Hindawi Publishing Corporation
Journal of Nanotechnology
Volume 2015, Article ID 861092, 10 pages
http://dx.doi.org/10.1155/2015/861092

Review Article

Harmful Effects of Nanoparticles on Animals

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Received 7 July 2015; Revised 29 September 2015; Accepted 30 September 2015

Academic Editor: Zhongwei Zhu

Since several years nanoparticles (NPs) are produced by industries and used in several fields of activities. They are finally found in aquatic and terrestrial environments, where they are ingested by living organisms in which they accumulate, before being eliminated. In organisms, NPs represent foreign elements with their own physicochemical properties due to their small size. So NPs may interfere with the normal physiological mechanisms of the embryos, growing animals, and adults, and it is indispensable to understand their potentially direct or indirect harmful effects on living organisms. It has been already shown that NPs could be toxic to bacteria, algae, invertebrates, and vertebrates. In this review, several examples of recent studies are given. We will examine successively the effects of NPs on terrestrial and semiaquatic and aquatic vertebrate and invertebrate animals.

1. Introduction

Nanoparticles (NPs) are substances the diameter of which does not exceed 100 nm. This size provides them with physical and chemical properties different from materials usually found in environment [1, 2]. Several types of NPs can be distinguished. Natural ones are found in soils, natural waters, or volcanic dust. They are generated by both geological and biological processes. Even when toxic, numerous organisms can adapt and evolve in environments rich in natural NPs [3]. Since several years NPs are produced by industries and used in agriculture, electronics, medicine, pharmacy, cosmetology, and so forth [4]. Studies carried out in USA and Europe showed that Ag-NPs, TiO_2-NPs, and ZnO-NPs from sewage treatment may be toxic for aquatic organisms [5].

NPs are finally found in aquatic and terrestrial environments, where they are ingested by living organisms in which they accumulate, before being eliminated by the immune system or other pathways. In organisms, NPs represent foreign elements with their own physicochemical properties, so they may interfere with the normal physiological mechanisms of the embryos, growing animals, and adults. In embryos, NPs sometimes disrupt the development, bringing on malformations which can be lethal. Not only do the chemicals constituting NPs react according to well-known mechanisms, but the size of NPs itself confers some particular properties which certainly interfere with physical, chemical, and biological activities of the organism [3]. Consequently, due to their small size, NPs can easily penetrate across the cell membrane, avoiding defense mechanisms. So, NPs then migrate into the cell and reach organelles such as mitochondria, modifying the cell metabolism and provoking cell death. If NPs are not enough small to penetrate into the cell, they can interfere with the cell membrane, disrupting the membrane functions such as ion transport or signal transduction. The chemical composition and physical properties of NPs can be cytotoxic. Positive electric charges of NPs can destroy membrane lipid bilayers. Surface coating of NPs can also interfere with cell structure [6]. In addition, NPs effects could be influenced by other substances such as pollutants. NPs can also adsorb materials having harmful effects on the organisms [3].

Since several years, some works were devoted to discover and understand these effects, but most of them still remain to be discovered. So, in front of the omnipresence of NPs today, it became indispensable to understand their potentially direct or indirect harmful effects on living organisms. It has been already shown that NPs could be toxic to bacteria, algae, invertebrates, and fish species, as well as mammals [3]. Today, several living models are used in order to measure the impacts of NPs on the organisms. Studies concerning mammals, such as mouse, or bony fishes, such as the zebrafish, showed that nanoparticles exerted harmful effects on the reproduction and embryonic development [7, 8]. Some experiments using *in vivo* and *in vitro* cultured animal tissues showed that Ag-NPs caused an oxidative stress characterized with well reactive molecules containing free oxygen radicals (reactive oxygen species or ROS), genotoxicity with DNA break, or cell apoptosis [9]. Studies regarding the environmental impact of engineered nanoparticles (ENPs) are hampered by the lack of tools to localize and quantify them in water, sediments, soils, and organisms. Neutron activation has been also used so as to study the effects of Co-NPs in the earthworm *Eisenia fetida*. Using scintillation counting and autoradiography, 4 nm Co-NPs constituting a nanopowder (59 m^2/g) were detected in spermatogenic cells, cocoons, and blood [10].

