ACTA VET. BRNO 2021, 90: 331–347; https://doi.org/10.2754/avb202190030331

A review of the effects of metallic nanoparticles on fish

Hana Čaloudová, Jana Čaloudová, Zdeňka Svobodová

University of Veterinary Sciences Brno, Faculty of Veterinary Hygiene and Ecology,
Department of Animal Protection and Welfare and Public Veterinary Medicine, Brno, Czech Republic

Received January 16, 2021
Accepted August 31, 2021

Abstract

Many important discoveries have been made in the field of nanotechnology in the last 40 years. Since then, nanoparticles became nearly ubiquitous. With their spreading use, safety concerns have warranted extensive research of nanotoxicity. This paper offers information about the occurrence, transport, and behaviour of metallic nanoparticles in the aquatic environment. It further summarizes details about parameters that dictate the toxicity of nanoparticles and discusses the general/common mechanisms of their toxicity. This review also focuses on fish exposure to nanoparticles, including the possibility of trophic transport through the food chain. Information on some of the most frequently used metallic nanoparticles, such as silver, gold, and titanium dioxide, is further elaborated on.

Toxicity, environment, silver, gold, titanium dioxide

The dawn of nanoscience and nanotechnology is connected to the invention of the Scanning Tunneling Microscope (STM) in 1981, which allowed scientists to observe individual atoms for the first time (Binnig et al. 1982). Since then, breakthrough discoveries have been made in almost every scientific field, including physics, chemistry, biology, engineering, or medicine. The basic definition states that nanoparticles (NPs) are particles of a size between 1 and 100 nm (ISO 2011). The size range is restricted arbitrarily, there is no clear cutoff in the properties of particles at 100 nm (Geertsma et al. 2015). Moreover, NPs are usually not entirely round, homogenous, unbound particles. Those aspects are taken into consideration by the Commission Recommendation on the definition of nanomaterial (2011/696/EU) which states that nanoparticles are not only particles in an unbound state but also aggregates or agglomerates where for 50% or more of the particles in the number size distribution, one or more external dimensions are in the size range of 1–100 nm. In specific cases, this threshold can even be lowered to 1%.

This might seem like a completely new area of materials that humans have never encountered, but the opposite is true. Nanoparticles were contained in a colloidal solution called "soluble gold" which was used for medicinal purposes more than 2000 years ago. During the Roman Empire and later in the Middle Ages, Au and Ag NPs were used to stain coloured glass (Horikoshi and Serpone 2013). Furthermore, NPs are not only human-made but also arise from natural processes, such as the photobiogeochemical reaction leading to the formation of Ag NPs in the aqueous environment by reducing silver ions with organic matter (Hou et al. 2013). Nanoparticles are also formed during combustion and are present as ultra-fine particles in the atmosphere. They are also formed by cells as products of bacterial metabolism. Due to this long-term presence of nanomaterials in ecosystems, it can be assumed that biological systems have established mechanisms at the level of cells and organisms to deal with their presence (Monteiro-Riviere and Tran 2007).

Address for correspondence:
Hana Čaloudová
Department of Animal Protection and Welfare and Public Veterinary Medicine
Faculty of Veterinary Hygiene and Ecology
University of Veterinary Sciences Brno
Palackého třída 1946/1, 612 42 Brno, Czech Republic

Telephone: +420 541 562 788
E-mail: H17345@vfu.cz
http://actavet.vfu.cz/

Yet, the ever-widening range of products and applications of NPs is associated with the increasing frequency and quantity of their unintentional release into the environment. The release may occur at all stages: during the manufacturing, use, and disposal of NPs-containing products. This brings uncertainty about possible environmental and health risks, especially in relation to substances such as metals that are a well-known current threat to the environment (Kovářík et al. 2020; Svoboda et al. 2020). Currently, metallic NPs represent the second largest market share of all NPs with an estimated value of 16 billion USD in the year 2020, and is projected to more than double the market value by 2027, growing at a compound anual growth rate of 14.2%. The highest growth is predicted for the pharmaceutical and healthcare sector, followed by electrotechnology and catalyst sectors (Global Industry Analysts, Inc. 2020; Grand View Research, Inc. 2020). Therefore, this review further focuses predominantly on metallic NPs.

Classification of metallic NPs

Metallic NPs can be divided into four categories based on their composition. The first group are pure metal-based NPs, usually comprised of noble metals (Ag, Au, Pt, etc), the second group are metal oxide NPs, which can have magnetic (Fe_3O_4, Fe_2O_3, etc) or semiconductor properties (TiO_2, ZnO, etc). The third group are chalcogenide NPs, represented by sulphides, selenides, and tellurides (PbS, ZnSe, CdTe). The fourth group are doped metal/metal oxide/metal NPs, which combine beneficial properties of both metals (Zn-Ag, Pt-Ni, etc) (Khanna et al. 2019; Yaqoob et al. 2020).

The occurrence of metallic NPs in the aquatic environment

The exact volumes of metallic NPs in the environment are not known; only estimates of the environmental concentrations of certain NPs are available. Currently, research is focused on the use of NPs in pesticides, fertilizers, and water and soil remediation products, which could be a significant source of contamination in the future. Mainly carbon NPs, metals, and their oxides are investigated (Khot et al. 2012).

