Chapter 5

Environmental Fate of Zinc Oxide Nanoparticles: Risks and Benefits

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Abstract

Zinc oxide nanoparticles (ZnO-NPs) are among nanoscale materials displaying exponentially growing production due to their applications in the field of cosmetology, medicine, as antibacterial agent and catalyst. The ZnO nanomaterials release into the aquatic ecosystems through domestic and industrial wastewaters has the potential to induce pernicious effects on fish and other organisms. Increasing concerns on the environmental hazard to aquatic biota have been highlighted by the toxic potential of some metal-based nanomaterials. Several characteristics of ZnO-NPs (e.g. size, shape, surface charge and agglomeration state) play a central role in biological effects such as genotoxic, mutagenic or cytotoxic effects. Overall, Zn bioaccumulation, histopathological, and hematological changes with oxidative and cellular stress have been reported in ZnO-NPs exposed animals.

This chapter provides an overview on applications of ZnO-NPs followed by a brief outline on methods of synthesis and characterization, and the current knowledge on the ZnO-NPs interaction with fish as they are valuable models in ecotoxicology, sensitive to many contaminants, representing a potential source of food for humans. This chapter intends to provide information for a critical overview of the pros and cons of using these particles, factors influencing their effects, and potential human health implications.

Keywords: zinc oxide nanoparticles (ZnO-NPs), nano-ecotoxicology, fish, human health

1. Introduction

1.1. Applications of zinc oxide nanoparticles

Zinc oxide nanoparticles (ZnO-NPs) are commonly used in several domains of human activity such as cosmetics, paints, optoelectronics, and pharmaceuticals, due to their low cost and interesting properties (e.g., conductivity, chemical stability, catalytic properties, photonics and optoelectronics, antibacterial, antifungal, and UV filtering properties). ZnO-NPs are highly used in the cosmetic industry, typically in sunscreens and facial creams [1]. In the biomedical field, ZnO-NPs have been applied in cell imaging [2, 3], drug delivery, and have demonstrated promising results in cancer research (for review see [4, 5]).

ZnO-NPs have shown to decrease the viability of cultured cell lines derived from human cancers. ZnO-NPs induced a 50% reduction in cell viability in MCF7 (breast cancer) and A549 (lung cancer) cell cultures, at a very low concentration (31.2 $\mu g\ ml^{-1}$), with size-dependent effectiveness [6]. A high toxicity on T98G (brain cancer) cells, moderate toxicity on KB (skin cancer) cells, and low toxicity on normal human HEK cells have also been reported [7]. ZnO-NPs have been proposed as genotoxic since they induced micronucleus in those cells. Apoptosis and intracellular production of reactive oxygen species (ROS) have been reported on melanoma cancer cells after exposure to different doses of ZnO-NPs [8]. These nanomaterials also exhibited activity against HepG2 (liver cells) cells depending on the dose [9]. A time-dependent reduction in the viability of murine cancer cells after exposure of ZnO-NPs was recently documented by [10]. ZnO-NPs strong protein adsorption properties may also lead to its use in other biomedical applications. ZnO-NPs may be used to modulate metabolism and cellular responses, and have been proven useful for the detection of low levels of biomarkers (e.g., proteins/peptides [11]). ZnO-NPs have shown promising results as cholesterol sensors, controlling diabetes and hyperglycemia, modulation of some allergic reactions, via inhibition of mast cell degranulation [12] as well in tissue engineering scaffolds to enhance angiogenesis [13].

As for other nanoparticles, ZnO-NPs may also be toxic for some microorganisms, making them potential antibacterial, antifungal, and antiviral agents. This is an important feature of these nanomaterials considering the increasing concerns related to the proliferation of pathogenic microorganisms that are multiresistant to conventional antibiotics. ZnO-NPs may interact with the bacterial surface and/or with the bacterial core, exhibiting different bactericidal mechanisms. Antimicrobial properties of ZnO-NPs have been demonstrated on bacteria such as *Bacillus subtilis, Escherichia coli* and *Pseudomonas fluorescens* [14], *Staphylococcus aureus* and *Salmonella typhimurium*, as well as on the fungi *Aspergillus flavus* and *Aspergillus fumigatus* [15]. In a study aiming to evaluate the immunological and antibacterial mechanisms of ZnO-NPs against human pathogens, Rashmirekha et al. [16] reported a higher effect of ZnO-NPs against *Staphylococcus aureus* when compared to *Mycobacterium bovis-BCG*. ZnO-NPs were able to disrupt bacterial cell membrane integrity, decrease cell surface hydrophobicity, and downregulate the transcription of oxidative stress-resistance genes in bacteria. The intradermal administration of ZnO-NPs reduced the skin infection, bacterial load, and inflammation in mice. ZnO-NPs treatment also increased the bacterial killing by inducing ROS. Virostatic

potential of micro-/nanofilopodia-like ZnO structures against herpes simplex virus-1 was reported by Mishra and colleagues [17]. Antoine and colleagues [18] synthesized 200 nm to 1 μm ZnO tetrapod-like structures, by flame transport method, to check the antiviral properties of ZnO micro- and nanostructures against HSV-2. ZnO tetrapods blocked the HSV-2 entry into the target cells and stopped the virus dispersal among already infected cells. The prophylactic treatment showed a decrease in HSV-2 internalization in both UV-treated and in nontreated conditions. The decreased internalization supports the preventive function of ZnO tetrapod nanoparticles against the ability of viruses to enter into the susceptible cells. ZnO tetrapods treatment decreased the cell fusion and syncytial formation of CHO-K1 cells.

ZnO-NPs synthesized by Nohynek et al. (2007), using wet chemical methods, revealed a high antibacterial activity, due to its inherent ability to absorb UV irradiation and optical transparency. This makes ZnO-NPs an important compound for the cosmetic industry, namely in formulations for sunscreens and facial creams [1]. The antibacterial activities of ZnO-NPs, as mentioned above, significantly contribute to its value in food processing industry, as a potent sanitizing agent for disinfecting and sterilizing food industry equipment and containers against foodborne pathogenic bacteria. ZnO-NPs are able to disrupt *E. coli* and *S. aureus* cell membrane causing cytoplasmic leakage and able to inhibit and kill the foodborne pathogens [19].

At industrial level, ZnO-NPs have various applications in catalysis and electronics [20]. ZnO-NPs can be used in infrared and chemical sensors, in the manufacture of rubber and cigarettes (used as filter) and preparation of creams and ointments used to treat skin diseases. The range of possible applications of ZnO-NPs also includes agriculture. Studies have shown potential beneficial effects of ZnO-NPs on seed germination, water purification, and soil remediation. Peanut seeds treated with 25 nm ZnO-NPs (1000 ppm) displayed high germination, seeding vigor, and plant growth [21], while decreased ryegrass germination has been reported after ZnO-NPs exposure [22]. Furthermore, the potential of ZnO-NPs to reduce microbial biomass and diversity [23] must also be taken into account.

1.2. Synthesis and characterization of ZnO nanoparticles

ZnO-based materials have been the subject of several reviews in the past years. A detailed survey on the literature concerning the synthesis and properties of nanosized ZnO can be found elsewhere [24]. ZnO is an inorganic crystalline compound with a band-gap energy located in the UV region that widens as the size of the particles decreases below a threshold of a few nanometers. Both in the bulk form and as a nanoscale material, ZnO is an important material for several applications including in electronics and optical devices [24]. ZnO-NPs may be synthesized by a variety of methods, selected based on the desired application, morphology, and size. Chemical and physical parameters (e.g., solvent type, precursors, pH, and temperature) are of high relevance in the synthesis protocols. A variety of shapes (nanorods, nanosphere, nanotubes, nanowires, nanoneedles, nanorings, spirals, drums, polyhedrons, disks, flowers, stars, boxes, and plates) may be produced, each displaying morphological-dependent physicochemical properties [25] that allow the exploitation of a variety of applications.