Nanoparticles such as Ag-, ZnO-, or CuO-NPs are frequently used as bactericides. But after releasing into wastes and environment, toxic effects can also affect nontarget organisms. A review of literature pointed out that such effects were observed in protozoa, nematodes, crustaceans, fish, or cell cultures [11]. Effects were different from that obtained after inhalation [12, 13]. But, presently, the knowledge about the harmful effects of NPs remains such as a patchwork. Consequently, Kahru and Dubourguier proposed several ways to systematize this study field: (1) identify the most harmful effects of NPs on the most sensitive biological groups and (2) gather ecotoxicological information in order to evaluate the risks considering the NP type, such as NiO_2-NPs, ZnO-NPs, CuO-NPs, Ag-NPs, single wall nanotubes (SWNTs) or single walled carbon nanotubes (SWCNT-NTs), multiwall nanotubes (MWCNTs), and C60 fullerene, and experiment in significant organisms such as bacteria, algae, yeast, protozoa, nematodes, earthworms, crustaceans, fish, amphibians, and mammals [14]. Kong et al. also expressed the question of experimental conditions to understand the cytotoxic effects of NPs, considering the choice of target-cell types, the preparation of NP samples, and the type of cytotoxic effects [15].

It is possible to consider the toxicity affecting the organisms living in all the environments (air with inhalation, fresh or marine water, and terrestrial environment). In order to search for toxic effects of NPs, experiments and analysis have been performed in several types of organisms: in protozoa, in numerous invertebrates, in vertebrates, and in adults as well as in embryos.

In this review, several examples of recent studies are given. Some animal models such as rodents, fish, or amphibians were often used. But other animals sometimes less conventional such as sea urchin, earthworms, or still mussels or oysters were also used in well interesting and useful studies. We will examine successively the study of the effects of NPs on terrestrial, semiaquatic, and aquatic vertebrate and invertebrate animals. In the text logic, the animal model and its natural environment were the starting point, but NP types also may be this starting point.

2. Effects of NPs on Terrestrial Animals Species

2.1. In Mammals. Mammals and especially humans are exposed to NPs which can penetrate in the body with inhalation, and a lot of works concerning medical effects have been published [16]. NPs can also penetrate the body in a transcutaneous manner. In all the cases, NPs size permits endocytosis to penetrate a cell and transcytosis to penetrate several cells, one after the other. So, when they are inhaled, NPs are able to reach nervous ends of the olfactive epithelium and then upstream the axons going to the olfactive bulbs in the brain where they affected neurons. In another way, NPs attempted the lungs, then reached blood, and attempted the blood-brain barrier. They also can reach other organs such as bone marrow, lymph nodes, spleen, or heart. It has been shown that NPs can provoke inflammation and both prooxidant and antioxidant activities, oxidative stress, and modification of mitochondrial distribution. These effects were dose-dependent and exerted according to the NP type [16].

Some experiments performed with rats and mice showed true harmful effects of NPs on brain. In the rat, an exposition to Cu-NPs (40 and 60 nm diameter) provoked the proliferation of endothelial cells of brain capillaries when they were administered to low concentrations (about 1.5 μg/mL). Higher concentrations (about 50 μg/mL) induced an increase of prostaglandine E2. Extracellular levels of TNFα and ILβ were significantly high, and the toxicity finally affected the blood-brain barrier [17]. Another study also performed on rat showed that a 24-hour exposition to Ag-NPs measuring 25, 40, or 80 nm affected the blood-brain barrier, inducing a proinflammatory reaction which could develop a brain inflammation accompanied with neurotoxic effects [18]. Cytotoxic effects were induced by the smallest NPs (25 and 40 nm) compared to largest ones (80 nm) [18]. Ag-NPs measuring 7.5 nm originated the emaciation of adult rat, with a decreasing of its locomotor activity [19]. Other works, performed on rat or mouse brain, confirmed the harmful effects of NPs, sometimes at a strong level, on the permeability of blood-brain barrier, thus affecting brain blood fluxes, with consequently the formation of cerebral edema. Morphological effects could provoke neurons injuries, modification of the activity of some glial cells, and the loss of myelin fiber due to the activation of HSP (hotshock proteins). The effects of Cu-NPs and Ag-NPs measuring 50 to 60 nm were more important than Al-NPs measuring also 50 to 60 nm [20]. It was also noticed that the effects were more important in rat than in mouse [20]. Comparable works, performed on the pig, showed pathogenic effects of 25, 40, and 80 nm Ag-NPs, 40 and 60 Cu-NPs, and 3 and 5 nm Au-NPs on

the blood-brain barrier, with an attenuated effect of Au-NPs [21]. In the rat, 45 nm Ag-NPs affected the action of acetylcholine with a production of NO (nitric oxide) inducing hyperactivity of the tracheal smooth muscle [22]. In mouse, injection of 25 nm Ag-NPs (100 or 500 mg/kg) originated an oxidative stress. NP aggregates were observed in spleen red pulp, lungs, and kidneys and in the nasal airway, without any significant morphological variations except in the nasal cavity [23]. The effects of Au-NPs (5 and 15 nm diameter) have been examined on a culture of mouse fibroblasts. NPs penetrated into the fibroblasts where they remained stocked. Only the smallest NPs (5 nm) presented some toxic effects on the shape of cells which became narrow and contracted, disrupting the cytoskeleton actin. Cells exposed to Au-NPs during 72 hours presented a degradation of the heavy chain of clathrin, a cytoskeleton protein [24].