The aquatic environment can be contaminated by the direct entry of NPs into water (for example during remediation, leaching of NPs during the use of the products, or accidental leaks), through emissions from wastewater treatment plants, air deposition, and flushings from contaminated soil (Gottschalk et al. 2009; Impellitteri et al. 2013; Gottschalk et al. 2015). Landfills are especially significant sources of contamination of ecosystems (Gottschalk et al. 2015).

Wastewater treatment plants can remove a significant part of NPs, for example, 97% of Ag NPs are removed from the water column into sewage sludge (Impellitteri et al. 2013). The methods of handling the sewage sludge differ among countries, in some places, they can be applied to the soil for their beneficial high contents of organic matter (Kwak and An 2016).

Application of sewage sludge can be hazardous due to high predicted concentrations of NPs, ranging in hundreds to thousands of $mg \cdot kg^{-1}$ for TiO_2 NPs, tens of $mg \cdot kg^{-1}$ for ZnO NPs, and dozens of $\mu g \cdot kg^{-1}$ for Ag NPs (Gottschalk et al. 2009; Sun et al. 2014; Gottschalk et al. 2015; Sun et al. 2016). According to the predictive models, European surface waters contain tens to thousands of $ng \cdot l^{-1}$ of TiO_2 NPs, tens to hundreds of $ng \cdot l^{-1}$ of ZnO NPs, and units to tens of $ng \cdot l^{-1}$ of Ag NPs (Gottschalk et al. 2009; Sun et al. 2014; Gottschalk et al. 2015; Sun et al. 2016; Peters et al. 2018).

Behaviour and stability of metallic NPs in the aquatic environment

Metallic NPs are not fully soluble in water and therefore models valid for soluble chemicals cannot be used to assess their behavior and toxicity. Instead of dissolving, we

have to take into consideration the formation of stabilized suspensions. Most metallic NPs are hydrophilic and often have low solubility, which can be perceived positively because metal ions tend to be their most toxic form (Batley et al. 2013). An example is the silver ion Ag^{+}, which is more toxic than silver bound in the form of compounds and Ag^{0} within NPs (Hogstrand and Wood 1998; Ribeiro et al. 2014). The fate of dissolved ions is influenced by the composition of the environment, Ag^{+} in natural waters rapidly forms sulphides and chlorides, which are insoluble, have lower toxicity, and are deposited in sediments (Kaegi et al. 2013). Sedimentation is another important way of loss of NPs from the water column. It is usually preceded by particle aggregation, the extent of which depends on pH, the presence of cations, ionic strength, and the size, shape, and charge of particles (Batley et al. 2013; Schaumann et al. 2015). The sea and brackish regions have high ionic strength, so lower concentrations of NPs can be expected due to rapid aggregation in those environments (Pamies et al. 2014; Li et al. 2016).

Both homoaggregation (NPs form aggregates with each other) can occur, or more often heteroaggregation, when they bind to naturally occurring colloids, which are present in waters in significant concentrations, namely 1–10 $mg \cdot l^{-1}$. These colloids consist of inorganic (e.g. metal oxides and sulphides, amorphous silicon), organic (formed primarily by humic substances and fibrous material, which may be polysaccharides and proteins formed by microorganisms), and biological components (viruses, bacteria). The fibrous material facilitates the aggregation of NPs, while humic substances can stabilize the dispersion of NPs by creating a coating that stabilizes the charge of the particle (Batley et al. 2013; Kaegi et al. 2013). Nanoparticles can also undergo redox reactions that change the chemical properties of the particle surface and can affect its environmental behaviour.This is typical for Fe^{0} which is used for water remediation. Iron particles oxidize rapidly in the environment, reducing the pollutant, such as trichloroethylene (Liu et al. 2005).

Determining properties of metallic nanoparticles

The toxicity of metallic NPs is dictated by multiple parameters, which must be taken into consideration as a complex of information, that includes their chemical composition, crystal structure, and purity of the sample, method of synthesis, size, and surface area of the nanoparticle (Warheit 2008; Wijnhoven et al. 2009) Larger particles are usually found to be less toxic, due to a smaller surface area. At the same volume, they have fewer atoms in the surface layer available for interaction with other compounds or ionization (Angel et al. 2013). At the same time, the nanosize provides opportunity to cross biological barriers and cell membranes (Nel et al. 2006). A study by Lee et al. (2007) demonstrates the passage of Ag NPs with Brownian diffusion through the chorion pore canals into the chorionic space and inner mass of zebrafish (*Danio rerio*) embryos *in vivo*. Ivask et al. (2014) show the size- and concentration-dependent effects of citrate-coated Ag NPs (10 nm, 20 nm, 60 nm, and 80 nm) on bacteria, yeast, algae, crustaceans, and mammalian cells *in vitro*. The shape of the particle plays an important role, as well. Hua et al. (2014) found that exposure of zebrafish embryos to ZnO nanosticks had more serious effects on the survival rate and hatching of the embryos than ZnO nanospheres or cuboidal submicron particles. Another aspect is surface chemistry, such as their ability to form reactive species, catalytic and photocatalytic activity, leading to reactive oxygen species (ROS) production when irradiated by ultraviolet rays, as has been described for TiO_2 and ZnO particles (Clemente et al. 2013; Sharma et al. 2020; Valério et al. 2020). The presence of coatings and addition of functional groups to the NPs surface alter their surface properties, improve their overall function, biocompatibility, or dispersion in aquatic media or reduce photocatalytic ROS production (Warheit 2008). For example, polyvinylpyrrolidone

(PVP) coating of Au NPs is considered safer and more biocompatible than citrate coating (Zhou et al. 2009; Iswarya et al. 2016).

