frac{1}{N(t)} = \frac{1}{N(0)} - \frac{4kT}{3\eta t} ,$$

where $N(0)$ is the initial concentration of nanoparticles in solution, η is the viscosity of the solution, k is the Boltzmann constant, and T is the temperature. For example, less than one minute is required for half of a suspension of 1 mg/L of CeO_2 nanoparticles to aggregate. This should be compared with the characteristic time for adsorption onto cells. On the other hand, it is very likely to be short compared with the modifications in the metabolism. This will affect the ecotoxicity of CeO_2 nanoparticles. Indeed, these suspensions prove to be toxic for *Escherichia coli* when their stability is maintained by working in a medium of low ionic strength. But at higher ionic strengths, CeO_2 nanoparticles aggregate and the toxic effect is no longer observed. However, we shall see later in the chapter that the colloidal stability of nanoparticles does not alone guarantee a toxic effect.

Carbon nanotubes are also prone to very strong interactions with many biological molecules, especially proteins. In fact, DNA is commonly used to stabilise carbon nanotube suspensions [14]. Moreover, it has been shown that carbon nanotubes interact with the immune system, not only in the blood complement [15], but also in the respiratory system through pulmonary surfactants [15]. Consequently, the state of aggregation of carbon nanotubes may

vary in time after exposure, and in different ways depending on the target organ. Likewise, the presence in the environment of industrial surfactants such as waste water, or natural surfactants such as humic acids, is likely to significantly modify their dispersion [16].

14.1.2 Chemical Stability of Nanoparticles

Similarly to aggregation, the chemical stability of nanoparticles, e.g., with regard to dissolution, oxidation, reduction, and generation of reactive oxygen species, plays an important role with respect to ecotoxicity. For example, nanoparticles are often made from soluble materials such as ZnO or CdS, which can salt out toxic ions. This is the case with Zn^{2+} ions released when ZnO nanoparticles are dissolved, and this underlies their bactericidal effects [17, 18]. Furthermore, the solubility of materials in the form of nanoparticles can be higher than that of the bulk material due to their higher specific surface area, but also their higher surface reactivity (see Chap. II).

The effect of specific surface area on the solubility of ZnO nanoparticles, and hence on their toxicity, has been demonstrated [19, 20]. Nanoparticles of diameter 100 nm are significantly toxic at concentrations above 12 mmol/L [20], whereas nanoparticles with diameters 10–15 nm are bactericidal from 1.3 mmol/L [19]. On the other hand, identical toxic effects are observed for 30 nm ZnO nanoparticles, ZnO microparticles, and dissolved $ZnCl_2$ salts [17, 18].

Nanoparticles can also generate reactive oxygen species, e.g., TiO_2, ZnO, Fe^0, Fe_3O_4. This is due to the properties of the material, and can be enhanced by the specific properties of the nanoparticles. Reactive oxygen species can also be generated under the effects of UV radiation. This is exemplified by TiO_2 and ZnO nanoparticles which exhibit an increased bactericidal effect under irradiation [9, 21]. Reactive oxygen species are produced by reactions of the following type:

$$\begin{aligned}
\mathrm{TiO_2} + h\nu &\longrightarrow \mathrm{TiO_2(h^+ + e^-)}\ , \\
\mathrm{e^-} + \mathrm{O_2} &\longrightarrow \mathrm{O_2^-}\ , \\
\mathrm{O_2^-} + 2\mathrm{H^+} + \mathrm{e^-} &\longrightarrow \mathrm{H_2O_2}\ , \\
\mathrm{H_2O_2} + \mathrm{O_2^-} &\longrightarrow {}^{\bullet}\mathrm{OH} + \mathrm{OH^-} + \mathrm{O_2}\ , \\
\mathrm{H^+} + \mathrm{H_2O} &\longrightarrow {}^{\bullet}\mathrm{OH} + \mathrm{H^+}\ .
\end{aligned}$$

Reactive oxygen species are also produced by Fenton reactions involving Fe^{2+} emitted during oxidation and dissolution–recrystallisation of iron-containing nanoparticles:

$$\begin{aligned}
\mathrm{Fe^0} + \mathrm{O_2} + 2\mathrm{H^+} &\longrightarrow \mathrm{Fe^{2+}} + \mathrm{H_2O_2}\ , \\
\mathrm{Fe^0} + \mathrm{H_2O_2} &\longrightarrow \mathrm{Fe^{2+}} + 2\mathrm{OH^-}\ , \\
\mathrm{Fe^{2+}} + \mathrm{H_2O_2} &\longrightarrow \mathrm{Fe^{3+}} + {}^{\bullet}\mathrm{OH} + \mathrm{OH^-} \quad \text{(Fenton reaction)}\ .
\end{aligned}$$

For example, nanoparticles containing only the oxidised form Fe^{3+}, e.g., maghemite, are stable and non-toxic towards *E. Coli* [22]. In contrast, those containing the reduced forms Fe^0 or Fe^{2+}, e.g., iron metal or magnetite, oxidise in solution and are highly bactericidal. On the other hand, it is silver nanoparticles containing the oxidised form Ag^+ rather than the purely metallic form Ag^0 which turn out to be toxic [13]. Moreover, for a given mass, the toxicity increases when the size of silver nanoparticles is reduced, and this is directly correlated with the increase in the fraction of Ag^+ ions at the particle surface.

Carbon nanotubes are different in this respect, because they are extremely hydrophobic and insoluble in the vast majority of solvents. However, residues of catalysts used to synthesise them (mainly transition metals like Fe, Co, and Ni) may nevertheless lead to the release of metal ions during exposure to carbon nanotubes.

14.1.3 Functionalised or Passivated Nanoparticles

Among the applications predicted for nanoparticles, some require the nanoparticle surface to be modified in order to increase their bioavailability, facilitate their dispersion in matrices, or deliver them to specific organs (as in the case of drug delivery). This happens in particular with iron oxide nanoparticles, widely used in the biomedical field. Owing to their zero charge point close to the physiological pH, these nanoparticles aggregate significantly in biological media (see Fig. 14.1). One way to limit aggregation is to create negative charges artificially, in order to generate sufficiently strong repulsive forces to keep them dispersed. A very effective molecule here is 2,3-dimercaptosuccinic acid [COOH–CH(SH)–CH(SH)–COOH] [23]. With its two thiol (–SH) functions, this molecule adsorbs strongly onto the surface of iron oxide nanoparticles via Fe–S bonds, while the $–COO^-$ groups confer a negative charge upon the nanoparticles, thereby limiting electrostatic attractions [24]. These strong chemical bonds survive prolonged suspension of iron oxide nanoparticles in biological media.

However, these surface modifications can cause drastic changes in the physicochemical properties and fate of nanoparticles in living organisms. For example, gold nanorods functionalised by specific bacterial antibodies exhibit a high level of toxicity, whereas non-functionalised gold nanorods have no toxic effect on the same bacteria [25]. In this case, bactericidal effects require direct exposure of the bacterial wall to the nanoparticles and light activation.