Inversely, 80 to 200 nm Se-NPs used usually to protect the endotracheal tube in some medical conditions against bacterial infection did not present any cytotoxic effect on the fibroblast of rat dermis [25].

2.2. In Earthworms. Engineering nanomaterials (ENMs) frequently used are able to salt out NPs in the environment and more especially within the soil, where they may affect the burrowing organisms. NPs may also be accidently recycled into the soil or carried with the wind. In parallel to their potential toxicity, penetration of NPs into the soil associated with incorporation to the soil solution modifies their bioviability conditions [26].

Earthworms are essential for the incorporation and fragmentation of organic debris, mineralization of organic matter, and recycling of mineral nutriments. Their burrowing activity is also crucial for water filtration and stabilization of erosion effects. In addition, earthworms constitute a large part of the soil biomass (from 60 to 80%) and their biology is widely admitted indicator of the soil health. Thus, some experiments were programmed in order to detect the effects of NPs on their biology. Several methods were used to consider these effects, one of them targeting the apoptotic process. Nevertheless, different earthworm species cannot be considered as equivalent models in these types of experiments. *Lumbricus terrestris* is an anecic worm, living deeply in the ground but eating at the soil surface; *Eisenia fetida* is an epigeic species spending its entire life cycle within the litter of the soil surface, or within compost. Thus, these animals are not submitted to the same toxic influence [26].

2.2.1. Tests in Eisenia Genus. A lot of tests have been performed using *Eisenia fetida*. Some experiments using 20 nm Ag-NPs in suspension in water (1 to 100 mg/mL) showed that the number of apoptotic cells increased with the concentration of Ag-NPs which never aggregated. In *Eisenia fetida*, these NPs were eliminated in 24 hours. The effects of NPs were weaker in the soil than in water. Apoptotic cells were observed in cuticle and intestinal epithelium, which are the parts of body directly exposed to NPs. In consequence, it might be concluded that NPs affected the extended barrier constituted with mucus and antibacterial molecules; they also affected the absorption of nutrients and the immune protection offered by the chloragogenous tissue [26]. *Eisenia fetida* exposed during seven days to 4 nm Co-NPs retained the NPs during eight weeks during which only about 20% of ingested NPs were excreted. These NPs were found in blood and cocoons and spermatogenic cells [10].

TiO_2 enters in the composition of several soil layers and is considered to be inert. This molecule is widely used in many industrial products, such as sunscreens. Toxic effects of TiO_2-NPs may be related to the formation of free radicals with water in the presence of sun light. Their toxic effects were measured on human cells and on vertebrate and invertebrate animals. TiO_2-NPs provoked DNA damage with or without light; it is known that they originated apoptosis in hamster fibroblasts with stretched micronuclei [27, 28]. Independently, the effects of TiO_2-NPs have been also investigated in earthworms. In *Eisenia fetida*, 10 to 20 nm TiO_2-NPs at concentrations higher than 1 g/kg of soil affected enzymatic activities, damaged mitochondria, and induced apoptosis [28]. *Eisenia andrei* and *Eisenia fetida* were exposed to 5, 10, and 20 nm TiO_2-NPs. Several tests concerning mortality, reproduction, avoidance of substances, and growth of juveniles were performed on natural and artificial soils. These soils received TiO_2-NPs in water solution or powder or were amended with TiO_2-NPs. When soil contained 200 to 10,000 mg NPs/kg, no significant effect was observed. Nevertheless, the worms avoided amended soils containing 1000 to 5000 TiO_2-NPs/kg showing that earthworms were able to differentiate soils with 10,000 mg TiO_2-NPs/kg from soils with particles (1 μm or several μm in size). These results showed that earthworms were able to differentiate particles and nanoparticles through a mode of detection which remains still unknown [29].