This information must be considered in relation to the characteristics of the exposure media (pH, presence of ions, and ligands, as mentioned above) which in reality differ greatly for *in vitro* and *in vivo* settings. The precise particle and exposure characterization is a key factor of each experiment (Nel et al. 2006; Warheit 2008; Wijnhoven et al. 2009).

Toxicodynamics of metallic nanoparticles in fish

There are several distinct toxic mechanisms by which NPs can impact aquatic organisms, which often act together and lead to similar results.

The most significant mechanism is the excessive production of ROS, overwhelming the cell antioxidant mechanisms, which can cause oxidative stress, leading to protein destabilization, lipoperoxidation of the cell membrane, DNA damage, causing genotoxic effects and cell death (Nel et al. 2006; Manke et al. 2013; Fu et al. 2014; Fard et al. 2015).

In many of the metallic NPs, the release of ions is a significant cause of adverse effects. The cytotoxic action of metal ions is caused by the complexation of metal cations with thiol groups of proteins and enzymes, which causes their inactivation (Sevcikova et al. 2011). Thiol groups are present in enzymes responsible for dealing with oxidative stress, such as lactate dehydrogenase and glutathione, which complicates the distinction of the two above mentioned toxicological mechanisms, when the enzyme activity is measured (Ulrich and Jakob 2019). In the case of silver ions, there is also a more pronounced specific toxicological mechanism in fish. Specific inhibition of Na^+/K^+-ATPase leading to blockage of active uptake of Na^+ and Cl^- on gill basolateral cells, leading to disruption of osmoregulation and death (Morgan et al. 1997; Hogstrand and Wood 1998). However, Yue et al. (2016) show, that the inhibition of Na^+/K^+-ATPase was caused by both $AgNO_3$ and particulate Ag NPs (dissolution rate of Ag^+ was 2.37%, thus ions can be only accounted for 16% of observed effect).

The mechanical effect is often attributed to particles with a greater aggregation rate. In fish, the adhesion of NPs to the gills and a subsequential mechanical restriction of gas exchange was described by Ma and Lin (2013). In fish embryos, aggregation of NPs on a surface of the chorion can block the chorion pore canals and cause hypoxia, which manifests with a decreased hatching rate (Shih et al. 2016; Caloudova et al. 2018).

Due to the larger surface area, small dimensions, and high sorption capacity, NPs can also act as a Trojan horse, binding contaminants to their surface, transporting them into organisms, where they can be released (Deng et al. 2017). This effect was reported by Zhu et al. (2011) in *Haliotis* sp. embryos exposed to tributyltin, and coexposed to TiO_2 NPs and tributyltin. While the TiO_2 NPs alone had no negative developmental effect at 2 $mg \cdot l^{-1}$, the same concentration increased the toxicity of tributyltin 20-fold, causing a drop in the hatching rate and an increase in the presence of malformations compared to exposition of embryos to tributyltin only.

In the case of metallic nanoparticles with antimicrobial properties, such as ZnO NPs, TiO_2 NPs or Ag NPs, the adverse effect on the diversity and composition of the microbiome of the fish should be taken into consideration (Azizi-Lalabadi et al. 2019; Chupani et al. 2019).

Toxicokinetics of metallic nanoparticles in fish

It has been shown that NPs can be taken up by aquatic organisms and can adversely affect them at the level of algae, invertebrates, and fish (Gaiser et al. 2011; Skjolding et al. 2016). Possible routes of uptake of NPs in fish include absorption through the gill epithelia, the gut epithelia during dietary exposure and drinking, as well as skin epithelia

(Handy et al. 2008; Nam et al. 2014). From the above mentioned, the skin is thought to have the smallest impact, due to the secretion of protective mucus which can chelate charged NPs, as well as the lack of metal transporters compared to gill (Coello and Khan 1996; Handy et al. 2008). Yue et al. (2016) show endocytic uptake of Ag NPs and their storage in endosomes and lysosomes of cells of RTgill-W1 cell line. Nanoparticle localization was different from Ag^+, ions were found predominantly in cytosol, associated with metallothionein-like protein fractions.

The theory of the gut as a viable route of uptake is supported by Gaiser et al. (2011) who exposed common carp for 21 days to nano- and microsized particles of silver and subsequently analyzed silver concentrations in the gills, intestine, blood, liver, gallbladder, kidney, and brain. Significant uptake was detected in the intestine, liver, and gallbladder. Higher concentrations of silver were observed in groups exposed to smaller particles. Chronic dietary intake of ZnO NPs did not cause accumulation of zinc in common carp (*Cyprinus carpio*) tissues, however, adverse effects on immune system and homeostasis, as well as nephrotoxic and hepatotoxic effects were connected to the ZnO NPs exposure (Chupani et al. 2017; 2018a). Whether the fish digests food during the exposure plays an important role in the bioavailability and toxicity of ingested substances. Proteins and amino acids have been shown to complexate the Ag^+ and prevent adverse effects on rainbow trout gut cells (RTGC) in the case of Ag NPs, they were only partially effective in sustaining the metabolic activity and integrity of the cell and lysosomal membranes (Minghetti and Schirmer 2016). It is to be noted that any damage, such as erosion or inflammation of epithelia, disables natural barriers and enables facilitated transport of NPs to the blood circulation and internal organs. The said damage can be caused by the NPs themselves. Chupani et al. (2018b) found an increased rate of apoptosis in the intestinal epithelium as well as increased levels of proteins associated with cancerous cell survival in the muscular layer of the intestine of the common carp (*Cyprinus carpio*) exposed to a diet containing ZnO NPs for six weeks.