Carbon nanotubes are also often functionalised. There are two main types of carbon nanotube (see Fig. 14.2): single-walled carbon nanotubes (SWCNT) and multi-walled carbon nanotubes (MWCNT) made from one or more concentric tubes, respectively. Among the MWCNT, double-walled carbon nanotubes (DWCNT) are intermediary between SWCNTs and MWCNTs with regard to characteristics such as morphology and mechanical and electronic properties. DWCNTs have a major advantage over SWCNTs in that it is

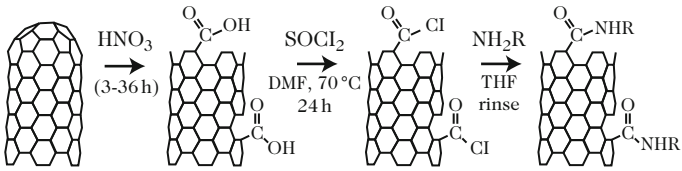

Fig. 14.2. Modification of nanoparticle surface properties. Different possibilities for functionalising the surface of carbon nanotubes following a primary oxidation stage

possible to modify their outer surface (by covalent grafting) without touching the inner tube. This means that they can be given useful surface properties, e.g., to facilitate their dispersion in a solvent, but without seriously damaging their mechanical properties (covalent functionalisation of SWCNTs partly destroys the carbon lattice) or electrical properties. Surface functionalisation of carbon nanotubes by oxygen functions can be achieved by reacting with an oxidising acid like concentrated nitric acid, for instance, or with mixtures of sulfuric acid and potassium permanganate, or other oxidising solutions. In this way, carboxylic acid and hydroxyl functions can be covalently grafted onto the surface, making the carbon nanotubes hydrophilic (see Fig. 14.2). These oxygen functions can serve as elementary building blocks for subsequent grafts of chemical functions, polymer chains, or molecules [26].

14.2 Ecotoxicity for Bacteria

There are many studies on the antibacterial properties of nanoparticles, e.g., [27]. For example, it is well established that silver and TiO_2 nanoparticles are efficient bactericides, used today to sterilise medical equipment. However, very few studies have directly investigated the harmful effects of nanoparticles on bacterial ecosystems. This is the subject of the present section.

The Cell: Basic Functional Unit of Life. One cell can function in complete autonomy in planktonic form or in a biofilm: this is the case of single-cell organisms, e.g., bacteria, archaea, micro-algae, protozoa, etc., or organisms integrated into a multicellular structure, e.g., fungal hyphae, tissues, etc.

Eukaryotic and prokaryotic cells share a highly organised structure made up essentially of four kinds of macromolecule: lipids, proteins, nucleic acids, and polysaccharides. It is the structure and organisation of these macromolecules on the cellular level that differentiates between the various organisms. A cell is always bounded by a membrane which isolates it from its surroundings and other cells. This membrane is structured in such a way as to retain chemical components and ions while at the same time allowing certain exchanges with the environment, namely the evacuation or entry of metabolites. This membrane bounds the compartment in which the essential functions of cell life take place, namely the cytoplasm. This in turn contains the nucleus or nucleoid, where the genetic information specific to the cell is stored, to be faithfully transmitted to the following generation. Most micro-organisms and plant

cells have a wall, in contrast to animal cells. This outer wall beyond the cytoplasmic membrane serves mainly to maintain the cell structure, whereas animal cells have an intracellular cytoskeleton.

Unlike prokaryotic cells which do not carry organelles, the cytoplasm of eukaryotic cells contains the nucleus which houses the genome, and mitochondria and chloroplasts (in the case of photosynthesising organisms) which provide the energy the cell needs to function.

14.2.1 Bacteria

Microbial cells constitute the main part of the terrestrial biomass despite their very small size. The number of bacteria is estimated to be around 5×10^{30} cells. Bacteria lie at the base of the food chain and are one of the main components of biogeochemical cycles, e.g., nutrients, minerals. They occur in most terrestrial and aqueous environments and can survive under extreme conditions, e.g., anaerobia, extreme temperature and pH, high metal concentrations, etc. They are highly flexible in morphological and physiological terms, with a great ability to adapt to and resist changing environmental conditions and all kinds of xenobiotic. Bacteria also exhibit the highest biological specific surface area, and this is in permanent interaction and exchange with the biotic and abiotic constituents of the environment. For this reason, any investigation of nanoparticle ecotoxicology must involve detailed study of nanoparticle–bacteria interactions, and the relevant toxicity mechanisms. Furthermore, bacteria can transform and 'metabolise' nanoparticles, modifying their mobility and bioavailability in the environment, important processes that need to be monitored in the context of environmental study.

It has been well established that nanoparticles have bactericidal effects. This suggests that nanoparticles may affect the viability and diversity of micro-organisms, and as a consequence, the functioning of the whole ecosystem, if they should occur in the environment at high concentrations and in a dispersed form.

In the environment, nanoparticles will begin by interacting with bacterial exopolymers, walls, and membranes. The cytoplasmic membrane plays a decisive role in the transport of nutrients and the wall in the protection of the cell against osmotic lysis:

- The cytoplasmic membrane comprises a double phospholipid layer about 8 nm thick, which is a permeable barrier. Many proteins, called intramembrane proteins, are encased in this membrane, most being involved in the transport of nutrients, secretion of other proteins, or rejection of toxic substances. The membrane is also where respiration takes place and the scene of the electron transfer chain.
- The wall is a rigid structure made up of peptidoglycans. The structure of the wall distinguishes between Gram-negative and Gram-positive bacteria. The wall of Gram-negative bacteria is the more complex, comprising

several sheets, while that of Gram-positive bacteria has a simpler composition but is often thicker.

- Bacteria also produce a wide range of exopolysaccharides which differ by their structure and function. These exopolysaccharides serve mainly to protect bacteria from hydric stress, the defence system of the host in the case of pathogens, and toxic substances, allowing them to colonise different media and arrange themselves in biofilms.

It is essential to take into account the kinds of interactions between nanoparticles and these bacterial constituents when carrying out nanoecotoxicity studies. Bacteria also provide a useful model because they operate an extracellular electron transport system which allows them to oxidise or reduce substrates that prove too large to be internalised, such as humic acids or iron oxides. Bacteria can metabolise these substrates by shuttles which are reduced in the membrane and oxidised in the substrate and vice versa, or directly by contact with enzymes or cytochrome located in the membrane. Other bacteria produce filaments several micrometers long from proteins, called fimbriae or pili, which can reduce iron oxides. This is the case of 'nanowires' of *Geobacter sulfurreducens* [28]. It is important to consider these reactions, initiated directly by enzyme activity or indirectly by production of oxidising or reducing agents, in the transformation of nanoparticles in the environment, e.g., redox, dissolution.

14.2.2 Effects of Nanoparticles on Bacterial Viability

In most studies today, the exposure conditions of the bacteria (solid or liquid media), the toxicity tests used, e.g., colony counts, growth curves, or membrane permeability, the types of nanoparticles, e.g., size, shape, dispersant, and the bacteria chosen for study, differ widely from one research group to another. For example, the toxicity of zinc oxide nanoparticles has been studied in a gelled solid medium [20, 29], in a liquid medium [17–19, 30], and by immersion of fabrics impregnated with nanoparticles in ultrapure water with the bacteria [31]. The same goes for TiO_2 nanoparticles investigated for their bactericidal effect in a liquid suspension [10, 32], dispersed in a gelled medium [9], adsorbed on cotton fibres [21], or adsorbed onto functionalised thin films [33].