A study focused on the uptake, excretion, and biodistribution of 4 nm Co-NPs and 20 nm Ag-NPs, soluble Co, and Ag salts in *Eisenia fetida* showed that Co ions and Co-NPs were accumulated and that both Ag ions and Ag-NPs were quickly excreted. Only 32% of accumulated Co ions and Co NPs were excreted. High accumulation of cobalt was observed in blood and digestive tract. Co NPs showed a release of ions while Ag ions and NPs appeared more inert [30]. Some individuals belonging also to the species *Eisenia fetida* were exposed during several weeks to 30–50 nm Ag-NPs coated with polyvinylpyrrolidone (PVP), a hydrophilic substance, or with oleic acid, a hydrophobic substance. Some of them were exposed to $AgNO_3$ (salt). Whatever the source of Ag, worms accumulated Ag with variations according to the concentration. Accumulation was higher with ions ($Ag^+NO_3^-$) than with NPs. No difference of toxicity was observed between 30 and 50 nm NPs coated with PVP or oleic acid [31]. In *Eisenia fetida* also exposed to Ag-HPs coated with PVP or to Ag NO_3 salt, the expression of several genes of oxidative stress, catalase activity, glutathione reductase inhibitors, phosphatase, and Na^+/K^+ ATPase varied again according to the concentration and the duration of exposure to $AgNO_3$ or 30 to 50 nm Ag-NPs [31]. Whatever the form in which Ag was administered, mechanisms of toxicity were comparable [32]. The toxic effects of ZnO-NPs and TiO_2-NPs were also investigated in *Eisenia fetida*. In natural soil, ZnO-NPs were quite toxic in opposition to TiO_2-NPs.

But when earthworms were exposed in a sandy soil, no toxicity was observed whatever the type of NP. After four months of exposure to artificial soils, earthworm reproduction was affected by both types of NPs. However, toxic effects of ZnO-NPs were more important than TiO_2-NPs ones [33, 34]. Measures of bioaccumulation of both labeled C-nanotubes and pyrene in *Eisenia fetida*, considered as a starting point of terrestrial trophic chain, showed bioaccumulation factors with nanotubes two times smaller than those measured with pyrene [35].

Several works concerned the effects of NPs on immune system of the earthworm *Eisenia fetida*. In this species, Ag-NPs were phagocyted and accumulated by coelomocytes. Consequently, an oxidative stress and an important alteration of the immune system were observed. Practically, no Ag^+ ion was salted out from Ag-NPs, these NPs being thus excreted as intact particles. In the same earthworm exposed to Co-NPs, a strong accumulation of Co was observed in both blood and digestive tract. [36].

2.2.2. Tests in Lumbricus Genus. *Lumbricus*, another earthworm genus, was also used to control some biological effects of NPs. In *Lumbricus terrestris* exposed to Ag-NPs, the measure of apoptosis constituted a tool to evaluate the toxicity of Ag-NPs often used in multiple uses such as antimicrobial substance for work clothes [37]. Several experiments showed apoptosis to affect the intestinal epithelium when directly in contact with NPs, especially in typhlosole in which apoptosis affected chloragogenous cells having a function comparable to that of liver in vertebrates or hepatopancreas in mollusks and arthropodia [38].

Again in *Lumbricus terrestris*, when 10×50 nm TiO_2-NPs coated with $Al(OH)_3$ and polydimethyl siloxane (PDMS) were added to food, no increasing of apoptotic cells was observed. If the same NPs were dispersed in the soil, the number of apoptotic cells increased in cuticle, like in animals exposed to NPs in water. Finally, the effects of TiO_2-NPs were not significantly different between water and soil. TiO_2-NPs induced effects weaker than Ag-NPs, and the same tissues were affected in both cases. In animals bred in water, TiO_2-NPs accumulated in gut lumen but never in tissues [27]. In *Lumbricus terrestris*, harmful effects of TiO_2-NPs were observed for lower concentrations (100 mg/Kg) than those in *Eisenia fetida* (1000 mg/kg) [27, 28].

In *Lumbricus rubellus*, a shortness of development and reduction of growth were observed after exposure to C60 fullerene-NPs [36, 39, 40]. Sublethal concentrations of fullerene-NPs decreased the gene expression of HSP. The worms exposed to these nanoparticles presented cuticle damage and pathological sign on epidermis, muscles, and intestinal barrier [36, 39].

Coelomocytes showed immunodepression after exposure to *in vitro* C60 fullerene-NPs [36, 39, 41]. By contrast with *Lumbricus rubellus*, no effects on the antioxidant enzyme both expression and activity and on any other toxical activity were observed in *Eisenia fetida* exposed to C60 fullerene-NPs [36, 39, 41].

3. Effects of NPs on Semiaquatic Animals (Amphibia)

Amphibians are divided into anurans, urodelans, and caecilians. As semiaquatic animals, their larval life is aquatic with adapted anatomy and physiology. They breath with gills, the gut is particularly elongated with loops for an herbivorous diet, the excretory system is adapted to a water life with a strong elimination of water, and they possess tail and caudal fin in order to move by swimming. When they become terrestrial, these animals are submitted to a metamorphosis which is a dramatic period of life. This phase is characterized with important modifications of respiratory system including disappearance of gills and development of lungs. Consequently the circulatory system is obviously modified. Gills disappearing, aortic arches become devoted to both small and large circulations. Kidneys become terrestrial organs of excretion with the possibility to economize water. The tail regresses in anurans but persists in urodelans and sometimes in caecilians. Anterior and posterior legs develop in anurans and urodelans. The animal is submitted to a destruction-reconstruction phase and is then very fragile. A lot of apoptotic cells are observed in tadpoles [42]. Metamorphosis is under thyroid and pituitary control. Thus, amphibians constitute excellent models to appreciate the toxic effects of several substances such as NPs on pituitary and thyroid.