Trophic transport of metallic nanoparticles

Bioaccumulation (accumulation of the NPs in the organism) and trophic transport have been shown to occur within the food chain, raising concerns about biomagnification (increased concentration of NPs in the food chain due to predation) within the ecosystems (Nam et al. 2014; Uddin et al. 2020). Ates et al. (2015) reported trophic transport between both CuO NPs and ZnO NPs contaminated crustacean (*Artemia salina*) and goldfish (*Carassius auratus*), resulting in accumulation of both CuO NPs and ZnO NPs in the intestine, liver, and gills of the goldfish. Similarly, Zhu et al. (2010) proved that TiO_2 NPs can be transferred from daphnids to zebrafish by dietary exposure, but biomagnification did not occur. On the other hand, Chen et al. (2015) provided the evidence of TiO_2 NPs transfer from an alga (*Scenedesmus obliquus*) to the water flea (*Daphnia magna*) with a biomagnification effect. Yoo-iam et al. (2014) evaluated the bioaccumulation and biomagnification of Ag NPs in a model food consisting of green algae (*Chlorella* spp.), water flea (*Moina macrocopa*), blood worm (*Chironomus* spp.), and silver barb (*Barbonymus gonionotus*). The highest bioaccumulation factor was found in the algae, the lowest bioaccumulation factor was determined in the fish. The trophic transfer occurred only between the algae and the water flea and there was no evidence of biomagnification.

Silver

Silver NPs are among the most frequently used and researched NPs, thanks to their excellent thermal and electrical conductivity, catalytic properties, nonlinear optical

properties, as well as antibacterial, antivirotic, antimycotic, antiinflammatory, and anticancer activity (Tian et al. 2007; Nowack et al. 2011; Gurunathan 2015; Shaalan et al. 2016). Silver NPs have a wide range of industrial and consumer applications in the production of electronics, construction, agriculture, food industry, healthcare, and medicine (in both treatment and diagnostics), as well as in household products, appliances, cosmetics, and textiles (Park et al. 2013; European Commission 2014; Geertsma et al. 2015; Zhang et al. 2016). The annual worldwide production of Ag NPs is estimated to be 320 tonnes (Nowack et al. 2011).

Silver ions belong amongst the most toxic metals to fish (Morgan et al. 1997; Hogstrand and Wood 1998), Ag^+ is attributed to a significant part of the toxic effect of Ag NPs, but not the whole, there is intrinsic toxicity of NPs (Bilberg et al. 2012; Lapresta-Fernández et al. 2012; Khan et al. 2015; Speshock 2018). Van Aerle et al. (2013) applied sequencing-based transcription-profiling on zebrafish embryos exposed to Ag NPs, bulk silver, and $AgNO_3$ for 48 h. Significant changes in gene expression were found in all exposition groups, with significant overlaps between the groups, particularly pathways associated with oxidative phosphorylation and protein synthesis. Ag NPs specific changes in gene expression were found, suggesting that Ag^+ is not the only originator of the adverse effect. This claim is supported by a study performed on adult zebrafish exposed to Ag NPs, and $AgNO_3$ for 48 h. Total silver body burden was significantly higher in groups exposed to Ag NPs and subsequent gene expression analysis revealed a variety of genes, that were differentially expressed only in Ag NPs expositions (Griffitt et al. 2009). The effect of Ag NPs exposition on gills has been studied thoroughly because gills are one of the main spots of Ag^+ toxicity. Garcia-Reyero et al. (2015) compared the effects of Ag NPs and $AgNO_3$ on gills of fathead minnow (*Pimephales promelas*). During the first 4 h, mucus production in all groups increased, which was followed by a decrease in mucus production after 24 h of exposure. Microarray analysis detected 109 differentially expressed genes shared by both $AgNO_3$ and Ag NPs, but there as many as 615 differentially expressed genes specific to Ag NPs. In a study by Hawkins et al. (2015), $AgNO_3$ and Ag NPs caused similar disruptions in gill structure and ionic regulation, including circulatory disturbances, and decreased activity of the Na^+/K^+-ATPase.

The developmental toxicity of Ag NPs to fish is well documented (Yeo and Kang 2008; Massarsky et al. 2013; Park et al. 2013; Xia et al. 2016). A dose-dependent increase in mortality, amount of non-hatched embryos, and presence of sublethal malformations, such as the presence of notochord deformations or pericardial oedema, bradycardia, and cardiac arrhythmia were found after 72 h exposition to Ag NPs. The distribution of Ag NPs was visualized by transmission electron microscopy in the brain, heart, yolk, and blood of the embryos (Asharani et al. 2008). Furthermore, the possibility of transgenerational transfer of silver has been shown in female zebrafish injected with 1 $mg \cdot l^{-1}$ 10 nm PVP capped, in which the passage to eggs has been proven. The highest load of Ag NPs was found in the liver of the fish, in which the upregulation of inflammatory gene interleukin-1β, tumour necrosis factor alpha (TNF-α), and pro-apoptotic caspase genes was measured (Speshock et al. 2016).