The main studies dealing with the bactericidal effects of nanoparticles are summarised in Table 14.1. It should be borne in mind that the wide range of methods used here makes it difficult to compare results. However, two paradigms arise in these ecotoxicity studies, and these will be discussed in Sects. 14.2.3 and 14.2.4.

14.2.3 Exposure of Bacteria to Nanoparticles

The first paradigm concerns the conditions under which bacteria are exposed to nanoparticles. The surface properties of cell membranes are a decisive factor

Table 14.1. Different studies investigating the bacterial ecotoxicity of nanoparticles

Nanoparticle	Biological species	Dose studied	Effects observed or parameters measured	Ref.
Ag^0	*E. coli*	10–60 mg/L	70% drop in bacterial survival above 10 mg/L. Increased membrane permeability	[12]
Ag^0	*E. coli*, *Pseudomonas aeruginosa*, *S. typhus*, and *Vibrio cholera*	25–100 mg/L	*P. aeruginosa* and *V. cholera* more resistant than *E. coli* and *S. typhus*. Above 75 mg/L, no growth observed in the four types of bacteria	[34]
Ag^0	*E. coli*	0.01–1 mg/L	Most toxic nanoparticles have triangular shape, for which 100% growth inhibition is observed for 0.6 mg/L, compared with 0.2 mg/L for spherical nanoparticles	[35]
Fe^0	*E. coli*	7–700 mg/L, 1 h	20% drop in bacterial survival above 70 mg/L and oxidative stress. Toxicity associated with oxidation of Fe^0 to Fe^{2+} and Fe^{3+}	[22]
Fe_3O_4	*E. coli*	7–700 mg/L, 1 h	20% drop in bacterial survival above 350 mg/L and oxidative stress. Toxicity associated with oxidation of Fe^{2+} to Fe^{3+}	[22]
γ-Fe_2O_3	*E. coli*	7–700 mg/L, 1 h	No significant drop in bacterial survival. Nanoparticles chemically stable	[22]
MgO	*E. coli*, *Staphylococcus aureus*	1 mg/L	Bacterial survival rate depends on particle size	[36]
MgO	*Bacillus subtilis*	0.50 g MgO, 24 h	Bactericidal effects increase with decreasing particle size. Nanoparticle surface generates high concentrations of O_2^-	[36]
ZnO	*E. coli*	8–800 mg/L	Significant perturbation of cells for concentrations above 1.3×10^{-3} mol/L. Nanoparticle internalisation is observed	[19]

ZnO	*E. coli*	100–250 mg/L	Significant bacteriostatic activity, alteration of the membrane. Coating by polyethylene glycol or polyvinylpyrolidone do not affect antibacterial activity	[18]
CeO_2	*E. coli*	0.5–500 mg/L, 3 h	50% drop in bacterial survival above 7 mg/L. Toxicity associated with reduction of Ce^{4+} to Ce^{3+}	[38]
CeO_2	*Synechocystis*	0.5–500 mg/L, 3 h	50% drop in bacterial survival without pH buffer and above 25 mg/L. Toxicity is due to changed pH	[39]
TiO_2	*E. coli*, *Bacillus megaterium*	1–400 mg/L	Growth inhibition in both bacteria in ambient lighting conditions	[40]
Pt/TiO_2	*E. coli*, *S. aureus*, *Enterococcus faecalis*	1 000 mg/L	Bactericidal effects under UV radiation: *E. coli* > *S. aureus* > *E. faecalis*. Pt(IV) increases bactericidal effects in darkness	[41]
CNT	*E. coli*	1–50 mg/L	Significant antimicrobial activity and alteration of cell membrane	[42]

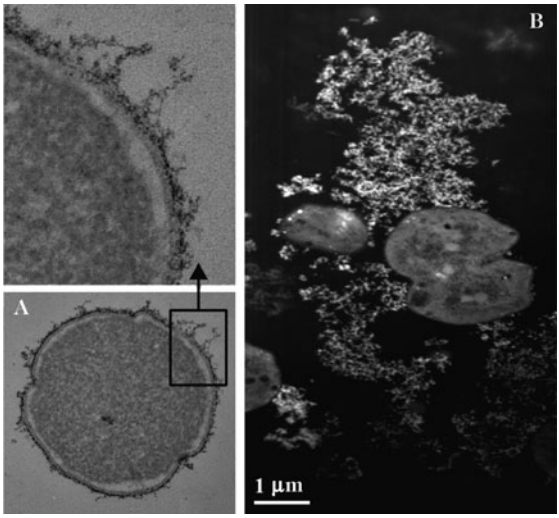

Fig. 14.3. Different conditions of exposure of bacteria to CeO_2 nanoparticles. (**A**) Adhesion onto the cell wall of *E. coli*. The nanoparticles form a monolayer covering the surface of the *E. coli*. (**B**) Adhesion in the exopolysaccharide layer of *Synechocystis*. In this case, little direct contact is observed between the nanoparticles and the bacterial wall

in nanoparticle toxicity [43]. For example, it turns out that C_{60} nanoparticles associate more strongly at the surface of Gram-negative bacteria, e.g., *E. coli*, than at the surface of Gram-positive bacteria, e.g., *B. subtilis*. It also turns out that, when nanoparticle toxicity is due to 'direct' redox effects, the proximity of the nanoparticles and the bacterial walls plays an important role (see Fig. 14.3) [38, 44]. This 'direct' redox toxicity can be inhibited or limited when the exposure of the cells to nanoparticles is modified. If the nanoparticles have aggregated and/or if their surface charge has been modified, the close contact interaction may not be able to occur and this form of toxicity is then significantly reduced. In this case, the area ratio between target cells and nanoparticles is large and one would no doubt observe effects due to size or the state of aggregation. On the other hand, when toxicity is due to an 'indirect' effect, such as the salting out of potentially toxic ions, e.g., Zn^+, Cd^{2+}, Ag^+ [17] or a change in pH [39], the exposure conditions are no longer fundamental. The important measurement for understanding toxic effects is then the nanoparticle concentration. In this case, the state of agglomeration will not be the key, even though several studies have suggested such a connection.

Some studies have also investigated bacterial communities in natural soils. Tong et al. [45] assessed the impact of adding 1 mg of C_{60} per gram of soil by carrying out DNA and fatty acid analyses, finding only a small impact on the structure of these communities. It thus turns out that C_{60} nanoparticles are less toxic under natural soil conditions [45] than under controlled laboratory

conditions [43]. However, the impact of C_{60} on the physiology and functions of soil bacteria remains unknown.

14.2.4 Oxidative Stress

The second paradigm concerns nanoparticle-induced oxidative stress. Nanoparticles that are chemically unstable in biological media can produce reactive oxygen species in the vicinity of bacteria and induce significant oxidative stress. It seems that metallic nanoparticles are the most sensitive to oxidation or reduction, e.g., Fe^0, Fe_3O_4, CeO_2, and have the most marked effect on bacteria [46].