Some preparative methods include chemical vapor deposition, precipitation in aqueous solution, hydrothermal synthesis, sol-gel method, and synthesis using microemulsions and mechanochemical processes. These methods allow the production of particles differing in shape and size. Some available reviews present a thorough explanation of the principles and techniques involved in the different procedures [26]. Briefly, the mechanochemical method is based on high-energy dry milling; the controlled precipitation method involves hydrolysis of a Zn(II) solution, in conditions that limit uncontrolled growth of particles, eventually followed by a thermal treatment to improve crystallinity; the hydrothermal method is a simple and environmentally friendly technique that involves the thermal treatment of Zn(II) aqueous solutions under auto-generated pressure, by using an autoclave as the reaction vessel. ZnO-NPs have also been prepared using a Zn(II) precursor in the presence of plant extracts [27, 28] as cost-effective approaches, with promising results in terms of bioapplications. Selected examples of synthesis methods for ZnO-NPs are presented below.

ZnO-NPs were synthesized by Aneesh et al. [29] by hydrothermal treatment of $Zn(CH_3COO_2)$ $2H_2O$ (0.1 M) solutions. ZnO-NPs were prepared by varying the growth temperature and concentration of the Zn(II) precursor. X-ray diffraction (XRD) performed on powdered samples revealed nanoparticles of *wurtzite*-type structure. This synthesis yielded particles of sizes between 7 and 24 nm. Overall, particle size increased with growth temperature and decreased with concentration of precursor. Ramimoghadam et al. [30] synthesized ZnO-NPs also by a hydrothermal method, using palm olein as biotemplate. Different morphologies including flake-flower and 3D star-like structure were obtained. The concentration of palm olein has an effective role on observed morphological changes of the synthesized nanoparticles. These changes are possibly due to the reaction between carboxylic groups of palm olein and hydroxyl groups at the surface of ZnO. The biotemplates could be also used to modify the surface properties of ZnO-NPs.

Soni and Koser [31] used a hydrolysis method for the synthesis of ZnO-NPs, with different concentrations of a surface-protecting agent (thioglycerol). UV-VIS spectroscopy revealed blueshifts in the absorption bands of the samples, as compared to the spectrum of typical bulk ZnO, as an indication for the presence of nanosized ZnO. The absorption band edge was observed in the UV region at wavelength 365, 362, and 364 nm for ZnO-NPs synthesized using 0.12, 0.3, and 0.5 ml of capping agent, respectively. The samples were composed of particles with average sizes between 3.5 and 3.9 nm, depending on the amount of molar concentration of the capping agent. Increasing the concentration of capping agent, the average particle size decreased and the respective band gap widens due to quantum size effects [24]. Also using a hydrolysis method, Wang et al. [32] have synthesized nanometric ZnO using cetyltrimethylammonium bromide (CTAB) as surfactant. These authors reported high-crystalline nanoparticles of 50 nm average diameter. CTAB affected the process of nucleation and growth of crystallites during the synthesis also preventing the formation of ZnO agglomerates.

Giri et al. [33] synthesized hexagonal ZnO-NPs and nanorods by low-temperature oxidation of metallic Zn powder in the presence of acetic acid and trifluoroacetic acid. The final colorless powders were a first indication for the presence of ZnO. In this method, acetic acid and

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trifluoroacetic acid induced the growth of hexagonal-type ZnO-NPs and ZnO nanorods, respectively, whose crystalline nature was confirmed by XRD. Transmission electron microscopy (TEM) and scanning electron microscopy (SEM) images displayed hexagonal cross section of the nanoparticles and nanorods. The samples showed strong UV absorption peaked at 378 nm. A green synthesis method was used by Oudhia et al. [34] for ZnO nanotubes aiming biomedical application. For this purpose, neem leaf extract as biotemplate was used. The XRD pattern indicated the *wurtzite*-type structure of ZnO-NPs. The average crystalline size of the synthesized nanotubes was estimated as 25 nm by using Debye-Scherrer equation applied to the XRD patterns of the samples.

Barreto et al. [35] synthesized ZnO-NPs by microwave-assisted method and checked the effect of precursor reagent, temperature, irradiation time, and additives on the morphology of synthesized nanoparticles by using ($Zn(NO_3)_26H_2O$, $Zn(CH_3COO_2)2H_2O$, or $ZnCl_2$) as precursor. Radiation temperature of 80–140°C and increase in the irradiation time give high purity and homogenous size and shape of nanoparticles. The final pH is another important variable which causes significant changes in the morphology of the final particles. The addition of the anionic surfactant (AOT, sodium di-2-ethylhexyl sulfosuccinate) to the reaction medium allowed the synthesis of smaller particles. Kumar and Rani [36] synthesized ZnO-NPs by using microemulsions as nanoreactor for the synthesis of ZnO using $ZnSO_4$ salt. The stable reverse micelle microemulsion was prepared by mixing a nonionic surfactant, Triton X-100, PVP (used as co-stabilizing agent), cyclohexane, and distilled water. XRD diffraction analysis shows the typical hexagonal *wurtzite*-type structure of ZnO. TEM revealed nanoparticles of 10–12 nm in average size and rod shaped, and UV-VIS spectroscopy was use to estimate the optical band gap of the samples.

Tsuzuki and Cormick [37] synthesized nanocrystallites of ZnO of 26 nm size by a mechanochemical method using $ZnCO_3$ as the precursor. It was observed that a milling time of 4 h was enough for the synthesis of ZnO-NPs. Song et al. [2] synthesized ellipsoidal ZnO-NPs with high crystal quality by another mechanochemical method. It was observed that depending on the solvent used, the ZnO-NPs remained dispersed with a mean diameter of 21 nm (nonpolar solvents), whereas in more polar solvents the nanoparticles gradually aggregated to a diameter of about 200 nm. Photoluminescence spectra of ZnO-NPs have been reported.

The routine methodologies used to characterize ZnO-NPs (colloids and powders) are those commonly applied to characterize other types of nanoparticles and include dynamic light scattering (DLS) techniques, UV-VIS absorption spectroscopy, selected area electron diffraction (SAED), and powder X-ray diffraction (XRD). The size and shape of the nanoparticles are directly analyzed by using microscopy (transmission electron microscopy—TEM, atomic force microscopy—AFM, or scanning electron—SEM). The crystalline phase (typically *wurtzite* type) can be identified by using XRD and the surface charge of the colloidal NPs through zeta potential measurements. A number of processes involving NPs are mediated by the surface. Although its characterization is not straightforward, important information can be acquired by using Fourier transform infrared spectroscopy (FT-IR), Raman spectroscopy, and X-ray photoelectron spectroscopy (XPS) among other techniques.