In several works, the effects of NPs on stress and thyroid hormones have been investigated using *in vitro* cultures of caudal fins in *Lithobates catesbeianus* (*Rana catesbeiana*) tadpoles [43, 44]. In this species, the effects of Ag-NPs, Ag-NPs aggregates, and ZnO-NPs on the expression of several genes with or without 3,3′,5′-triiodothyronine (T3) were recorded using qPCR. Results were compared to that obtained for tissues exposed to $AgNO_3$, Ag particles measuring several μm, and Cd telluride particles also measuring several μm. Ag-NPs and small aggregates affected the expression of transcripts linked to T3, with several stress molecules. ZnO-NPs did not perform any effect. Small concentrations of Ag-NPs disrupted T3 signal without inducing any stress [43].

Other experiments concerning the effects of TiO_2-NPs were also performed on *in vitro* cultures of *Lithobates catesbeianus* tadpole fins. The effects were measured through transcription of genes encoding for thyroid hormones receptors (*thra* and *thrb*) implicated in metamorphosis, for larval keratine type I (*rlkI*), stress proteins (*hsp30*), superoxide dismutase (*sod*), and catalase (*cat*). The levels of transcription were not affected whatever the TiO_2 form. Significant effects have been observed after exposure to 20 nm NPs [44]. A study with epithelial strains from *Xenopus laevis* showed a toxicity of Cu under three different forms: Cu^{++}, 6 nm CuO-NPs, and 100 nm aggregates of CuO-NPs. Cytotoxic effects were different according to the kind of compound, with effects depending on the stage of cell cycle. Mitotic cells treated with all the three kinds of substances stopped their division with a significant increasing of the number of apoptotic cells, after a time depending on the kind of Cu substance. After 48 h as well as 6 and 7 days, treatment with 6 nm CuO-NPs or Cu^{++}, respectively, provoked a significant decreasing of cell

proliferation and an increase of apoptosis. Some treatments were identical on differentiated cells, but after a shorter time of exposure [45].

A study about embryonic development of amphibians showed a weak lethal effect on the embryos but early at the highest concentrations. CuO-NPs, TiO_2-NPs, and ZnO-NPs did not provoke embryo death but performed teratogenic effects particularly on intestine when concentrations were higher than 50 mg/L. ZnO-NPs triggered the most severe effects on the intestinal barrier, thus allowing NPs to reach connective tissue. TiO_2-NPs were weakly teratogenic with perhaps hidden physiological effects. Dissolved ions coming from CuO-NPs could be responsible for some effects but not ZnO [46]. The evaluation of toxic effects of $TiSiO_4$-NPs, measuring lesser than 50 nm, showed a mortality lesser than 11% in tadpoles of *Pelophylax perezi* (*Rana perezi*). Inversely, important effects were observed on lactate and melanin, with consequently an increasing of oxidative stress. $TiSiO_4$-NPs certainly performed long-time effects on these animals [47].

Contrary to general harmful effects of NPs, some experiments showed that ZnO-NPs (more than 40 nm) can improve several visual functions. Electroretinograms (ERG) highlighted that these NPs significantly increased the wavelength amplitude in toads adapted to obscurity, when they were exposed to light. In addition, ZnO-NPs improved the visual sensitivity and shortened the time of rhodopsin regeneration, a pigment implicated in the sensitivity to the light [48].

An interesting study was devoted to the trophic transfer of NPs along the trophic chains [49]. Indeed, the authors exposed earthworms *Eisenia fetida* to 10–20 nm Au-NPs. Worms were then eaten by bullfrogs *Lithobates catesbeianus*. Results were compared to bullfrogs directly eaten with the same NPs. Presence of Au-NPs was found in several frog organs: liver, kidney, spleen, muscle stomach, and intestine. They represented about 0.09% when NPs were directly ingested and 0.12% when bullfrogs were fed with earthworms. These results clearly demonstrated the possibility of a trophic transfer of Au-NPs.

4. Effects of NPs on Aquatic Animals

Large quantities of NPs are found in both marine and freshwater aquatic environments. Several works performed on aquatic organisms allowed knowing the effects of these NPs. However, these effects are variable. Recent studies considered NPs as a new nature of pollutant, the effects of which depending on their size, which are still not well-known. Several laboratory studies suggested harmful effects on fishes and invertebrates after exposure. Conventional or lesser conventional animal models were used to assess the nature of these potential effects [4]. Thus, some works focused on bony fishes, more especially the zebrafish *Danio rerio*, or the trout *Onchorynchus mykiss*. Other studies targeted Daphnia and other crustaceans, sea urchin, mollusks, or still plankton.