Using bacterial strains deficient in superoxide dismutase, an antioxidant, it has been shown that oxidative stress is one of the main toxicity mechanisms. For iron-containing nanoparticles, reactive oxygen species are generated through Fenton reactions, which produce hydroxyl radicals from the emitted Fe^{2+}. For example, magnetite (Fe^{2+}/Fe^{3+}) nanoparticles of radius 6 nm are highly toxic to *E. coli* from 0.7 g/L of Fe_3O_4. It has been shown using X-ray absorption spectroscopy that the surface of magnetite nanoparticles oxidises to maghemite (Fe^{3+}) after contact with the bacteria [22]. This change of phase occurs via desorption of Fe^{2+} from the structure and the creation of surface vacancies [47]. Fe^0 nanoparticles are much more sensitive to oxidation and generate toxicity at 10 times lower doses, viz., 0.07 g/L of Fe^0 [22]. They are entirely transformed into lepidocrocite (Fe^{3+}) and magnetite (Fe^{2+}/Fe^{3+}). This oxidation follows a dissolution–recrystallisation process producing a hydroxide of Fe^{2+} and Fe^{3+}, called green rust.

For CeO_2 nanoparticles, reactive oxygen species are produced in redox cycles $Ce^{4+} \longrightarrow Ce^{3+} \longrightarrow Ce^{4+}$, which occur on the nanoparticle surface [48]. These cycles underlie the catalytic properties of CeO_2 nanoparticles and are accompanied by significant electron transfer, ion transfer, and the creation of vacancies in the surface structure. In biological media, these redox cycles can induce the oxidation of certain compounds at the interface with the bacterial walls. Thill et al. (2006) showed that 50% of the *E. coli* population does not survive the presence of 0.003 g/L of CeO_2 (Ce^{4+}) nanoparticles of diameter 7 nm adsorbed on their walls [38]. This toxicity is associated with the reduction of 30% of their surface atoms into Ce^{3+}.

One consequence of the production of reactive oxygen species is that they can trigger a chain of destructive radical reactions such as lipid peroxidation, in the bacterial lipopolysaccharide layer. This happens with reactive oxygen species generated during oxidation of TiO_2 and ZnO nanoparticles [49]. In particular, Sunada et al. [33] have observed the destruction of the outer then inner membrane in *E. coli* in the presence of TiO_2 nanoparticles. Finally, an interesting example is C_{60} [50], which induces a modification in the synthesis of bacterial fatty acids. This is a mechanism for protecting the cell membrane against reactive oxygen species. *Pseudomonas putida* reduces the synthesis of conventional fatty acids in favour of cyclopropane fatty acids,

while *Bacillus subtilis* synthesises more monosaturated fatty acids. Membrane fluidity is increased in both cases.

The Cell: A Chemical Factory. A cell interacts with its environment to obtain the nutrients it transforms (metabolism) in order to extract energy and to produce the macromolecules it needs to keep the cell machinery working and maintain the cell structure. It also produces metabolites that it must release into its environment.

A cell transforms chemical compounds to generate another living organism by reproducing, doubling its contents to give rise to a cell that generally has the same properties and characteristics as the mother cell. Cell division involves a stage in which the genetic material is doubled by replication of the chromosomes. The genes essential to cell division are transcribed from the DNA to make RNA, which is in turn translated into proteins with the help of ribosomes, particles composed of RNA and proteins.

All these operations are orchestrated by regulators which allow the cell to 'sense' its environment and adapt its responses and its way of life to external conditions by expressing suitable genes. The cells also communicate with one another via chemical mediators. They can move toward environments where conditions are more favourable, because most living organisms are endowed with mobility and able to move in an autonomous way, with the exception of plants, which are sessile. Living organisms evolve through genetic rearrangements which allow them to acquire new properties. This evolution takes place over several generations and can be studied in micro-organisms. Single-cell micro-organisms have the property of reproducing quickly and autonomously, reaching high population densities under laboratory conditions and producing several generations over a reasonable lapse of time, which makes them good models for studying the cell machinery and its adaptations, evolution, and limitations in the face of environmental stress.

14.3 Ecotoxicity for Aquatic Organisms

The available ecotoxicological data is rather incomplete and insufficient to draw global conclusions about the impact of nanoparticles on the aquatic environment. One particular difficulty is to evaluate the concentrations of nanoparticles which might occur in aquatic environments and which could be qualified as realistic from an environmental standpoint. This section presents the results of recent studies carried out on aquatic vertebrates and invertebrates in order to assess the ecotoxicity of carbon nanoparticles (see Sect. 14.3.1) and metal and metal oxide nanoparticles (see Sect. 14.3.2).

14.3.1 Carbon Nanoparticles

Most available studies concern the fullerenes C_{60}. These studies, summarised in Table 14.2, demonstrate the ingestion of C_{60} and its associated toxicity in several model organisms, viz., the freshwater crustacea *Daphnia magna* and *Hyalella azteca*, along with the fish *Pimephales promelas*, *Oryzias latipes*, *Danio rerio*, *Micropterus salmoides*, and *Carassius auratus* [3, 51–57].

Table 14.2. Studies on the ecotoxicology of C_{60} in crustacea, fish, and earthworms

Biological species	Concentration	Effects observed and parameters measured	Ref.
Freshwater crustacea			
Daphnia magna	0.5–1–2.5–5 mg/L for 48 h and 21 days, and 30 mg/L for 2 days	Moulting delayed after 21 days and reduced reproduction at 2.5 and 5 mg/L, respectively, in invertebrates. Reduced expression of the protein PMP70 (peroxisomal lipid transport) in *P. promelas*, but not in *O. latipes*, suggesting modifications in acyl-CoA pathways	[53]
Hyalella azteca	7 mg/L for 96 h		
Marine copepods	3.75–7.5–15–22.5 mg/L for 96 h		
Pimephales promelas	0.5 mg/L for 96 h		
Oryzias latipes	0.5 mg/L for 96 h		
Daphnia magna	0.5–1.5–10–25–50–100 mg/L for 48 h	Increased immobilisation and mortality from low doses. EC_{50} (immobilisation) = 9.3 mg/L and LC_{50} (mortality) = 10.5 mg/L	[56]
Daphnia magna and the fish *Pimephales promelas*	0.5 ppm of THF–nC$_{60}$ and water–nC$_{60}$ for 48 h	LC_{50} for THF–nC$_{60}$ = 0.8 mg/L and water–nC$_{60}$ > 35 mg/L. 100% mortality in fish exposed to THF–nC$_{60}$, but not in fish exposed to water–nC$_{60}$. Lipid peroxidation in brain and gills. Increased expression of isoenzymes CYP2 in liver of individuals exposed to water–nC$_{60}$	[55]
Daphnia magna	0.26 mg/L of nC$_{60}$ and C$_{60}$H$_x$C$_{70}$H$_x$	Increased heart rate and predation, and reduced reproduction above 0.26 mg/L	[52]
Daphnia magna	0.04–0.18–0.26–0.35–0.44–0.51–0.7–0.88 mg/L (filtered THF–nC$_{60}$) and 0.2–0.45–0.9–2.25–4.5–5.4–7.2–9 mg/L (sonicated nC$_{60}$)	Increased mortality from lowest doses. LC_{50} 48 h = 0.46 mg/L, LOEC = 0.26 mg/L and NOEC = 0.18 mg/L (THF-nC$_{60}$). LC_{50} 48 h = 7.9 mg/L, LOEC = 0.5 mg/L and NOEC = 0.2 mg/L (sonicated nC$_{60}$)	[52]