2. Nanoparticles in aquatic systems

Increase in production and applications of ZnO-NPs is expected to result in its increased release into the environment. Ultimately, the aquatic ecosystems will probably be the main recipients, mainly as a result of industrial and domestic wastewaters [38]. As one of the most produced nanoparticles in the European Union, with an estimated production of around 1600 t [39], ZnO-NPs environmental release may occur as early as their production, production of products containing nanoparticles, during their use and end of life of those products. Despite the knowledge that nanoparticles are increasingly being used in different fields of human activity, the quantification of their release in the environment, at any given time, is quite challenging due to the limited data on their current and expected prevalence in commercial products [40–42]. The technical difficulties associated with quantification of ZnO-NPs levels in the environment led to the need to predict environmental concentrations based on market penetration of nanomaterials, known usage of the products as well as fate/behavior. According to the available data, the theoretically predicted average environmental concentration of ZnO-NPs in European surface waters is 0.09 μg L^{-1} (with 85% confidence intervals: 0.05–0.29) [39]. No standards have yet been established for permissible levels of nanoparticles in the environment. Nonetheless, in addition to dose, physicochemical properties of nanoparticles (e.g., size, shape, chemical composition, aggregation) as well as ionic strength and pH of receiving media play determinant roles on their behavior, bioavailability, and biological effects of nanoparticles [43]. Once released into the environment, ZnO-NPs may display different behaviors. Nanoparticles in the environment may stay in suspensions as individual particles; dissolve; aggregate; form larger particles and ultimately sediment; adsorb onto water constituents (e.g., dissolved organic matter); transform chemically (e.g., due to redox reactions) or biologically (e.g., in the presence of microorganisms) in the marine environment [44]. Once in the environment, most ZnO-NPs are likely to precipitate due to its poor colloidal stability [45]. The available studies indicate that aggregation of ZnO-NPs, as for other nanoparticles, increases with ionic strength. In high ionic strength environments, reduction in electrostatic repulsion forces between the nanoparticles occurs, promoting aggregation and sedimentation. However, the presence of natural substances such as humic acids may help to steric and electrostatic stabilization of ZnO-NPs, aiding in their transport, mobility, and dispersion [46]. Other highly relevant alterations that may occur in the environment and lead to toxic effects of ZnO-NPs are the dissolution and redox transformations. ZnO-NPs may dissolve, releasing Zn ions which may induce toxic effects [47], with reported faster dissolutions at smaller sizes [48]. Redox reactions on ZnO-NPs' surface may lead to the production of ROS, which are able to oxidize organic compounds and lead to oxidative stress. Thus, for risk assessments of nanoparticles such as ZnO-NPs, different factors have to be taken into account based on the wide variety of reactivities and properties of a particular type of nanoparticle [49].

The available studies on the behavior of ZnO-NPs in water systems have been performed under laboratory conditions, focusing mostly on freshwater. Available data indicate that dissolution is dependent on concentration, with the lowest dissolution percentage at the highest ZnO concentrations [50].

3. Interaction of nanoparticles with aquatic organisms

Among aquatic species, fish have been considered as the ideal sentinels to detect toxicological effects, due to their wide distribution, known physiology, and sensitivity to exposure to contaminants via food or water. In this chapter, some of the available studies with fish at different life stages (embryos, juveniles, and adults) are presented in **Table 1**.

Primary particles	Study aim	Test organisms	Exposure protocol	Assessed endpoints	Main effects	References
Size: <100 nm	To assess developmental toxicity, oxidative stress, and DNA damage	*Danio rerio* (Zebrafish)	Waterborne exposure to 1, 5, 10, 20, 50, and 100 mg L^{-1} up to 144 h postfertilization	Embryo/larvae survival, hatching, and malformation rates; ROS measurement; DNA damage; antioxidant enzymes; lipid peroxidation mRNA levels of genes encoding antioxidant proteins and regulation of ROS production	Reduction of hatching rate and induction of malformations; ROS generation; DNA damage	[51]
Size: 25 nm	To identify potential mechanisms of cardiorespiratory effects of ZnO-NPs and characterize the ecophysiological importance of ZnO-NPs toxicity	*Catostomus commersonii* (White sucker)	Waterborne exposure for 25 h to 10 mg L^{-1}; for 15 and 30 h to 1 mg L^{-1}	Gill morphology, cardiorespiratory function	Damage to the gill epithelium; decreased heart acetylcholinesterase activity; reduction of aerobic capacity	[52]
Size: 25 nm	To assess the effects of ZnO-NPs exposure in the liver of a freshwater fish	*Catostomus commersonii* (White sucker)	Waterborne exposure for 29.5 h to 1 mg L^{-1}	Biomarkers of oxidative stress and antioxidant response	Changes in levels of hepatic enzyme activities, antioxidants, and	[53]

Primary particles	Study aim	Test organisms	Exposure protocol	Assessed endpoints	Main effects	References
					lipid peroxidation products	
Size: 20 nm	To study stress proteomic responses	*Oryzias melastigma* (Medaka fish)	Waterborne exposure to 4 and 40 mg L^{-1} for 96 h	Molecular biomarkers (SOD, MT and HSP70)	Upregulation of HSP70	[54]
Size: 30 nm	To evaluate bioaccumulation and subacute toxicity compared with bulk particles	*Cyprinus carpio* (Juvenile carp)	Waterborne exposure (5–1000 mg L^{-}) for 30 days	Accumulation in different tissues (gill, liver, intestine, muscle, and brain); histopathological changes; enzyme activities (e.g., Na^{+}/K^{+}ATPase, and SOD) nonenzymatic antioxidants and oxidative damage	Higher bioaccumulation, oxidative effect, and histopathological changes than bulk ZnO	[55]
Size: 30 nm	To study Zn accumulation and the mechanism of hepatic detoxification in comparison with bulk ZnO and Zn^{2+}	*Carassius auratus* (Gold fish)	Waterborne exposure for 30 days to 2 mg L^{-1}	Zn concentration and its subcellular distribution gills, liver, gut, and muscle	Tissue-specific bioaccumulation dependent on the exposed material	[56]
Size: 15–350 nm	To compare the effects of zinc compounds in the form of nano-, microparticles and ions	*Brachydanio* (Danio) *rerio* (zebrafish)	Waterborne exposure to 0.2, 2, 10 and 20 mg L^{-1} for 120 h	Embryo/larvae survival, hatching, and malformation rates	Retardation of hatching and deviations in embryonic development; adherence of the particles on the egg surface at high ZnO-NPs concentrations	[57]
Size: 50–60 nm	To compare the effect of different	*Ctenopharyngodon idella* (Grass carp)	Diet exposure (4% body weight	Lethality, growth performance, food	Improvement in growth performance	[58]

Primary particles	Study aim	Test organisms	Exposure protocol	Assessed endpoints	Main effects	References
	forms zinc (ZnO, $ZnSO_4$, ZnO-NPs) on growth and hematological indices		for 90 days	conversion ratio and efficiency, hepatosomatic index; blood parameters	and red blood cell count with supplementation with ZnO-NPs compared to oxide and sulfate form	
Size: <100 nm	To evaluate the acute toxicity and hematological effects	*Oreochromis mossambicus* (Tilapia)	Waterborne exposure to 30, 50 and 70 mg L^{-1} for 96 h	Blood parameters	96 h LC_{50} of ZnO-NPs between 100 and 110 mg L^{-1}; chromosomal damages, changes in blood parameters	[59]
Size: <100 nm	To evaluate the long-term effects of 3 sublethal concentrations	*Cyprinus carpio* (Carp)	Exposure for 21 days of three sublethal concentrations from the 96 h LC50 value (4.897 mg L^{-1})	Histopathological changes in the liver	Dose-dependent histological alterations generally associated with the response of hepatocytes to toxicants	[60]
Size: <100 nm	To study the acute toxicity (LC_{50}) and gill histopathology	*Cyprinus carpio* (Carp)	Waterborne exposure for 96 h to 2, 4, 8, and 16 mg L^{-1}; waterborne exposure to sublethal concentrations for 21 days	Lethality; gill histopathology	A 96 h LC_{50} of 4.897 mg L^{-1}. Histopathological alterations in the gills at sublethal concentrations at higher concentrations	[61]
Size: 30 nm	To quantify the trophic transfer of ZnO-NPs by feeding *D. rerio* with *D. magna* exposed to ZnO-NPs prior to the feeding experiments	*Danio rerio* (Zebra fish) and *Daphnia magna* (Water flea)	Fish exposure through diet for 14 days to *D. magna* (4–5 days old) preexposed to ZnO-NPs and ZnO-octyl NP (1.0 mg Zn L^{-} for 24 h)	Zn content on fish body burden	Uptake of both ZnO-NPs and ZnO-octyl NP with values exceeding by tenfold the levels obtained through aqueous exposure in other studies	[62]

Primary particles	Study aim	Test organisms	Exposure protocol	Assessed endpoints	Main effects	References
Size: 50 nm	To determine lethal concentration and histopathological lesions	*Cyprinus carpio* (Carp)	Waterborne exposure for 24 h to 10–50 mg L^{-1}	Lethal concentrations and histopathological alterations in gills, liver, kidney, and pancreas	Histopathological lesions in the kidney and gills; necrosis in liver, and hemorrhage in pancreas	[63]

Abbreviations: HSP70: heat shock protein 70; MT: metallothionein; NPs: nanoparticles; ROS: reactive oxygen species; SOD: superoxide dismutase.