4.1. In Fish. Young salmons were exposed to Ag-NPs commercial suspensions and prepared ones with $AgNO_3$ reduced with $NaBH_4$. The size of both commercial and prepared colloidal Ag-NPs was set between 3 and 220 nm. Gills of fish accumulated Ag-NPs in all the experiments except when concentration of NPs was the lowest (1 μg/L). Responses were concentration dependent and triggered an increase of stress molecules in gills such as plasmatic glucose or presence of HSP 70. An inhibition of Na^+/K^+ ATPase depending on concentration indicated a default of osmoregulation. At the highest concentrations (100 μg/L), Ag-NPs induced a necrosis of gill lamellae, and 73% of individuals died. These experiments highlighted the impact of NP mode of preparation on toxic effects [50].

On the other hand, NPs may be constituted in a single metal or a single metallic oxide or in several metals. Thus, chemical characteristics and behavior of metallic-NPs are linked with both the dynamics of aggregates and the equilibrium of metal ions, and the acute toxicity of metal-NPs could be linked to the presence of these free metal ions. Biological effects of NPs may be linked to the presence of ion carriers and to the capacity of endocytosis. Some works showed that NPs could induce lethal effects on fishes when concentrations varied from the order of μg/L to mg/L in the environment. In several fish species, metallic-NPs can be more toxic than dissolved forms. In young zebrafish, LC-50 was reached at 0.71 mg/L for Cu-NPs and 1.78 mg/L for dissolved Cu. Unknown mechanisms are certainly implicated. It is obvious that these NPs cause sublethal effects such as breath toxicity, disruption of tissue elements, inhibition of Na^+/K^+ ATPase, and an oxidative stress [51].

Pathologies affecting a lot of organs, such as gills, liver, intestine, and brain, suggested that the response to NPs and metal salts presented some common points. Several effects on development were also observed. Ag-NPs went across the chorion, the envelope surrounding the egg or the developing embryo. Cu-NPs and ZnO-NPs would be more toxic for embryos and juveniles than the corresponding salt. It remains possible that metal-NPs interfered with corresponding stress responses, maybe by stimulation [51]. Mechanical effects of NPs and other nanomaterials on fish began to be studied since the end of 2000s. These studies focused on the mechanisms of absorption, distribution, metabolisation, and excretion of substances in fishes by comparison with other chemical substances. These aspects have been examined for TiO_2-NPs and C60 fullerene in several organs: gills, digestive tract, liver, and adrenals. NPs penetrated the tissues by endocytosis more intensively than with ionic carriers, like for the corresponding metal ion, or by diffusion. In fishes, NPs were rarely excreted by kidneys but could be eliminated with bile. However, data remain still incomplete to date [52].

The effects of ZnO-NPs were investigated on *in vitro* cultures of hepatocyte strains coming from human and fish. These NPs aggregated, which strongly contributed to the toxicity on fish cells. For human cells, the toxicity would be caused by the dissolved salts released by NPs [53]. A comparative study performed on *in vivo* zebrafish and *in vitro* culture of tumoral human hepatocytes Huh7 showed that the Ag-NPs (120 nm in diameter) penetrated into the hepatocytes inducing an oxidative stress characterized with the presence of ROS, INFα expression, and endoplasmic reticulum (ER) disruption. Transcription alterations of *p53*

and *Bax* genes implicated in apoptosis were also observed. Yet, some differences were noted between fish and human hepatocytes, suggesting that there were several mechanisms of stress linked to the modification of ER. Development of zebrafish embryos submitted to Ag-NPs was also affected, inducing malformations [54].

In vitro studies on zebrafish showed that Ag-NPs induced neurotoxic effects different from Ag^+ ones. The effects of Ag-NPs with different sizes (12 and 28 nm), Ag-NPs coated with PVP (45, 63, 65, and 324 nm), and Ag ions were also different on the embryonic development. Ag^+ slowed the development of swim bladder, implicating also several malformations; Ag-NPs effects were lower. Fish behavior was also modified in front of light stimuli: the smallest PVP-coated NPs originated hyperactivity and the largest PVP-coated ones originated a hypoactivity [55]. A deep analysis highlighted that the effects of 1–20 nm Ag-NPs introduced in zebrafish embryos were found in adult nervous system. Ag ions released by Ag-NPs could provide increasing mortality and malformations. Ag-NPs could act on cell differentiation with the intermediary of cell inhibition with acetylcholine [56]. Also in zebrafish embryos Ni-NPs were responsible for mortality and induced malformations. The intestine became very thin on the contact with Ni-NPs, contrarily to the solution of Ni with which no effect was found. Skeletal muscles were affected by 30, 60, and 100 nm Ni-NPs and also by soluble Ni. So, toxicity of 30, 60, or 100 nm Ni-NPs was very little different from soluble nickel one. Inversely, large particle clusters of aggregated 60 nm Ni-NPs with a dendritic structure were particularly toxic on both intestine and skeletal muscles. Thus, the configuration of Ni (NP, aggregates, or ion) was more important than its size [57]. 10 nm Au-NPs moved in all the parts of body in zebrafish embryos. These particles accumulated in aggregates the sizes of which being function of the concentration, but the effects on the development were not proportional to concentrations. Malformations obtained could be due to the random distribution of Au-NPs within cells during development. With the toxicity depending on chemical properties, Au-NPs were lesser toxic than Ag-NPs. Thus, zebrafish embryos can be used as *in vivo* models, especially concerning biocompatibility of materials [58].