(*Continued*)

Table 14.2. (Continued)

Biological species	Concentration	Effects observed and parameters measured	Ref.
Fish			
Micropterus salmoides	0.5 and 1 mg/L of THF–nC$_{60}$ for 48 h	Lipid peroxidation in brain at 0.5 mg/L. Glutathione depletion in gills at 1 mg/L. Increased limpidity of exposure water due to bacterial activity	[3]
Danio rerio (embryos)	1.5 and 50 mg/L of THF–nC$_{60}$ and THF–nC$_{60}$(OH)$_{16–18}$ for 96 h	Delayed hatching and development of larvae, reduced survival and hatch rate, and pericardial edema at 1.5 mg/L of C$_{60}$	[57]
Juvenile carp *Carassius auratus*	0.04–0.20–1 mg/L of nC$_{60}$ for 32 days	Induction of the antioxidant enzymes superoxide dismutase and catalase in gills and liver. Reduced glutathione in all tissues tested. Reduced lipid peroxidation in gills and brain except at 1 mg/L in the liver. Inhibited growth at 1 mg/L	[54]
Danio rerio (embryos)	0.1–0.2–0.3–0.5 mg/L of DMSO–nC$_{60}$ and nC$_{60}$(OH)$_{24}$ under illumination for 120 pfh (malformations and mortality) and 24 pfh (sublethal effects)	Mortality, altered expression of genes involved in oxidative stress. Reduced mortality, malformations and pericardial edema at 0.2 and 0.3 mg/L with light reduction. Reduced mortality and pericardial edema in embryos coexposed in the presence of glutathione precursor. Increased sensitivity of embryos coexposed to GSH inhibitors. Increased mortality in embryos coexposed in the presence of H_2O_2	[58]
Earthworms			
Eisenia veneta	1 000 mg of C$_{60}$ per kg of food (dry weight) for 28 days	No effect on hatching or mortality at 1 000 mg of C$_{60}$ per kg of food	[59]

DMSO dimethyl sulfoxide, EC_{50} efficient concentration for 50% of individuals exposed, pfh post-fertilization hours, LC_{50} lethal concentration for 50% of individuals exposed, LOEC lowest observed effect concentration, NOEC no observed effect concentration, TFH tetrahydrofurane

There has been little work on the ecotoxicology of carbon nanotubes in aquatic organisms (see Table 14.3). Petersen et al. (2008) demonstrated that the freshwater oligochaetes *Lumbriculus variegatus* ingest SWCNTs associated with sediment particles, identifying them in the intestine but not establishing whether they are absorbed in the tissues [60]. Roberts et al. (2007) demonstrated the ingestion of SWCNTs coated with lysophospholipids by the freshwater crustacea *Daphnia magna*, and observed mortality associated with high concentrations [61]. Templeton et al. (2006) found increased mortality and reduced fertilization rate in the estuarine copepod *Amphiascus tenuiremis*, depending on the SWCNT mixtures used [62]. Recently, Kennedy et al. (2008) identified reduced viability in the cladoceran *Ceriodaphnia dubia* exposed to raw MWCNTs, while this was not observed when these same MWCNTs were functionalised [63]. In the amphipods *Leptocheirus plumuloss* and *Hyalella azteca* exposed via sediments, they also observed that mortality increased as the size of the sediment particles decreased, although mortality here was lower for exposure to raw MWCNTs than for exposure to carbon black and active carbon. In the zebrafish *Danio rerio*, Cheng et al. found delayed hatching of eggs after exposure to SWCNTs and DWCNTs [64], and exposure to MWCNTs functionalised by bovine serum albumin [65]. In the trout *Onchorhynchus mykiss* exposed to SWCNTs, Smith et al. (2007) observed various respiratory toxicological effects and gill pathologies (hyperventilation, secretion of mucus), neuronal pathologies, and hepatic pathologies (apoptotic bodies, abnormal cell division) [66].

Two studies published recently investigate the effects of raw DWCNTs on amphibians. Amphibians and especially their larvae are excellent indicators for the health of ecosystems at the land–water interface. Studies on larvae of the axolotl *Ambystoma mexicanum* (see Fig. 14.4) revealed no sign of toxicity or genotoxicity, despite massive ingestion of DWCNTs [67]. In the xenopus *Xenopus laevis* (see Fig. 14.5), results show that, despite the mortality and growth inhibition measured at high DWCNT concentrations, associated with massive ingestion [68], no genotoxicity was observed.

In terrestrial organisms (earthworms), Scott-Fordsmand et al. (2008) showed that the exposure of *Eisenia veneta* to carbon-based nanoparticles by feeding affects neither hatch rate nor mortality at 1 000 mg C_{60}/kg dry weight of food and up to 495 mg of carbon nanotubes/kg dry weight of food [59]. In contrast, reproduction in these worms is affected from 37 mg carbon nanotubes/kg of food. Petersen et al. (2008) showed that exposure of *Eisenia foetida* to carbon nanotubes in soil induced a bioaccumulation factor twice as small as for exposure to pyrene, the chosen control molecule [69]. The authors identified carbon nanotubes in the intestine, associated with ingested soil particles. However, absorption of carbon nanotubes by tissues was not demonstrated for these organisms.

Table 14.3. Ecotoxicology of carbon nanotubes in aquatic invertebrates and vertebrates, and earthworms

Biological species	Carbon nanotube	Exposure mode	Concentration	Effects observed and parameters measured	Ref.
Invertebrates					
Freshwater oligochaete annelid	C^{14}-MWCNT	Contamination of sediment, exposure of worms for 7, 14, and 28 days	0.37 and 0.037 mg/g of dry sediment	Bioaccumulation factor lower than pyrene control. Nanotubes found in intestine, associated with ingested sediment particles. Absorption of nanotubes by tissues not demonstrated	[60]
Lumbriculus variegatus	C^{14}-SWCNT		0.03 and 0.003 mg/g of dry sediment		
Daphnia magna	SWCNT	Exposed 48 h in solution	0.1–0.5–1.5–10–50–100 mg/L	Increased immobilisation and mortality from lowest concentrations. EC_{50} (immobilisation) = 1.306 mg/L and LC_{50} (mortality) = 2.425 mg/L	[56]
	MWCNT			EC_{50} (immobilisation) = 8.723 mg/L and LC_{50} (mortality) = 22.751 mg/L	
Daphnia magna	SWCNT with lysophosphatidylcholine	Exposed 48 and 96 h in solution	2.5–5–10–20 mg/L with food. 0.1–0.25–0.5–1–2.5 mg/L without food	Ingestion of SWCNT coated with lysophospholipids. Daphnia can modify nanotubes by eliminating the coating lysophospholipids. 100% mortality after 48 h at 20 mg/L and 85% mortality after 96 h at 20 mg/L	[61]
Estuarine copepod *Amphiascus tenuiremis*	Purified SWCNTs and functionalised SWCNTs	Exposure in solution	0.58–0.97–1.6 and 10 mg/L	No significant effect on development and reproduction with purified SWCNTs. Increased mortality, reduced fertilization rate, reduced success in development of nauplii with functionalised SWCNTs	[62]
Estuarine copepod *Amphiascus tenuiremis* polychaete *Streblospio benedicti*	SWCNTs associated with sediment and C^{14}-SWCNTs	Coexposure to sediments and organic contaminants (COH) and aromatic hydrocarbons (PAH)	5 mg/g for 14 days	Reduced bioaccumulation of COH in *S. benedicti*. No impact of SWCNTs on accumulation of PAHs in *A. tenuiremis*. Neither model assimilates nanotubes in its tissues, although *S. benedicti* ingests C^{14}-SWCNTs (activity of C^{14} in excrement the same as in sediment)	[70]