Table 1. Examples of research studies on the effects of ZnO-NPs on fish.

As shown in **Table 1**, examples of recent research on ZnO-NPs interaction with fish have essentially focused on freshwater organisms such as *Cyprinus carpio, Oreochromis mossambicus, Tilapia zillii, Oreochromis niloticus, Ctenopharyngodon idella, Carassius auratus*, and *Danio rerio*. Several techniques and approaches were used in those studies including, mainly, analytical methods for detection of metal in tissues as well histopathological, hematological, and oxidative stress endpoints to study the effects of ZnO-NPs, and histopathological, hematological, and oxidative stress as relevant endpoints to evaluate the possible interrelationships.

The effects of ZnO-NPs have been associated with two main mechanisms: oxidative stress and nanoparticle-protein interactions [52]. Although some of the effects have been associated with the release of Zn ions as a result of particle dissolution, not all studies confirm that the toxic effects are due to dissolution. Higher bioaccumulation and effects have been observed after exposure to nanoparticle form when compared to bulk form, confirming the complexity and specificity of mechanisms associated with experimental conditions. Nonetheless, it is clear that more studies are needed, with lower concentrations and longer exposure periods, representing a more environmentally relevant scenario. There is also a clear need for studies on combined effects of abiotic factors variation (e.g., temperature, salinity, UV radiation), classical environmental contaminants (e.g., organic compounds, pesticide and pharmaceuticals), and ZnO-NPs, to represent an environmentally relevant scenario. There is also a need to assess the effects of ZnO-NPs in organisms present in high ionic strength environment (e.g., estuaries and marine environments). The information obtained in trophic transfer studies supports the concerns of potential effects of nanoparticles, to higher trophic levels in which humans may also be a target.

Considering the tested endpoints, the available data revealed that histological and hematological responses occur. After ZnO-NPs exposure, both juvenile and/or adult fish have shown its accumulation on tissues such as brain, liver, muscle, and gills [55, 56]. Hao et al. [55] reported ZnO-NPs accumulation on tissues of juvenile carp (*Cyprinus carpio*), and cellular oxidative stress response was denoted as the main toxic mechanism of nano-ZnO. The bioaccumulative

behavior of ZnO-NPs and their potential trophic transfer from *Daphnia magna* to zebrafish (*Danio rerio*) was reported by Skjolding et al. [62].

Several parameters such as hematological approaches have been used for the monitoring of health conditions of fish [58, 59]. Blood parameters as red and white blood cells count, hemoglobin content, and hematocrit value, and red blood cell indices are usually assessed on some toxicological studies. Although enhancement on red blood cell count was reported on the grass carp *Ctenopharyngodon idella* with supplementation of NPs from [58], deleterious changes on blood parameters were documented on Tilapia *Oreochromis mossambicus* exposed for 96 h to ZnO-NPs (100–110 mg L^{-1}) [59].

Additionally, histology is also an important tool for the evaluation of fish health, showing the initial signs of lesions not easily noticeable during the macroscopic observation of tissues and organs [55]. Gills are vital organs for respiration and osmoregulation. Gill histopathological alterations can be considered as indicators of the ZnO-NPs–induced toxicity in the common carp [60]. Among other relevant organs, hepatic histopathological changes were documented as a result of exposure to ZnO-NPs [55, 61, 63].

As reported in **Table 1**, the ecotoxicological impacts of ZnO-NPs on fish include in most studies histopathological, hematological, and oxidative stress under different doses, protocols, and exposure [53, 55]. Tomilina et al. [57] reported decreased motility and increased curvature of tail in *Brachydanio* (*Danio*) *rerio* embryos exposed for 24 h to 0.01 mg L^{-1} ZnO-NPs and affected dynamics of hatching of *Brachydanio* (*Danio*) *rerio* prelarvae at higher concentrations.

In contrast, scarce data have demonstrated positive biological effects of ZnO-NPs. Reports on fish (*C. idella*) growth performance improvement after 90 days ZnO-NPs exposure, through diet, compared to oxide and sulfate form of Zn were recently published [58], suggesting a potential application of these particles on aquaculture.

4. Potential impacts on public health

The hazard potential of ZnO-NPs to humans in comparison with microparticulate and dissolved Zn has been evaluated in the context of major accident prevention [64]. Based on the analysis of endpoints of subtoxic events (inflammation, oxidative stress response, or gene expression profiling) over different timescales, the authors concluded that the hazard potentials of nano- and microparticles of ZnO are identical during acute (medium) and chronic (low) toxicity. Inhalation of ZnO fume and dust over the permissible exposure limit of 5 mg m^{-3} appears to be the riskiest toxic exposure, since Zn fume fever could be lethal [65]. ZnO is quite soluble in acids and alkalies, thereby the toxicity of ZnO-NPs was also compared to that of zinc(II) ions. When considering the concentration of dissolved Zn, no significant differences between exposure to ZnO and ZnO-NPs have been found in EC_{50} and LC_{50} values, using *Daphnia* and fish, respectively, as testing organisms. Clinical reports on human intoxication by ZnO-NPs are hardly any in the literature. Conversely, information regarding the toxicity of Zn ions for humans has been gathered during the last 60 years, since the first clinical reports on Zn fume fever [66].

Zn is an essential trace element and micronutrient for humans. Under physiological conditions, it exists as a redox neutral divalent cation that is reactive as a Lewis acid. The continuous external supply of this metal ion is vital for many metabolic pathways, given that there is no body storage depot for zinc and its exchange between tissues is limited. The man body Zn content is approximately 2 g, and the recommended daily intake is 8–11 mg (tolerable upper intake level, 40 mg day^{-1}). Bones and skeletal muscles contain more than half of the total body Zn, while the highest concentrations (>1 mg g^{-1} dry weight) are achieved in prostate gland [67]. Around 0.1% of the total body zinc is replenished daily through diet, and it is equivalent to the percentage that is hold in blood serum (90 μg dL^{-1}) [68].

Zinc homeostasis is tightly controlled at the whole body down to the subcellular level. In cells, half of the Zn content is in the cytoplasm, while nucleus and plasma membrane accounts for, respectively, 35 and 10% of the total cellular Zn [69]. Since this metal ion is mainly bound to proteins (i.e., metallothioneins) and sequestered in organelles (i.e., mitochondria, endoplasmic reticulum, Golgi apparatus, secretory granules, and other vesicular compartments), the cytosolic free Zn concentration is in the picomolar/nanomolar range. In the cytosol, Zn concentrations fluctuate in wave and spark manners, of which regulatory mechanism still not completely understood. Zinc transporters (ZnTs) are of outstanding importance for the cellular and subcellular zinc homeostasis (**Table 2**), since ions cannot be synthesized or broken down by cells. ZnT transporters (ZnT1-ZnT10) belong to the Solute Carrier Family 30A (SLC30A). This protein "Family" also comprises another group of proteins that translocate Zn across membranous barriers, the ZIP transporters (ZIP1-ZIP14). However, ZIP transporters are involved not only in Zn transport but also in the homeostasis of cadmium, manganese, iron, and calcium [70].