Impairment of the neurological development, manifesting morphologically as a hypoplastic hindbrain, reduction of the eye and overall head size, plus cardiac defects, accompanied with alteration of the neural development-related genes expression was observed in zebrafish embryos exposed to Ag NPs up to 96 h post fertilization; no free Ag^+ was detected in exposure solutions. Also, higher accumulation of Ag was observed in the head area of the embryo (Xin et al. 2015). Another study brought evidence of reduction of the acetylcholinesterase (AChE) activity in the brain of adult zebrafish, exposed to 0.1 ppm of Ag NPs for 15 days. This leads to continuous muscle stimulation, manifested in altered responses and behaviour of the fish. Neurological changes were accompanied by necrotic and degenerative changes

in the liver, as well as decreased activity of liver antioxidant enzymes, catalase, and superoxide dismutase, as a result of oxidative stress (Devi et al. 2015). Induction of oxidative stress was measured in zebrafish liver cells exposed to 120 nm Ag NPs for 24 h. This was accompanied by upregulation of the expression of a variety of endoplasmatic reticulum specific stress marker genes, pro-apoptotic gene Bax, and TNF-α. On the other hand, the expression of the tumour suppressor gene p53 was downregulated (Christen et al. 2013). The summary of studies carried out into the toxicity of Ag NPs to fish is listed in Table 1.

Gold

Gold NPs are popular due to their relatively easy synthesis, stability, catalytic and optoelectronic properties, low toxicity, and high biocompatibility. They are used in technical applications such as electronics, photovoltaics, catalysts, probes, and sensors. Gold NPs also play an important role in medicine, both in diagnostics (for example in tumour detection and imaging) and therapeutic applications – in drug delivery, or as photothermal agents (Chandra et al. 2013; Mahapatra et al. 2015; Patibandla et al. 2018). The estimated worldwide production of Au NPs ranges from 1–3 tonnes per year (Pulit-Prociak and Banach 2016).

Although the toxicity of Au NP is significantly lower than Ag NPs (Bar-Ilan et al. 2009; Lapresta-Fernández et al. 2012; García-Cambero et al. 2013), major adverse effects have been described as well. In a study by Patibandla et al. (2018), exposure of zebrafish embryos to gold nanorods lead to increased mortality, decreased hatching, and a decreased heart rate. A significant increase in the expression of oxidative stress genes as well as increased apoptosis occurred, suggesting an important role of ROS in the mechanism of Au NPs toxicity.

Similar adverse effect on the circulatory system manifested by the presence of heart oedemas and decreased heart rate was observed in zebrafish embryos exposed to Au NPs stabilized with PVP (Hlavkova et al. 2020). Upregulation of oxidative stress gene expression, as well increased levels of protein biomarkers, such as catalase, superoxide dismutase, and metallothioneins were found in adult zebrafish exposed to Au NPs for 96 h. Furthermore, decreased swimming speed and abnormalities in the swimming behaviour were observed (Botha et al. 2019). In addition, Dedeh et al. (2014) reported modulation of the expression of genes involved in oxidative stress, mitochondrial metabolism, and DNA repair, as well as altered neurotransmission due to increased brain and muscle AChE activity in adult zebrafish, exposed to Au NPs contaminated sediment for 20 days.

Genotoxic effects of Au NPs have been observed even at concentrations as low as 4 $\mu g \cdot l^{-1}$ in gilthead seabream (*Sparus aurata*) exposed to citrate and PVP coated Au NPs for 96 h. Nanoparticles have induced DNA damage (erythrocyte DNA strand breaks), the frequency of erythrocytic nuclear abnormalities was also increased (Barreto et al. 2019). Alteration of gene expression levels, mutations, and mitochondrial dysfunction was observed in zebrafish exposed to citrate capped Au NPs (sized 12 nm and 50 nm) in their diet, over the period of 36 and 60 days. More severe effects were observed in groups fed the smaller particles (Geffroy et al. 2011). The summary of studies carried out into the toxicity of Au NPs to fish is listed in Table 2.

Titanium dioxide

Of the group of metal oxides, titanium dioxide is the most commonly used. Piccinno et al. (2012) estimate its annual global production volume to be 3 000 tonnes. Titanium dioxide

Table 1. Summary of studies carried out into the toxicity of silver nanoparticles to fish.