Invertebrates (Cont.)					
Amphipods (sediment) *Hyalella azteca*, *Leptocheirus plumulosus*	Raw MWCNT, MWCNT-OH, MWCNT-COOH stabilised with organic matter	Exposure in solution	0.4–1.1–3.3–9.9 and 30% in sediment (dry weight)	Ingestion of raw, hydroxylated, and carboxylated MWCNTs, sometimes causing mortality and immobilisation. *C. dubia* EC_{50} at 48 h = 50.9 raw MWCNT	[63]
Cladoceran (water column) *Ceriodaphnia dubia*			32 and 120 mg/L of MWCNT-OH, 39.5 mg/L of raw MWCNT, and 88.9 mg/L of MWCNT-COOH		
Vertebrates					
Zebrafish *Danio rerio* (embryos)	Raw MWCNT	Exposure in solution at stage 8–16 cells for 24, 48, and 72 pfh	2.5–5–10–20–30–40–50–60–70–100–200–300 mg/L	NOEC = 40 mg/L and LOEC = 60 mg/L. Lethal effects (mortality at 72 pfh) and sublethal effects (delayed hatching, lowered blood flow rate) from 60 mg/L. 100% and no hatching above 200 mg/L. Teratogenic effects and apoptosis above 100 mg/L. Caudal and notochordal malformations at 60 and 70 mg/L. Inflammatory response by production of mucus at 60 mg/L	[71]
		Microinjection at 8 cell stage	5 ng/ml	35% mortality. Malformations from 60 mg/L	
	Raw DWCNT, raw SWCNT	Exposure in aqueous medium at stage 4 pfh for 4–96 h	SWCNT: 20–40–60–120–240–360 mg/L. DWCNT: 120–240 mg/L	Delayed hatching of eggs from 120 mg/L (SWCNT) between 52 and 72 h after fertilization and 240 mg/L (DWCNT). Delay probably induced by the Co and Ni used to synthesise the SWCNTs and which remains as a trace even after purification. Embryonic development not affected	[64]

(*Continued*)

Table 14.3. (Continued)

Biological species	Carbon nanotube	Exposure mode	Concentration	Effects observed and parameters measured	Ref.
Vertebrates (Cont.)					
Zebrafish *Danio rerio* (embryos)	Functionalised MWCNT (BSA-MWCNT) and labelled with FITC	Microinjection in egg at 1 cell stage and 72 pfh. Microangiography (venous sinus injection)	About 2 ng per embryo	No mortality or developmental defect for micro-injected eggs. In vivo biodistribution in developing eggs equivalent to control (without microinjection). Intracellular biodistribution not equivalent: accumulation of FITC-BSA-MWCNTs in nuclei of blastoderm cells; cell division and proliferation rate faster in the presence of MWCNTs. Biodistribution of FITC-BSA-MWCNTs throughout the organs and tissues of the larva after microangiography. Preferential accumulation in natatory bladder. Disappearance of signal beyond 96 h in relation with excretion of MWCNTs. Immune response induced in early stages in the presence of MWCNTs. Recruitment of lysosomal vesicles in the blastoderm. Lower survival rate of the second generation after 14 days of fertilization in the presence of MWCNTs (effect on reproductive potential)	[65]
Trout *Onchorhynchus mykiss*	SWCNT in presence of SDS	Exposure in solution at juvenile stage for 10 days	0.1–0.5 and 0.25 mg/L	Respiratory toxicological effects. Gill and neuron pathologies. Liver affected. Abnormal cell division. Raised ventilation rate and secretion of mucus. Increased activity of ATPase Na^+L^+ in gills and intestine. Reduced thiobarbituric acid in the brain, gills, and liver. Increase in the overall level of glutathione in the gills and liver	[66]

Vertebrates (Cont.)					
Amphibian axolotl *Ambystoma mexicanum*	Raw DWCNT	12 day exposure in solution at two-finger stage	1–10–100–125–250–500 and 1 000 mg/L	No sign of acute toxicity (mortality, growth) or genotoxicity (induction of micronuclei), despite massive ingestion of nanotubes by larvae	[67]
Amphibian xenopus *Xenopus laevis*	Raw DWCNT	12 day exposure at stage 50 of the Nieuwkoop–Faber table	10–100–500 mg/L	Mortality and delayed growth measured at high DWCNT concentrations, associated with massive ingestion of DWCNTs. No associated genotoxicity (induction of micronuclei)	[68]
Earthworms					
Eisenia foetida	C^{14}-MWCNT, C^{14}-SWCNT	Mixed with soil for 1, 7, 14, and 28 days	0.03 mg/g of dry soil and 0.3–0.03 mg/g of dry soil	Bioaccumulation factor only half the value for the pyrene control. Nanotubes present in intestine, associated with ingested soil particles. It was not shown that nanotubes were absorbed in the tissues	[69]
Eisenia veneta	DWCNT	Exposure via food for 28 days	0–50–100–300–495 mg DWCNT/kg of food (dry weight)	Reproduction affected (EC_{10}) above 37 mg DWCNT/kg of food ($EC_{50} = 176 \pm 150$ mg DWCNT/kg). No effect on hatch rate or on mortality up to 495 mg DWCNT/kg of food	[59]

BSA bovine serum albumin, FITC fluorescein isothiocyanate, pfh post-fertilization hours, NOEC no observed effect concentration, SDS sodium dodecyl sulfate

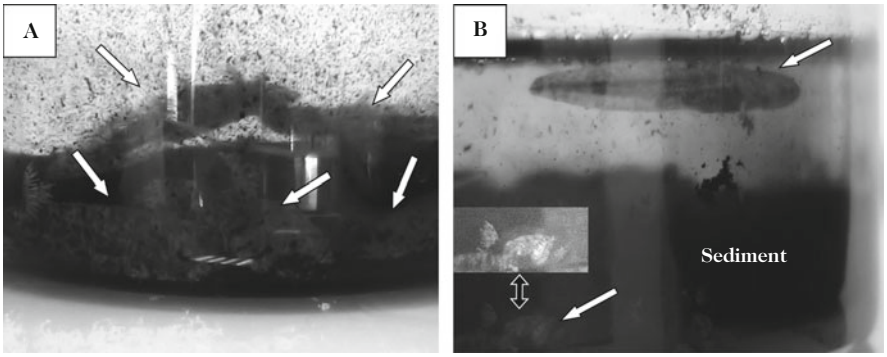

Fig. 14.4. Exposure of axolotl larvae to carbon nanotubes. Axolotl larvae (*white arrows*) in the presence of carbon nanotubes (**A**) at the beginning and (**B**) at the end of exposure (12 days). The larvae bury themselves in the 'sediment' of carbon nanotubes (**A**) and swim through the water column (**B**), probably in search of oxygen at the end of exposure. When they are at this level, they are not covered with carbon nanotubes. No toxicity is observed (mortality, growth) in larvae exposed to a broad range of concentrations of raw carbon nanotubes (1–1 000 mg/L). See colour plate