Transporters		
Name	**Main functions at subcellular level**	**Entries: Protein/Gen***
ZnT1	Zn^{2+} efflux through plasma membrane. Negative regulation of Zn^{2+} and Ca^{2+} transmembrane import and neurotransmitter secretion	Q9Y6M5 (ZNT1_HUMAN)/ SLC30A1
ZnT2 (2 isoforms)	Zn^{2+} transmembrane transport (accumulation in endosomes, lysosomes, and secretory vesicles in mammary epithelial cells). Regulation of sequestering of and response to Zn^{2+}	Q9BRI3 (ZNT2_HUMAN)/ SLC30A2
ZnT3	Zn^{2+} transporting ATPase (accumulation in synaptic vesicles, late endosomes, and lysosomes). Regulation of sequestering of and response to Zn^{2+}	Q99726 (ZNT3_HUMAN)/ SLC30A3
ZnT4	Zn^{2+} transmembrane transport (transport out of the cytosol—accumulation in endosomes, lysosomes, secretory vesicles, and trans-Golgi network)	O14863 (ZNT4_HUMAN)/ SLC30A4
ZnT5 (4 isoforms)	Zn^{2+} transmembrane transport into lumens of the Golgi apparatus and early compartments of the secretory pathway such as COPII-coated vesicles (putative transporter of Zn^{2+} into β cells in order to form insulin crystals). Required with	Q8TAD4 (ZNT5_HUMAN)/ SLC30A5

Transporters		
Name	**Main functions at subcellular level**	**Entries: Protein/Gen***
	ZnT7 for the activation of Zn-requiring enzymes, alkaline phosphatases, and ZnT6 and ZnT7 for the activation of TNAP	
ZnT6 (4 isoforms)	Zn^{2+} efflux transporter that allocates it to trans-Golgi network and vesicular compartment. Regulation of sequestering of and response to Zn^{2+}	Q6NXT4 (ZNT6_HUMAN)/ SLC30A6
ZnT7	Zn^{2+} transmembrane transport into lumens of Golgi apparatus and vesicular compartments. Required for activation of alkaline phosphatases and with ZNT5 and ZNT6 for the activation of TNAP	Q8NEW0 (ZNT7_HUMAN)/ SLC30A7
ZnT8 (4 isoforms)	Zn^{2+} efflux transporter which allocates it to intracellular vesicles (i.e., accumulation into insulin granules in pancreatic β cells, providing Zn^{2+} to insulin maturation and/or storage). Regulation of sequestering of and response to Zn^{2+}. Responsiveness to glucose, γ-interferon, and interleukin-1	Q8IWU4 (ZNT8_HUMAN)/ SLC30A8
ZnT9	Role in the p160 coactivator signaling pathway that mediates transcriptional activation by nuclear receptors. Transcriptional activation of Wnt-responsive genes	Q6PML9 (ZNT9_HUMAN)/ SLC30A9
ZnT10 (3 isoforms)	Zn^{2+} transmembrane transport into Golgi apparatus and early endosomes. Regulation of sequestering of and response to Zn^{2+}	Q6XR72 (ZNT10_HUMAN)/ SLC30A10
ZIP1 (2 isoforms)	A major Zn^{2+} uptake transporter in many cells; responsible for the rapid uptake and accumulation of physiologically effective Zn in prostate cells	Q9NY26 (S39A1_HUMAN)/ SLC39A1
ZIP2 (2 isoforms)	Zn^{2+} transport through the plasma membrane (uptake mediated by Zn^{2+}-HCO_3^- symport). It is involved in contact inhibition of normal epithelial cells, and loss of its expression is related to tumorigenesis	Q9NP94 (S39A2_HUMAN)/ SLC39A2
ZIP3 (2 isoforms)	Zn^{2+} transport through the plasma membrane (influx to cytosol). It is involved in cell morphogenesis and T cell homeostasis	Q9BRY0 (S39A3_HUMAN)/ SLC39A3
ZIP4 (2 isoforms)	Zn^{2+} transmembrane transport (influx to cytosol) It is involved in the regulation of cellular Zn homeostasis in response to Zn^{2+} availability (cycles between endosomal compartments and the plasma membrane)	Q6P5W5 (S39A4_HUMAN)/ SLC39A4
ZIP5	Zn^{2+} transmembrane transport (serosal to mucosal) through basolateral cell membrane in polarized cells	Q6ZMH5 (S39A5_HUMAN)/ SLC39A5
ZIP6 (2 isoforms)	Zn^{2+} transport through the plasma membrane (influx to cytosol)	Q13433 (S39A6_HUMAN)/ SLC39A6
ZIP7	Zn^{2+} transmembrane transport from the endoplasmic reticulum/Golgi apparatus to the cytosol that is stimulated by growth factors (EGF), $Ca^{2+}$and exogenous Zn^{2+}	Q92504 (S39A7_HUMAN)/ SLC39A7

Transporters		
Name	**Main functions at subcellular level**	**Entries: Protein/Gen***
ZIP8 (3 isoforms)	Zn^{2+} transport through the plasma membrane and endosomal and lysosomal membranes (influx and release to cytosol)	Q9C0K1 (S39A8_HUMAN)/ SLC39A8
ZIP9 (3 isoforms)	Zn^{2+} transport through the plasma membrane and trans-Golgi network membrane (influx and release to cytosol)	Q9NUM3 (S39A9_HUMAN)/ SLC39A9
ZIP10 (2 isoforms)	Zn^{2+} transport through the plasma membrane (influx to cytosol). Positive regulation of B cell proliferation, B cell receptor signaling pathway, and protein tyrosine phosphatase. Negative regulation of B cell apoptotic process	Q9ULF5 (S39AA_HUMAN)/ SLC39A10
ZIP11 (3 isoforms)	Zn^{2+} transport through the trans-Golgi network membrane (release to cytosol)	Q9NUM3 (S39A9_HUMAN)/ SLC39A9
ZIP12 (5 isoforms)	Zn^{2+} transport at the plasma membrane, nucleus, and Golgi apparatus (influx and release to cytosol). Regulation of microtubule polymerization, neuron projection development, and signal transduction	Q504Y0 (S39AC_HUMAN)/ SLC39A12
ZIP13 (2 isoforms)	Zn^{2+} transmembrane transport in the Golgi apparatus (release to cytosol)	Q96H72 (S39AD_HUMAN)/ SLC39A13
ZIP14 (3 isoforms)	Zn^{2+} transport through the plasma membrane (influx to cytosol). Broad-scope metal ion transporter with a preference for Zn^{2+} uptake (cellular uptake of nontransferrin-bound Fe)	Q15043 (S39AE_HUMAN)/ SLC39A14

Zn-finger proteins		
Family; subfamily	**Representative protein and its function**	**Protein entry***
ZNF593/ BUD20 C2H2	Zinc finger protein 593 negatively modulates the transcriptional regulatory activity of Oct-2	O00488
Teashirt C2H2	Teashirt homolog 2 is a putative transcriptional regulator in developmental processes, acting as transcriptional repressor	Q9NRE2
Sp1 C2H2	Transcription factor Sp9 positively regulates FGF8 expression in the apical ectodermal ridge and contributes to limb outgrowth in embryos	P0CG40
Snail C2H2	Transcriptional repressor scratch 1 binds E-box motif CAGGTG and modulates the basic helix-loop-helix transcription factors during neuronal differentiation	Q9BWW7
Sal C2H2	Sal-like protein 4 is an important transcription factor in the maintenance and self-renewal of embryonic and hematopoietic stem cells	Q9UJQ4