Characterization of the NPs	Model	Stage	Duration	Effect	Reference
Spherical, citrate-capped Ag NPs, sizes: 10 nm ± 2 nm and 100 nm ± 8 nm	*Danio rerio*	embryo	120 h	Accumulation of Ag in embryos, delayed hatching, bradycardia, . pericardial oedema	Park et al. 2013
Spherical Ag NPs, size 10 nm	*Danio rerio*	embryo	48 h	Down-regulation of gene pathways associated with oxidative phosphorylation and protein synthesis.	van Aerle et al. 2013
Ag NPs size: 10-20 nm	*Danio rerio*	embryo	72 h	A dose-dependent decrease in hatching rate, abnormal notochord, weak heartbeat, damaged eyes, curved tail, increased catalase activity.	Yeo and Kang 2008
Polyacrylate sodium stabilized Ag NPs, size: 8.39 ± 0.98 nm	*Danio rerio*	embryo	96 h	Increased mortality and morphological deformities, bradycardia, hatching delay. Increased ROS production, depleted glutathione levels. Co-exposition with cysteine reduced toxicity.	Massarsky et al. 2013
PVP coated Ag NP, size: 10–20 nm	*Danio rerio*	embryo	48 h	A dose-dependent increase in mortality, hatching delay, pericardial oedema, deformed notochord. Down-regulation of marker genes involved in early embryo development (sox17, gsc, ntl, otx2).	Xia et al. 2016
Starch and bovine serum albumin stabilized Ag NPs, size: 5–20 nm	*Danio rerio*	embryo	72 h	A dose-dependent increase in mortality, amount of non-hatched embryos, and presence of sublethal malformation (notochord deformations, pericardial oedema, bradycardia, and cardiac arrhythmia). Accumulation of Ag in the brain, heart, yolk, and blood.	Asharani et al. 2008
PVP stabilized Ag NPs, size: 58.4 ± 8.9 nm, maltose and gelatine stabilised Ag NPs, size: 30.7 ± 0.6 nm	*Danio rerio*	embryo	96 h	Maltose and gelatine stabilized Ag NPs: LC_{50} = 4.31 mg·l^{-1}. PVP stabilized Ag NPs: LC_{50} > 100 mg·l^{-1}. Decreased hatching rate following PVP stabilized Ag NPs exposure, minimal sublethal defects. Presence of heart and yolk sac oedemas and spine deformations following maltose and gelatine stabilized Ag NPs exposition.	Caloudova et al. 2018
Ag NPs, sizes: 4 nm and 10 nm	*Danio rerio*	embryo	96 h	Small head and eyes, hypoplastic hindbrain, cardiac defects. Greater accumulation of Ag in the head area than trunk area, more efficient accumulation of smaller NPs. Down-regulation of neuraldevelopment-related genes (gfap, huC, ngn1), up-regulation of metallothionein genes.	Xin et al. 2015
Spherical Ag NPs, size: 120 nm	*Danio rerio*	embryo	120 h	Decreased hatching rate, morphological defects at high concentrations. Up-regulation of endoplasmic reticulum stress-related genes (BiP and 33 Synv) and pro-apoptotic genes (Noxa and p21).	Christen et al. 2013

Table 1. Summary of studies carried out into the toxicity of silver nanoparticles to fish (continued).

Characterization of the NPs	Model	Stage	Duration	Effect	Reference
Spherical Ag NPs, sizes: 3, 10, 50, and 100 nm	*Danio rerio*	embryo	120 h	Size-dependent increase in mortality and sublethal effects, higher toxicity of smaller NPs. Ag NPs were more toxic than Au NPs.	Bar-Ilan et al. 2009
Citrate stabilized Ag NPs, size: 26.6 ± 8.8 nm	*Danio rerio*	adult/ embryo	48 h	LC_{50} = 7.07 mg·l^{-1} in adult fish, LC_{50} = 7.20 mg·l^{-1} in embryos. Lower toxicity compared to ionic Ag.	Griffitt et al. 2008
PVP coated Ag NPs, size: 81 nm	*Danio rerio*	adult	48 h	Increased rate of operculum movement and surface respiration. LC_{50} = 84 μg·l^{-1}.	Bilberg et al. 2012
PVP coated Ag NPs, size: 10 nm ± 2 nm	*Danio rerio*	adult	24 h	Intramuscular application caused damage and Ag accumulation in the liver, heart, gallbladder, spleen, and kidney. Up-regulation of inflammatory genes for interleukin -1β, tumor necrosis factor alpha, and pro-apoptotic caspase genes.	Speshock et al. 2016
Ag NPs, size: 26.6 ± 8.8 nm	*Danio rerio*	adult	48 h	No morphological changes in the gill. Accumulation of Ag in gill and whole body. Exposure to Ag NPs and ionic Ag produces a distinct gene expression.	Griffitt et al. 2009
Citrate coated Ag NP, size: 20 nm, PVP coated Ag NP, size: 20 nm	*Pimephales promelas*	adult	96 h	Increased mucus production at 4 h, decreased after 28 h of exposure. Cardiac hypertrophy and necrosis, renal necrosis, positive acute-phase proteins.	Garcia-Reyero et al. 2015
Citrate coated Ag NP, size: 20 nm, PVP coated Ag NP, size: 20 nm	*Pimephales promelas*	adult	96 h	Histopathological changes in gill including degenerated goblet cells, decreased Na^+/K^+-ATPase activity. Citrate-coated Ag NP had higher toxicity than ionic Ag.	Hawkins et al. 2015
Spherical, PVP capped Ag NP, size: 22–26 nm	*Danio rerio*	adult	15 days	Enhanced liver oxidative stress, alteration of detoxification enzymes, and decreased brain AChE activity. Sulphidation reduced toxicity.	Devi et al. 2015

NPs – nanoparticles; PVP - polyvinylpyrrolidone

Table 2. Summary of studies carried out into the toxicity of gold nanoparticles to fish.