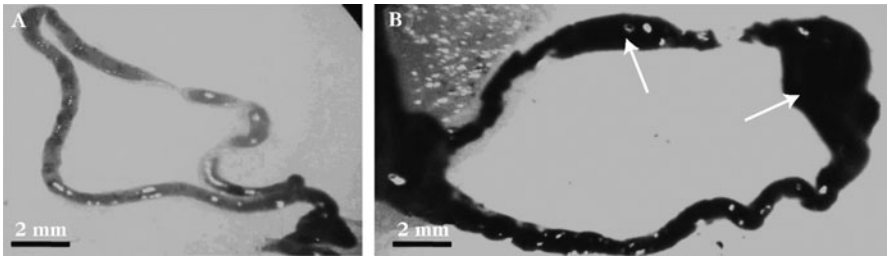

Fig. 14.5. Accumulation of carbon nanotubes in the intestine of xenopus larvae. Taken from [68], with the kind permission of Elsevier. (**A**) Control without carbon nanotubes and (**B**) after 12 days' exposure in the presence of 10 mg/L of carbon nanotubes. Dissection of the xenopus larvae revealed an accumulation of black clusters in the digestive system. The intestines of larvae exposed to carbon nanotubes in the medium have a swollen appearance (*white arrows*) (**B**) compared with the control (**A**). See colour plate

14.3.2 Metal and Metal Oxide Nanoparticles

Two interesting studies investigate exposure of the zebrafish (*Danio rerio*) to copper nanoparticles [72] and exposure of the rainbow trout (*Oncorhynchus mykiss*) to TiO_2 nanoparticles [73]. In both cases, toxicological effects were observed in the gills, with the proliferation of epithelial cells and the development of edemas in the gill filaments. However, the blood parameters of these fish were barely altered. Copper nanoparticles induced a slight vacuolisation of the hepatic cells but did not significantly modify the activity of

plasma alanine aminotransferase which reflects kidney and liver damage. On the other hand, in the case of TiO_2 nanoparticles, an increase in the activity of ATPase with respect to Na^+ and K^+ and a decrease in the concentration of thiobarbituric acid reactive substances attest to possible effects on osmoregulation and oxidative stress in the fish gills. These effects are also observed in the intestine, and to a lesser extent in the brain, but not in the liver.

According to these results, both exposure routes seem to bring about accumulation of nanoparticles, although the direct route predominates over the trophic route. These studies do not inform as to the origins of the nanoparticles observed in the animals' internal organs. They may be carried there via the blood, following translocation through directly exposed gill cells, or after crossing the epithelium of the gastro-intestinal tract following trophic exposure.

14.3.3 Latex Nanoparticles

A model study involving fluorescent latex nanoparticles, which have the advantage of being easily localised within the organism, showed that they distributed themselves in the gills, blood, intestine, liver, and the kidney of the medaka (*Oryzias latipes*) [74]. Some nanoparticles were also visible in the sexual organs and the brain, despite these being protected from xenobiotics by physiological barriers (the blood–brain barrier and the blood–testicle barrier for male sexual organs). The presence of nanoparticles in the exposed fish depends on their size and aggregation state. When the salinity of the biological medium is increased, the nanoparticles aggregate, and this seems to favour their presence in organisms [74]. It seems that nanoparticles with mean hydrodynamic diameter around 470 nm enter the fish more efficiently than smaller or bulkier nanoparticles.

14.3.4 Co-contamination by Nanoparticles and Metals or Organic Pollutants

The toxicity of nanoparticles observed in organisms can be intrinsic (due to the nanoparticle alone) or indirect (nanoparticle as potential carrier) owing to their proven adsorption potential, which means that there may be pollutants at their surface or within their structure whose toxic potential may be induced, repressed, or limited. Indeed, when they come into contact with the environment, the nanoparticles will be in permanent interaction with the other components of the medium, and in particular, the contaminants. In some cases, the nanoparticles may play the role of collector, e.g., by adsorption, for certain molecules, or a masking role wherein they immobilise a non-negligible fraction of the compounds that are potentially reactive for living matter. It is thus impossible a priori to predict the potential biological effects resulting

from the presence of nanoparticles in a complex environment such as a natural aquatic environment.

The literature shows that the adsorption potential of carbon-based nanoparticles like carbon nanotubes has been studied as a way of removing organic and inorganic pollutants from the air, including dioxin [75] and volatile organic compounds [76], but also from water or aqueous solutions, including fluoride [77], 1,2-dichlorobenzene [78], trihalomethanes [79], and divalent metal ions [80, 81]. However, while these studies demonstrate the efficiency of these nanoparticles for adsorbing pollutants, no study to our knowledge indicates whether this adsorption efficiency extends to biological effects, nor whether this adsorption can modify the direct effects of these pollutants in organisms.

It has been shown for carp (*Cyprinus carpio*) that coexposure to cadmium and TiO_2 nanoparticles causes a significant increase in the accumulation of cadmium in these fish [82]]. The overall accumulation of cadmium increases by 146% in the presence of 10 µg/L of nanoparticles, going from 9 to 22 µg of Cd^+ per gram of fish. The cadmium and TiO_2 nanoparticles accumulate mainly in the viscera, gills, skin, scales, and muscles. The bioconcentration factor of cadmium in the gills is 152 in the presence of TiO_2 nanoparticles, as compared with 34 in their absence. The co-accumulation of cadmium and nanoparticles in the viscera occurs either directly through the gill cell barrier, or indirectly by trophic exposure and accumulation in the gastro-intestinal tract, followed in some cases by relocation in the internal organs of the viscera.

14.4 Phytotoxicity and Translocation in Plants

Due to the fact that they do not move, plants have developed particularly effective transport systems enabling them to obtain nutrients (1) from the soil via the root system, and (2) from atmospheric gases via the stalk and leaves. These two routes for supplying nutrients can both be involved in the uptake of pollutants, and hence nanoparticles. Within the plant, nutrients are transported by xylem vessels (raw sap, rich in water and minerals) and the phloem vessels (phloem sap, rich in glucides). The main driving force for the flow of water from the root to the aerial parts of the plant is transpiration. Evaporation of water from the leaves of the plant creates a suction effect which causes a massive uptake of water, nutritive elements, and also potentially nanoparticles via the root system. The accumulation of nanoparticles in the roots and/or the aerial parts of plants may result in transient or long-lasting changes affecting the growth and development of the plant. This property is commonly referred to as phytotoxicity. But apart from their phytotoxicity, a further risk associated with the accumulation of nanoparticles in plants would be the possibility of their entering the food chain, mainly through cultivated plants.