Zn-finger proteins		
Family; subfamily	**Representative protein and its function**	**Protein entry***
Odd C2H2	Protein odd-skipped-related 1 is a transcription factor in the regulation of embryonic heart and urogenital development	Q8TAX0
Krueppel C2H2; ZFX/ZFY	Zinc finger Y-chromosomal protein is a transcriptional activator through binding to the consensus sequence 5′-AGGCCY-3′	P08048
Krueppel C2H2; ZFP57	Zinc finger protein 57 homolog is a transcription regulator that binds to a 5′-TGCCGC-3′ consensus sequence and recognizes the methylated CpG within this element. It is important for the maintenance of maternal and paternal gene imprinting through control of DNA methylation during the earliest multicellular stages of development at multiple imprinting control regions	Q9NU63
Krueppel C2H2; ZBTB18	Zinc finger and BTB domain-containing protein 18 is a transcriptional repressor that binds to the consensus DNA sequence 5′-[AC]ACATCTG[GT][AC]-3′ containing E-box core. It is involved in the recruitment of chromatin remodeling multiprotein complexes, the regulation of skeletal myogenesis, progenitor cell division, and postmitotic cortical neurons survival	Q99592
Krueppel C2H2; Hic	Hypermethylated in cancer 1 protein is a transcriptional repressor that recognizes and binds to the consensus sequence '5-[CG]NG[CG]GGGCA[CA]CC-3'. It regulates the Wnt signaling pathway, p53/TP53-dependent apoptotic DNA damage responses, and the transcription of CCND1/cyclin-D1 and CDKN1C/p57Kip2 in quiescent cells. May act as a tumor suppressor and is involved in development of head, face, limbs, and ventral body wall	Q14526
Krueppel C2H2	Krueppel-like factor 1 is a transcription regulator of erythrocyte development and switch factor during erythropoiesis. When sumoylated, acts as a transcriptional repressor by promoting interaction with CDH2/MI2β and represses megakaryocytic differentiation	Q13351
Ikaros C2H2	DNA-binding protein Ikaros has transcription regulator activity, via binding to γ-satellite DNA, which is isoform-specific and modulated by dominant-negative inactive isoforms. It increases normal apoptosis in adult erythroid cells and confers early temporal competence to retinal progenitor cells	Q13422
GLI C2H2	Both isoforms of zinc finger protein GLI1 are transcriptional activators that bind to the DNA consensus sequence 5′-GACCACCCA-3′, but activate different sets of genes. Isoform 1 plays a role in cell proliferation and differentiation, through SHH signaling pathway, whereas isoform 2 activates CD24 expression. Promotes cancer cell migration	P08151
EGR C2H2	E3 SUMO-protein ligase EGR2 is a transcription factor that binds to two sequence-specific DNA sites located in the promoter region of HOXA4. Supports SUMO1 conjugation to	P11161

Zn-finger proteins		
Family; subfamily	**Representative protein and its function**	**Protein entry***
	coregulators NAB1 and NAB2, whose sumoylation downregulates EGR2 own transcriptional activity	
DZIP C2H2	Zinc finger protein DZIP1 interaction with DAZ supports the participation in spermatogenesis and primary cilia formation through Hedgehog signaling pathway	Q86YF9
Delta-EF1/ ZFH-1 C2H2	Zinc finger E-box-binding homeobox 1 is a transcriptional repressor that positively regulates neuronal differentiation and promotes tumorigenicity	P37275
CTCF	Transcriptional repressor CTCF plays important roles in gene silencing, chromatin remodeling, interchromosomal association, regulation of epigenetic modifications, oocyte and preimplantation embryo development. It is also a putative tumor suppressor	P49711
AEBP2/jing C2H2	Zinc finger protein AEBP2 is a DNA-binding transcriptional repressor that stimulates PRC2 complex activity	Q6ZN18

Enzymes		
Recommended name	**EC number****	**Comments**
D-Lactate dehydrogenase (acceptor)	1.1.99.6	Alanine metabolism
Formaldehyde dismutase	1.2.98.1	Contains a tightly but noncovalently bound NADP(H) cofactor, as well as Zn^{2+} and Mg^{2+}
Peptide-methionine (R)-S-oxide reductase	1.8.4.12	Selenoprotein. Prevention of oxidative stress damage caused by reactive oxygen species by reducing the oxidized form of methionine back to methionine and thereby reactivating peptides that had been damaged
Superoxide dismutase	1.15.1.1	Degradation of reactive oxygen species and superoxide radicals
Histone acetyltransferase	2.3.1.48	Different specificities toward histone acceptors
RING-type E3 ubiquitin transferase	2.3.2.27	Degradation of misfolded protein
Protein geranylgeranyltransferase type I	2.5.1.59	Zn metalloenzyme. Zn^{2+} is required for peptide, but not for isoprenoid, substrate binding. Inhibition induces simultaneous p53-dependent apoptosis and autophagy in airway smooth muscle cells
Tyrosine transaminase	2.6.1.5	Involved in multiple metabolic pathways
Riboflavin kinase	2.7.1.26	Mg^{2+} is preferentially required for activity. Essential in recruiting Nox1 to death receptor4/5, critical role in the KD548-

Enzymes		
Recommended name	**EC number****	**Comments**
		Fc-mediated reactive oxygen species accumulation and coupling of TNF-receptor-1 to NADPH oxidase
Rhodopsin kinase	2.7.11.14	Inhibited by Zn^{2+}
β-Adrenergic-receptor kinase	2.7.11.15	Inhibited by Zn^{2+}
tRNase Z	3.1.26.11	Involved in both, nuclear and mitochondrial tRNA 39 end maturation and in the p53 signaling pathway
N-acetylphosphatidylethanolamine-hydrolyzing phospholipase D	3.1.4.54	Contains Zn^{2+} and is activated by Mg^{2+} or Ca^{2+}. It does not hydrolyze phosphatidylcholine and phosphatidylethanolamine
Aminopeptidase B	3.4.11.6	Exopeptidase strictly specific for the removal of N-terminal basic residues from peptides and proteins
Xaa-Trp aminopeptidase	3.4.11.16	Zn^{2+}containing glycoprotein from renal and intestinal brush border membranes
Aminopeptidase I	3.4.11.22	Activity is stimulated by both Zn^{2+} and Cl^{-}
N-acyl-aliphatic-L-amino acid amidohydrolase	3.5.1.14	Contains Zn^{2+} (completely inactivated by metal removal, whereas addition of Zn^{2+}, Mn^{2+}, or Fe^{2+} restores activity). It is involved in the hydrolysis of *N*-acylated or *N*-acetylated amino acids (except L-aspartate)
Cu^{2+}-exporting ATPase	3.6.3.4	Zn binds with a stoichiometry of 6–1 and induces a conformational change in the N-terminal domain that is different from those observed for Co binding, leading to a loss of secondary structure in the domain
Mitochondrial protein-transporting ATPase	3.6.3.51	A nonphosphorylated, non-ABC (ATP-binding cassette) ATPase involved in the transport of proteins or preproteins into mitochondria using the TIM protein complex
Porphobilinogen synthase	4.2.1.24	Contains Zn^{2+} at the active site. Essential for respiration and a primary target in Pb intoxication
Ubiquitin-protein ligase	6.3.2.19	Crucial role in the recognition and degradation of target proteins by 26S proteasomes

*UniProtKB/Swiss-Prot—European Bioinformatics Institute.
**Enzyme Database—BRENDA and IUBMB Enzyme Nomenclature.

Table 2. Illustrative examples of human Zn transporters, Zn-finger proteins, and enzymes that require Zn.

Zn ions are important for the regulation of central biochemical processes (gene transcription and the metabolism of lipids, proteins, and nucleic acids), which impacts a variety of physiological functions (e.g., neuronal, endocrine, skeletal, reproductive, immune, and healing). It is estimated that the 10% of the proteins (around 3000 proteins) encoded in the human genome are zinc proteins [71]. According to the last release of the UniProtKB/Swiss-Prot database, more than one hundred of human proteins are zinc finger (ZnF) macromolecules (**Table 2**). Apart from the structural role of Zn in ZNFs [72], which contributes to shape the zinc-binding repeats as molecular scaffolds for tight binding of their target molecules (DNA, RNA, other proteins, or lipids), this metal ion is also essential for enzyme catalysis and cell signaling (**Table 2**) [73, 74]. In enzyme catalytic centers, Zn often promotes substrate activation by stabilizing negative charges due to strong Lewis acid properties. The metal ion acts as endocrine, paracrine, autocrine, and intracrine mediator. In cells, the distribution of Zn is modified by the stimulation that triggers its release in the central nervous and neuroendocrine systems. Zn is also a ubiquitous cytosolic second messenger, leading to fast alteration of signaling enzyme activities (i.e., phosphodiesterases, mitogen-activated protein kinase, protein kinase C, protein tyrosine phosphatases, calcineurin, caspases) and afterward to the biosynthesis of proteins that control its cytosolic concentrations. Interestingly, Zn accumulation in specific subcellular compartments appears to occur during both physiological and pathological conditions. For instance, the Zn spark that follows the calcium wave during fertilization is thought to be crucial for further cell cycle resumption in eggs [75]. Accumulation of Zn in lysosomes is a common observation during neurodegenerative processes and intoxication [76].