Zebrafish is certainly a good model for NPs studies, but other fish species such as trout are also largely used. The effects of TiO_2-NPs and C-NPs were researched in trout hepatocytes. For that, the effects investigated concerned nanomaterials currently used such as C60 fullerene, MWNTs, SWNTs, with or without a define function, and TiO_2-NPs measuring 5 to 200 nm. Experiments showed that these substances were found showing ecotoxicological effects. But several analyses revealed a presence of Co residues, which did not enter in NPs constitution but found their origin in the manufacture proceeding. Trace elements were perhaps responsible for toxic effects. Indeed, mechanisms, such as a facilitated transport linked to the presence of NPs, could increase the toxic response comparatively to only an exposure to the same molecules in aqueous solution [59]. In *Onchorynchus mykiss*, the effects of 87 nm Cu-NPs and $CuSO_4$ were compared. Juvenile trouts were exposed to several concentrations of $CuSO_4$ and Cu-NPs. An accumulation of NPs and $CuSO_4$ was observed in gills, but with different proportions. No accumulation of both products was observed in spleen, brain, or muscle, but an increasing of Cu was noted with both NPs and salts. Whatever the kind of Cu, Cu-NPs were not toxic for hydromineral regulation, but a decrease of Na^+/K^+ ATPase was observed in brain and intestine. Finally, the toxic effects of Cu-NPs seem to be similar to that of $CuSO_4$, but at lower concentrations [60]. Exposition of *Onchorynchus mykiss* to about 20 nm TiO_2-NPs provoked several gills pathologies characterized by edema and thickness of gill lamellae. No perturbation of blood and blood cells was observed. Metal content in tissue was not affected except for Cu and Zn according to the NPs concentration and more especially in the brain. A decrease of Na^+/K^+ ATPase was observed in gills and intestine. Substances such as thiobarbituric acid (TBA) depending on concentration increased in gills and brain. Minor variations of lipids were observed in the liver in which several hepatocytes became apoptotic [61]. Other metabolism variations were also observed [61]. In *in vitro* culture of hepatocytes belonging to several species, Ag-NPs provoked a dose-dependent decrease of the membrane integrity and cell metabolism. Au-NPs increased ROS without any cytotoxic effect. With an addition of carbonic acid, no alteration of the cytotoxic potentiality of NPs and of the capacity to induce ROS was observed. Indeed, on culture of trout hepatocytes, effects of Ag- and Au-NPs were sometimes in opposition [62].

A work appreciated the effect of lesser than 25 nm TiO_2-NPs eaten by *Danio rerio* embryos and larvae on development. In a first experiment, these NPs were ingested when they were added to commercial food, and, in another experiment, fishes were fed with algae previously exposed to TiO_2-NPs. At low concentrations, hatching was premature, and the impact on juvenile animals was not strong. But, after exposure to contaminated food, the digestive physiology was altered after 14 days of exposure [63].

4.2. In Crustaceans. Crustaceans are usually used to appreciate the effects of toxic in polluted environments. So crustaceans such as *Daphnia magna* or *Thamnocephalus platyurus* have been also used to test the potential harmful effects of NPs, sometimes with comparison with corresponding metal toxic.