Characterization of the NPs	Model	Stage	Duration	Effect	Reference
Gold nanospheres, size 38.1 ± 2.8 nm, gold nanorods, nanorods coated with PSS and nanorods coated with both PSS and polyallamine hydrochloride, size: 12.7 ± 1.8 nm by 51.6 ± 8.2 nm	*Danio rerio*	embryo	72 h	No mortality or sublethal effects in nanospheres exposures. Increased mortality and expression of oxidative stress-related genes in nanorod exposures. Decreased hatching rate and bradycardia. Coating and double coating of nanorods reduced the toxicity.	Patibandla et al. 2013
Spherical Au NPs, sizes: 3, 10, 50, and 100 nm	*Danio rerio*	embryo	120 h	Minimal sublethal adverse effects, mortality under 3%.	Bar-Ilan et al. 2009
Citrate capped Au NPs, size: 1.16 nm, PVP capped Au NPs, size: 11.6 nm	*Danio rerio*	embryo	96 h	Low toxicity, cardiac oedemas, lack of movement at high concentrations.	Hlavkova et al. 2020
Citrate and PVP coated Au NPs, sizes: 40 nm	*Sparus aurata*	juvenile	96 h	DNA damage (erythrocyte DNA strand breaks), increased frequency of erythrocytic nuclear abnormalities.	Barreto et al. 2019
Bare and hyaluronic acid capped Au NPs, size: 12.5 nm	*Danio rerio*	adult	96 h	Accumulation of Au only in the gut lumen, no mortality or sublethal effects.	García-Cambero et al. 2013
Citrate capped Au NPs, size: 14 ± 2 nm	*Danio rerio*	adult	96 h	Up-regulation of catalase, superoxide dismutase, and metallothionein genes. Decreased swimming speed and altered behavioral swimming patterns.	Botha et al. 2019
Au NPs, size 14 nm	*Danio rerio*	adult	20 days	Exposed to Au NPs containing sediment. Increased brain AChE activity, modulation of gene expression of genes involved in oxidative stress, mitochondrial metabolism, and DNA repair.	Dedeh et al. 2014
Citrate stabilized Au NPs, sizes: 12 nm, 50 nm	*Danio rerio*	adult	36 and 60 days	Dietary exposure to low concentrations of Au NPs. No accumulation of gold in tissues. Up-regulation of pro-apoptotic and DNA repair genes (c-jun, p53, bax), mitochondrial dysfunction, DNA mutations. Smaller NPs were more toxic.	Geffroy et al. 2011

NPs – nanoparticles; PSS – polystyrenesulphate, PVP - polyvinylpyrrolidone

has good electrical properties and catalytic activity, high light reflectance, and a refractive index. It's stable and insoluble in water (Sungur 2020). There are three crystal structures of TiO_2, brookite, anatase, and rutile. Anatase is considered to be the most chemically reactive (Shi et al. 2013). TiO_2 is used as a photocatalyst in solar panels, in plastics, paints, pharmaceuticals, and even as a food colouring. Since TiO_2 is transparent and blocks UV radiation, it is frequently used in cosmetics, especially sunscreens (Klaine et al. 2008; Ray et al. 2009; Stark et al. 2015).

TiO_2 is generally thought to be a safe, non-toxic material. Griffitt et al. (2008) performed acute, 48-h tests with 30 nm TiO_2 NPs at concentrations of up to 10 $mg \cdot l^{-1}$ on both adult and larval zebrafish, alongside with adult crustacean *Daphnia pulex,* juvenile water fleas *Ceriodaphnia dubia*, and algae (*Pseudokirchneriella subcapitata*, 96-h test). TiO_2 did not cause adverse effects in any of these assays. Similarly, Jemec Kokalj et al. (2019) found no adverse effects on survival, hatching, and sublethal endpoints in zebrafish embryos during an acute 96-h assay, using three anatase TiO_2 NPs (sized 4.9 nm, 30 nm, and 5.1 nm) and three titania Magnéli suboxides (sized 192 nm, 507 nm, and 795 nm) at concentrations of up to 100 $mg \cdot l^{-1}$. Exposure of zebrafish embryos to TiO_2 NPs for 120 h showed no effect on the survival, hatching, and malformation rates, however, the average and maximum velocity and activity level of the larvae were affected already at 0.1 $mg \cdot l^{-1}$ (Chen et al. 2011). However, assays with chronic and subchronic duration bring evidence of adverse effects of TiO_2, such as reproductive toxicity, namely a 29.5% loss of eggs, which was found after a 13-week exposure of zebrafish to 0.1 $mg \cdot l^{-1}$ anatase TiO_2 NPs (Wang et al. 2011). Hepatotoxic effects, likely as an effect of oxidative stress, were found in goldfish (*Carassius auratus*) and zebrafish exposed to solutions TiO_2 NPs as low as 0.01 $mg \cdot l^{-1}$ for 21 days, with a subsequent 14 day depuration period. Results of this study show degeneration of hepatocytes and concentration-dependent increase of malondialdehyde, indicating lipid peroxidation and significant glutathione-S-transferase (GST) activity increase at day 7 of exposure, however, at 21 days the GST activity decreased in both species as a result of prolonged stress (Diniz et al. 2013). Similarly, acute (2 days) and subchronic (14 days) exposures of juvenile benthic fish *Prochilodus lineatus* to TiO_2 NPs lead to the presence of degenerative alterations of hepatocytes and increase of glutathione after both exposures and GST activity after subchronic exposure. Furthermore, a decrease of the red blood cell count and monocytosis after acute exposure was noted. In the case of subchronic exposure, both red blood cell and white blood cell counts including lymphocytes were decreased, which points to immunotoxic effects of TiO_2 NPs (Carmo et al. 2019). This claim is supported by another study, in which fathead minnow was exposed to environmentally relevant concentrations of TiO_2 NPs (2 $ng \cdot g^{-1}$ or 10 $\mu g \cdot g^{-1}$ of body weight), and subsequently challenged with bacterial pathogens *Aeromonas hydrophila* or *Edwardsiella ictaluri*. TiO_2 NPs were found to be accumulated in the kidney and spleen significantly more compared to the liver and rest of the body, resulting in histopathological damage of these organs. Neutrophil phagocytosis was significantly affected, increased mortality and morbidity during bacterial infection was observed (Jovanović et al. 2015). After subchronic (14 days) semi-static exposure of rainbow trout, histopathological changes in gills (oedema and thickening of the gill lamellae) and erosions of enterocytes, as well as decreased Na^+/K^+-ATPase activity in the gills and intestine were observed. Thiobarbituric acid reactive substances (TBARS) were increased in a concentration-dependent manner in the gills, intestine, and brain; the glutathione level was also increased in the gill, suggesting the effect of oxidative stress (Federici et al. 2007). The neurotoxic potential of TiO_2 has been shown by the decrease of muscular AChE activity observed in juvenile *Prochilodus lineatus* in two separate studies (Miranda et al. 2016; Carmo et al. 2019). The summary of studies carried out into the toxicity of TiO_2 NPs to fish is listed in Table 3.