In humans, symptoms of Zn deficiency include: severe anemia, persistent diarrhea, immune insufficiency ensuing recurrent inflammations and impairment of wound healing, growth retardation, hypogonadism, skin and eyes abnormalities, baldness mental lethargy, brain dysfunctions, and behavioral changes. Zn deficiency still continues a global public health concern, particularly in developing countries where it causes mortality among young children [77]. Conversely, minor Zn deficiency among elderly population and individuals who undergo gastric bypass surgery for obesity seems to be increasing in industrialized countries. Thereby, development of high efficient Zn-enriched nutritional supplements could be advantageous in decreasing the incidence of degenerative and immunodeficiency disorders, infections, and persistent diarrhea. Current Zn formulations better absorbed through supplements are in the form of picolinate or chelates of amino acids. Duodenum is the principal site for Zn absorption. Therefore, intestinal pathologies that cause poor micromineral absorption, such as Crohn's disease, can also induce Zn malabsorption. Other metal ions, such as Ca^{2+}, Fe^{2+}, and mostly Cu^{2+} compete with Zn^{2+} during translocation from the apical surface of the villae to the basolateral surface of enterocytes. In matter of fact, Zn intake close to the recommended dietary allowance (15 mg/day) may cause copper and iron deficiency and adversely affect HDL cholesterol concentrations. Overt symptoms of Zn poisoning (i.e., nausea, vomiting, epigastric pain, lethargy, and fatigue) usually occur only after exposure to extremely high Zn levels [78].

Zn-related diseases might be prompted either when its scarcity and overload go beyond the limited cellular Zn buffering capacity, which seems to be rather sensitive to environmental factors. Apparently, healthy individuals tolerate up to 10-fold changes of the recommended

daily intake of Zn [79]. Whether exposure to Zn concentrations outside the physiological range promotes salutary or injurious effects depends on Zn concentration. Different ranges of Zn concentration appear to be required for exhibiting beneficial properties as antioxidant, antiinflammatory, and antiapoptotic agent [80]. Intriguingly, both Zn excess and scarcity undermine the loosely equilibrium toward prooxidant, proinflammatory and proapoptotic actions.

Lung toxicity after inhalation of ZnO-NPs is likely the most documented deleterious effect in the literature. The severity of ZnO-NPs-induced inflammatory condition is significantly correlated with the mass and surface area of the nanoparticles, suggesting that the toxic effect of ZnO-NPs is mainly caused by the release of Zn ions [81]. It is also claimed that intact ZnO-NPs have a unique way of inducing inflammatory effects compared with dissolved Zn ions [82]. For example, ZnO-NPs may either stimulate the production of IFN-γ and subsequent macrophage activation, neutrophilic infiltration, and fibrosis (Th1 inflammatory response) or cause a mixed inflammatory cell immune response by triggering a Th2 response [83]. Inhaled ZnO-NPs, through the olfactory bulb–brain translocation pathway, could also induce neurotoxic effects by activation of astrocytes and microglia, which causes neuroinflammation [84, 85].

Recently, a battery of tests, including hemolytic and oxidative stress markers, *in vitro* ROS generation and the comet assay, has been applied to evaluate cytotoxic and genotoxic effects of ZnO-NPs on human erythrocytes and lymphocytes [86]. The authors concluded that, similarly to dissolved Zn, ZnO-NPs concentrations above 50 ppm. are cytotoxic and genotoxic, due to the enhancement of oxidative stress induced by ROS generation. In addition to disruption of cellular Zn homeostasis, alteration of multiple enzymatic activities, and interaction with biomolecules, exacerbation of oxidative stress is the most recognized mechanism through which ZnO-NPs induce toxic effects. Accordingly, it has been demonstrated that the cytotoxic effect of ZnO-NPs is more pronounced in human cells previously exposed transiently to sublethal doses of H_2O_2, a standard oxidative stress-inducing agent [87]. One important scenario of the consequences of human exposure to ZnO-NPs was anticipated by the authors: individuals who suffer from diseases associated with increased oxidative stress (i.e., asthma, atherosclerosis, cardiovascular diseases, chronic obstructive pulmonary disease, and neurodegenerative diseases) should be considered at additional risk upon exposure to ZnO-NPs.

The pharmaceutical industry makes use of Zn(II) compounds ($ZnCl_2$, ZnO, zinc pyrithione, $Zn(CH_3CO_2)_2$) as active substances, lubricants, and emollients for a long time [88]. Usually, zinc-containing medicines are intended to topic application as wound healings, anti-infectious, disinfectants, and lubricants (**Table 3**). Apart from ZnO-NPs use in pharmaceutical formulations (i.e., drug delivery) and medicine (i.e., bioimaging), they are also present in a large number of consumer products (protective sunscreens, hair care formulations, cosmetics, supplements, food additives, etc.), as already mentioned above (Section 1). Recently, the European Council on Cosmetic Products (The European Commission (2016), Commission Regulation (EU) 2016/621 of 21 April 2016) restricted the use of ZnO-NPs in spray products because it could lead to exposure of the consumer's lungs to ZnO-NPs by inhalation, and encouraged only oral and dermal use of ZnO-NPs, up to a maximum concentration in ready

for use preparation of 25%, with the following characteristics: (i) purity ≥96%, with *wurtzite* crystalline structure and physical appearance as clusters that are rod-like, star-like, and/or isometric shapes, with impurities consisting only of carbon dioxide and water, while any other impurities are less than 1% in total, (ii) median diameter of the particle number size distribution D50 (50% of the number below this diameter) >30 nm and D1 (1% below this size) >20 nm; (iii) water solubility <50 mg L^{-1}; and (iv) uncoated or coated with triethoxycaprylylsilane, dimethicone, dimethoxy diphenylsilane triethoxycaprylylsilane crosspolymer, or octyl triethoxy silane.

Up to date information highlights not only the genotoxicity and cytotoxicity of ZnO-NPs due dissolution, ROS generation, immunomodulatory, and apoptotic responses, but also their selective cytotoxicity [82, 89–93]. In general, cytotoxicity is thought to be a collateral effect to avoid. To evaluate the specific risks and benefits of human exposure to ZnO-NPs, its size, shape, degradability, agglomeration/aggregation propensity, adsorption ability, specific surface area, and interfacial chemical and physical reactivity should be considered intrinsic properties, which likely influence their biopersistence, cellular interactions, and bioactivities when compared with microparticulate and dissolved Zn during intentional and unintentional human exposures. For instance, ZnO-NPs sized 20–25 nm appear to exhibit higher antibacterial and antifungal activity than other ZnO forms [15]. Sublethal concentrations of ZnO-NPs (surface area of 10.7 ± 0.7 nm) reduce the mitochondrial membrane potential, leading to a dose-dependent increase in gluconeogenesis and glycogenolysis, which could not be only attributed to dissolution of ZnO-NPs in extracellular fluids [94]. Kao et al. [85] proposed that ZnO-NPs are mainly internalized by endocytosis and dissolved in endosomes, raising the cytosolic free Zn^{2+} concentration, which is further sequestrated by mitochondria leading to cell apoptosis, due to mitochondrial dysfunction and caspases activation. ZnO-NPs readily dissolve in artificial lysosomal fluid (pH 4.5), but form aggregates and precipitates in the slight alkaline interstitial fluid [95].