In *Daphnia magna*, $AgNO_3$ mainly affected the reproduction, and 20 nm Ag-NPs affected growth [64]. Several types of TiO_2-NPs used in industry presented photocatalytic effects on Daphnia and algae; that is, the effects of these substances were different according to the lighting and their nature [65]. Toxic effects of Ag-NPs when Ag is linked to PVP or collargol (Ag-NPs linked to proteins) were very strong for *Daphnia magna* and *Thamnocephalus platyurus*, compared to that of $AgNO_3$. Ag-NPs were 10 times more toxic than $AgNO_3$ in terms of EC50 [66]. Toxic effects of $AgNO_3$, colloidal solutions of 15 nm Ag-NPs, and Ag-NPs suspensions forming 25 to 100 nm aggregates were investigated in *Daphnia magna*. Teratogenic effects and repercussions on swimming were observed according to the concentration and the Ag form. Aggregates of Ag-NPs suspensions were the most toxic; NPs were fixed under the crustacean carapace, affecting directly

the animal swimming [67]. *Daphnia magna* exposed to algae contaminated with TiO_2-NPs showed an accumulation of Ti within the digestive tract. When the exposition was chronic, growth and reproduction decreased, and the toxicological impact of these nanomaterials could be modulated with the age of the NPs [68].

4.3. Other Aquatic Organisms. Since a long time, echinoderms and bivalve mollusks represent good animal models to appreciate the effects of toxics in salt water. So, after having been tested in presence of chemical pollutant, they are also used to understand the effects of NPs.

The effects of metallic oxide-NPs (SnO_2-NPs, CeO_2-NPs, and Fe_3O_4-NPs) were tested on immune cells in the sea urchin *Paracentrotus lividus*. NPs were found in coelomocytes, which constitute the immune cells of sea urchins. These cells presented a well reduced cholinesterasic activity, an underregulation of stress proteins, and variable morphological alterations of ER lysosomes [69].

In bivalve mollusks, immune function is targeted by NPs. The first essay showing immunomodulation induced by NPs in bivalves was performed in *Elliptio complanata* in 2008 [70]. Since this time, various works focusing on the effects of NPs on the immune system were performed with the mussel *Mytilus galloprovincialis*. Several NPs types were absorbed by the hemocytes, affecting several of their parameters, such as lysosomal activity, phagocytosis capacity, production of free radicals, or an increase of the apoptotic activity. A lot of NPs exerted an effect on immune system. In the oyster *Crassostrea gigas*, the exposition to several kinds of NPs also provoked effects on oxidative stress and lysosomal activity and apoptosis, more especially into the gills and digestive gland, and on the embryonic development. Accumulation of NPs was also observed in several tissues depending on the nature of NPs. In *Mytilus edulis*, TiO_2-NPs presented some *in vivo* immunomodulator effects. TiO_2-NPs were aggregated in water and then could paste on the gills, join directly the digestive gland, and penetrate cells with consequent lysosomal perturbation and modification of the expression of genes implicated in the antioxidant stress and in the immune response, especially lysozymes and antimicrobial peptides. NPs may be carried out of the digestive tract via the hemolymph and through hemocytes in which they induce functional modifications, in particular lysosomal function, phagocytosis, production of NO and ROS implicated in oxidative stress, with the apoptosis initiation, as well as in membrane and mitochondria. Significant variations of expression of antioxidant and immunity genes were also observed. Yet, TiO_2-NPs originated an underregulation of immune genes in the digestive gland and hyperregulation of these same gens in hemocytes [36].

Using metatranscriptomic analysis, that is, the analysis of RNA quantities extracted from a planktonic sample, consisting of a community of aquatic small organisms, some toxic effects of Ag^+ ions and free 6 nm Ag-NPs affecting the composition of planktonic sample and the metabolism of its members were searched. For that, authors used a metatranscripnomic analysis, that is, the analysis of RNA quantities extracted from the planktonic sample. Compared to controls, significant results were obtained after treatment with Ag-NO_3 (5 μg/L) but not with Ag-NPs. These results showed that ionic Ag was more toxic than Ag-NPs [71].

5. Conclusions

NPs are more and more used and they enter in the composition of different substances such as SIM cards of cell phones or sunscreens. After having consumed these products, free NPs can be released in the environment and in the air as well as in water or soils. Thus, NPs are new potentially toxic substances. They can induce effects directly linked to their chemical composition like a lot of other industrial products including pesticides, but, in addition, their own nature with generally a size smaller than 100 nm, intermediary between particles (several μm and more), and molecules and ions confer them particular properties which remain to be discovered. The effects of NPs begin to be known, but a great effort of investigation remains to be done, in particular on organisms used as bioindicator, and a characterization of highly toxic NPs remains to be established. To date, numerous works concerning NPs focus on the understanding of the effects of NPs on organisms living in soil, such as mammals or earthworms, semiaquatic organisms, such as amphibians, or freshwater or marine organisms, such as fishes, invertebrates, algae, and plankton.

A new research domain is now developing in order to measure the potential harmful effects of this useful NPs and to allow an efficient protection of the environment. Interdisciplinary investigations are, more than in other domains, certainly essential [72]. In addition, these studies will allow one to find new biological mechanisms, still unsuspected, which will give new knowledge about cell life.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Acknowledgment

The authors are grateful to Philomène Cuzin who helped them in bibliographic search.

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