14.4.1 Basic Tools for Studying Nanoparticle Phytotoxicity

At the present time there is little available data regarding the effects of nanoparticles on natural or cultivated plants. The only published studies were carried out under conditions simplified by exposing the plants to nanoparticles dispersed in a hydroponic solution or in water. The guidelines put forward by the Organisation for Economic Cooperation and Development (OECD) for phytotoxicity tests recommend the use of seedling emergence and growth tests [83], growth inhibition assays on duckweed or *Lemna sp.* [84], and vegetative vigour tests [85]. While there are still no guidelines relating to nanoparticle phytotoxicity, these tests could serve as a reference, to a first approximation. In practice the most widely used tests are the germination test, the foliar growth test, and the radicle growth test.

14.4.2 Phytotoxic Effects: Inhibition of Germination and Growth

Germination takes place in several stages, from seed imbibition to the growth of a radicle. The toxic effects of nanoparticles may appear at these two stages. Recent studies have shown that phytotoxicity varies with the type of nanoparticle, its physicochemical characteristics, and the exposed plant. The effects of aluminium (Al), alumina (Al_2O_3), zinc (Zn), and zinc oxide (ZnO) nanoparticles, and multiwall carbon nanotubes have been studied on various plants, including radish, colza, ray grass, lettuce, maize, and cucumber. It seems that the stage most affected is the growth of the radicle rather than imbibition of the seed [86]. Zinc (Zn) and zinc oxide (ZnO) nanoparticles are the most phytotoxic and disturb the root development of all the species studied. Zn nanoparticles delay germination of ray grass while ZnO nanoparticles have the same effect on radish. ZnO nanoparticles also delay the growth of the aerial parts of ray grass [87]. They are detected in the cells of the endodermis and the vascular cylinder of the roots. The nanoparticles presumably cross the epidermis and the root cortex by the apoplastic route, and then the endodermis via the protoplasts, to reach the central part of the root. The hypothesis put forward to explain this is that the nanoparticles create pores in the walls of the plant cells, as they do in bacteria, thereby allowing root uptake [87]. Note that, in this study, the nanoparticles are aggregated in the exposure solution, although some remain isolated. Many nanoparticle clusters are observed at the root surface, and these may mechanically alter their development and restrict the supply of nutrients to the plant.

Another point is that, for a given type of nanoparticle, the surface state governs phytotoxic effects. Al_2O_3 nanoparticles delay root elongation in maize, cucumber, soybean, cabbage, and carrot. On the other hand, when these nanoparticles are first put in contact with phenanthrene, a polycyclic aromatic hydrocarbon, they no longer exhibit phytotoxic effects [88].

14.4.3 Nanoparticle Translocation from Roots to Aerial Parts

Whether they have phytotoxic effects or not, nanoparticles are likely to accumulate in plants and thus be introduced into the food chain. At the present time, the translocation of nanoparticles from the roots to the aerial parts has been reported for several plants, but the phenomenon seems minor. In addition, the concentrations used for laboratory exposure are very high, hence far removed from realistic environmental contamination.

In the study on ray grass described in the last section [87], although ZnO nanoparticles reach the vascular region of the root, few nanoparticles are observed in the aerial parts. The transfer factor (ratio of the zinc concentration in the aerial parts to that in the roots) is very low, viz., 0.01–0.02 compared with 0.03–0.50 in the case of Zn^{2+} ions. On the other hand, another study showed that the aluminium concentration in ray grass leaves increases when the plant is cultivated in soil amended with aluminium nanoparticles [89]. Likewise for the pumpkin, a plant recognised for its ability to absorb a large amount of water, Fe_2O_3 nanoparticles are transferred from the roots to the leaves where they accumulate without inducing phytotoxicity [54]. The ability of the plant to extract water in large amounts from the soil thus seems relevant to the translocation of nanoparticles from the roots to the aerial parts. Presumably the flow of water to regions of transpiration carries the nanoparticles along with it.

14.5 Conclusion

The nanometric size of nanoparticles means they have special properties, quite different from those of the bulk material (see Chap. II). These properties can be exploited to engineer new materials, satisfying constraints of chemical reactivity, electrical conductivity, or optical sensitivity that could not otherwise be achieved. Nanoparticles thus confront us with novel and as yet unknown types of molecular behaviour. However, these new technologies are already being used in many commercial products and will no doubt see a huge development over the coming decades. For this reason, the question of the potential hazards of nanoparticles and the materials incorporating them has already been raised. The stakes are high. The problem is to keep pace with the regulatory measures needed to control the use and dissemination of these objects throughout our environment and our future way of life. The social issues seem enormous, given the vast range of applications expected over the coming years. While the current approach to risk assessment with regard to chemical substances in our environment is organised according to the new European regulation known as Registration, Evaluation and Authorisation of CHemicals (REACH), this does not apply to nanoparticles either directly or simply as-is. So today there is a regulatory vacuum that needs

to be filled by risk assessment methods characterised by and tailored for nanoparticles. But the first step here must be to obtain a better understanding of the potential hazards intrinsic to nanoparticles at all the organisational levels of living systems, from the subcellular level to the global level of the ecosystem.

Obtaining this understanding will be a cross-disciplinary exercise in which ecotoxicology must play a dominant role, investigating the potential hazards of these materials for the integrity of our environment. However, the wide range of nanoparticles, the many different forms in which they may turn up in natural environments, and the broad array of responses of living organisms, will make it a very difficult task to analyse their potential effects in this context. The results reviewed in the present chapter already suggest that a simple, fast, or easily generalisable response with regard to all the relevant organisms will probably prove impossible. Several paradigms can nevertheless be discerned:

- The importance of nanoparticle localisation which will dictate those organs or functions potentially affected by them.
- The importance of intrinsic nanoparticle reactivity, and in particular redox activity.
- Nanoparticle-induced oxidative stress seems to be a frequent issue common to many organisms.
- The toxicity and solubility of the chemical elements, e.g., Cd, Zn, making up nanoparticles.

At the present time, few research groups have yet begun to assess the ecotoxicological risks due to the presence of nanoparticles in the environment. Our current understanding of the probable effects of these nanoparticles, even for living beings taken individually, is incomplete to say the least. So what are the potential effects of such particles in the complex natural environments all around us? How will nanoparticles behave in these environments? What will be their distribution? What hazards do they represent for ecosystems?

The problem of hazards is not the only one that must be solved to achieve the overriding objective of managing the risks associated with the presence of nanoparticles in our environment. Understanding the life cycles of these products and the transfer of degradation products within the various environmental compartments, not to mention their behaviour within complex environmental media, are so many challenges that must be met and scientific bottlenecks that must be overcome in order to better control the effects of these nanomaterials within a framework of sustainable development. The answers to such questions could only be obtained by bring together the skills of many different disciplines in order to characterise the effects (if there are such) within organisms, populations, communities, or indeed the ecosystem as a whole.

Acknowledgements

The authors would like to thank the *Centre national de la recherche scientifique* (CNRS) and the *Commissariat à l'énergie atomique* (CEA) for financing the International Consortium for the Environmental Implications of NanoTechnology (GDRi iCEINT) directed by Jean-Yves Bottero, and also the National Science Foundation and the Environmental Protection Agency in the US for financing the Center for the Environmental Implications of NanoTechnology (CEINT) (EF-0830093) directed by Mark R. Wiesner. Some of the results presented in this chapter were obtained by the combined research group NAUTILE (nanotubes and ecotoxicology) CNRS/UPS/INPT–ARKEMA FR (specific collaboration agreement CNRS No. 48361, director L. Gauthier).

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