Zinc compound	Route of administration	Role in formulation	Target organ	Clinical recommendations
Zinc acetate	Topic	Active ingredient: antibacterial action	Skin	Treatment of inflammatory acne, characterized by bacterial involvement
Zinc chloride	Topic	Active ingredient: antibacterial, analgesic, and healing actions	Mouth/ oropharynx	Treatment of gingivitis and stomatitis; relief of toothache; oral hygiene
Zinc oxide	Topic	Active ingredient: adjuvant of healing	Skin	Treatment of diaper dermatitis
	Topic	Emollient/lubricant: antihemorrhoidal action	Anus/lower rectum	Symptomatic treatment of hemorrhoids
	Topic	Emollient/lubricant: soothing, smoothing, and moisturizing actions	Skin	Symptomatic treatment of dry and scaly lesions, especially of ichthyosis, psoriasis, and eczema Disinfection and

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Zinc compound	Route of administration	Role in formulation	Target organ	Clinical recommendations
				hygiene of the skin and mucous membranes, superficial wounds, and diaper dermatitis
Zinc pyrithione	Topic	Active ingredient: antibacterial and antifungal actions	Skin	Treatment of pityriasis versicolor, tinea pedis, psoriasis, seborrheic dermatitis, eczema, and vitiligo

Table 3. Commonly used medications that contain Zn for topical application.

In contract with small molecules that are translocated across the plasma membrane by passive diffusion or active transport, nanosized particles are internalized by cells mainly through endocytosis. This feature of NPs offers a myriad of opportunities for specific cellular targeting, controlled drug delivery, and bioimaging. The endocytotic capability varies significantly among cellular populations. Phagocytes (i.e., macrophages, monocytes, neutrophils, dendritic, and mast cells) are chemotaxing cells that move toward site of infections causing inflammation, play a central role in innate immunity response, and stimulate lymphocytes to produce antibodies (adaptive immunity) by antigen presentation. ZnO-NPs coating that favors interaction with opsonin, scavenger, or Toll-like receptors should enhance selective internalization by phagocytes. Uncoated ZnO-NPs should also be engulfed by lymphocytes, erythrocytes, fibroblasts, epithelial, and endothelial cells. Given that ZnO-NPs are microscopic particle with at least one dimension less than 100 nm, they can be internalized by any living cell by micro- and pinocytosis. The subcellular availability of ZnO-NPs should greatly depend on the specific endocytotic pathway (i.e., clathrin-dependent, caveolae-dependent, clathrin- and caveolae-independent, receptor-mediated) involved in the internalization process. For instance, ZnO-NPs loaded in caveolae vesicles may reach the endoplasmic reticulum and the nucleus, since caveosomes are pH neutral multivesicular bodies [96]. Conversely, internalization of ZnO-NPs through LDL receptor-mediated endocytosis should raise the cytosolic zinc ion significantly, due to dissolution of ZnO in the acidic lysosomal environment. Accordingly, it has been experimentally demonstrated by using ICP-MS and fluorescent-labeled ZnO dissolution occurs in endosomes, and that nondissolved ZnO-NPs enter caveolae in BEAS-2B cells (human bronchial epithelial cells) and enter lysosomes in RAW 264.7 cells (mouse leukemic monocyte macrophage cell) in which smaller particle remnants dissolve [90]. In the support of cell-specific behavior of stable aqueous solutions of monodispersed ZnO-NPs is the fact that ZnO-NPs doses exhibiting negligible cytotoxic effects to osteogenically differentiated mesenchymal stem cells were lethal to proliferating pluripotent mesenchymal stem cells [97]. As already mentioned above (Section 1), ZnO-NPs selectively induce apoptosis, mediated by reactive oxygen species via p53 pathway, in cancer cells (human hepatocellular carcinoma HepG2, human lung adenocarcinoma A549, and human bronchial epithelial BEAS-2B), but did not affect normal astrocytes and hepatocytes [98]. Thereby, to better explore therapeutic advantages and prevent unwanted cytotoxic effects and the potential of ZnNPs in terms of clinical diagnosis, it is important to perform a holistic analysis of the characteristics of ZnNPs,

the administration route, the zinc body burden, the target cells, and the relevant physiological processes in each specific case.

5. Conclusions, next steps, and opportunities

The ever increasing literature on ZnO-NPs clearly demonstrates the current value and applications of these particles and tremendous potential for future applications. There are a large number of challenges associated with its safe use, when compared with commonly tested substances. As for other nanoparticles, the advantages have to be carefully weight against potential pernicious effects. The currently available data clearly demonstrate the ability of ZnO-NPs to induce acute effects on fish, although at concentrations higher than those estimated to be present in the environment. Nonetheless, the long-term effects are yet to be explored. The considerable lack of information in terms of how these particles are released in the environment, at which levels, and in what form make the establishment of maximum allowed concentration a difficult task, based on available toxicity tests. More studies have to be conducted to explore the behavior of particles upon alterations of receiving media characteristics (e.g., ionic strength UV/radiation) and their fate. This information is essential for environmentally relevant ecotoxicological studies.

It is expected that, in the very near future, advances in analytical techniques allow quantification and accurate characterization of nanoparticles in environmental matrices which will allow the establishment of potentially impacted areas, monitoring of levels and effects on biota from those sites. Also, the need to the development of more effective wastewater treatments will potentially reduce the risk of the increased production of nanoparticle containing materials.

As can be seen from the literature, a broad range of applications of nanomaterials, in particular ZnO-NPs, exists on human activities. In this chapter, the benefits of ZnO nanomaterials are clearly recognized on a myriad of applications, having a great potential for the diagnosis, imaging, drug delivery, and treatment of several pathologies. Other areas within agricultural domain and energy resources have also relevant applications. Moreover, great potentials for their applications on aquaculture improving fish growth were documented.

The disposal and fate of ZnO-NPs into the environment may represent a risk to aquatic biota. This chapter highlights the significance in considering their fate and behavior into water bodies and its role on aquatic organisms, particularly fish. The published literature undoubtedly illustrates that ZnO-NPs have different toxic effects on microorganisms, rodents, human cells, and fish depending on their physicochemical features. In addition, the trophic transfer of these nanomaterials to humans through diet (i.e., by consuming contaminated fish) warrants special care. Therefore, disposal of ZnO-NPs deserves more attention since bioaccumulation of these elements may occur on aquatic species with impact on both human and environmental health. Precaution and more strict rules must be delimited for disposal of ZnO-NPs into the aquatic environments.

Zinc ion homeostasis is vital for humans and is closely linked with the homeostasis of other metal ions, particularly iron and copper. Nowadays, hypozincemia and hyperzincemia are two

pathophysiological conditions of which enduring prevalence is also related to malnutrition during aging and emerging lifestyle diseases (i.e., obesity) in industrialized countries. While the risks of using ZnO-NPs are not fully understood, the advantages of its emerging applications, including in the therapeutic and diagnostic areas, are already widely recognized. Probably, the toxicity of ZnO-NPs for man is not superior to the zinc ion itself, and nanoparticulate forms appear to enable interaction with specific cell cycle states (i.e., proliferating cells) and selective interference with important physiological processes, allowing not only selection of the administration route of ZnO-NPs but also the cellular internalization pathway and further intracellular distribution.

The balance of the positive aspects of these nanomaterials and risks caused in some aquatic species, particularly on fish, targeting possible implications for human health deserve a continuous monitoring. Although safety measures have been assumed during industrial production, storage, and removal of these nanomaterials, a constant monitoring of possible risks for aquatic life and ultimately humans is needed.

As a general conclusion, it is expected that in the near future, there is an increase in the use of ZnO-NPs for various purposes. Comprehensive understanding of their toxic effect is needed for their prolonged use.

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