Table 3. Summary of studies carried out into the toxicity of titanium dioxide nanoparticles to fish.

Characterization of the NPs	Model	Stage	Duration	Effect	Reference
TiO_2 NPs (100% anatase), 4.9 ± 1.0 nm, 30 ± 7 nm, sizes: 5.1 ± 1.2 nm	*Danio rerio*	embryo	96 h	No adverse effects observed up to 100 $mg \cdot l^{-1}$.	Jemec Kokalj et al. 2019
TiO_2 NPs, size: 20 nm	*Danio rerio*	embryo	120 h	No effect on survival, hatching, and development of malformations up to 10 mg/l. Decreased velocity and activity level of the larvae.	Chen et al. 2011
TiO_2 NPs (20% rutile and 80% anatase), size: 30 nm	*Danio rerio*	embryo/ adult	48 h	No adverse effects observed up to 10 $mg \cdot l^{-1}$.	Griffit et al. 2008
TiO_2 NPs (75% anatase and 25% rutile), size: 21 nm	*Oncorhynchus mykiss*	juvenile	14 days	Gill pathologies, erosions of enterocytes, decreased Na^+K^+-ATPase activity in the gills and intestine. Increased glutathione levels in the gills and thiobarbituric acid reactive substances in the gill, intestine, and brain.	Federici et al. 2007
TiO_2 NPs, size: 21 nm	*Prochilodus lineatus*	juvenile	5 days/30 days	Pathomorphological changes in the liver, decreased AChE activity in the muscle.	Miranda et al. 2016
TiO_2 NPs, size: 20.5 ± 6.7 nm	*Danio rerio*	adult	48 h	No morphological changes in gill, expression of genes involved in ribosomal function altered.	Griffitt et al. 2009
TiO_2 NPs (100% anatase), size: 240–360 nm	*Danio rerio*	adult	13 weeks	Reproductive toxicity, loss of eggs, decreased number of stage IV follicles, accumulation of Ti in ovaries, alteration of expression of genes involved in proteolysis, oxidative stress regulation, metabolism, insulin signalling, apoptosis, and oocyte maturation. Decreased survival of embryos found after 13-week exposure of female zebrafish to 0.1 $mg \cdot l^{-1}$ TiO_2 NPs.	Wang et al. 2011
TiO_2 NPs (80% anatase and 20% rutile), size: 78 nm	*Carassius auratus* and *Danio rerio*	adult	21 days, depuration 14 days	Pathomorphological changes in hepatocytes, increased levels of malondialdehyde, GST activity increased at day 7, decreased at day 21. Partial recovery during the depuration period.	Diniz et al. 2013
TiO_2 NPs (100% anatase), size: 585 nm	*Pimephales promelas*	adult	28 days	Intramuscular application of low concentrations. Accumulation of Ti and pathomorphological changes in the kidney and spleen. Decreased neutrophil phagocytosis and resistance to bacterial pathogens.	Jovanović et al. 2015

NPs – nanoparticles

Conclusion

With the rise of nanotechnology, the importance of thorough nanotoxicological research is growing, because the properties and behaviour of NPs differ from their bulk and ionic counterparts both *in vitro* and *in vivo*. A proper characterization of the determining properties of NPs, such as their chemical composition, crystal structure, size, shape, presence of coatings, surface chemistry, charge, and solubility, is a key step of every study. When assessing the overall safety of NPs, an important aspect to consider is the different behaviour of NPs in standardized test media and in the environment, in which NPs interact with the substances present – the natural organic matter and inorganic materials. The toxicity of NPs is thought to be generally lower in the environment than in the laboratory settings, but a cautious approach to nanotechnologies is warranted.

Acknowledgements

This work was supported by the grant IGA VFU 217/2020/FVHE. The authors thank Mr. Steven Sangsahachart for a language revision of the manuscript.

References

Angel BM, Batley GE, Jarolimek CV, Rogers NJ 2013: The impact of size on the fate and toxicity of nanoparticulate silver in aquatic systems. Chemosphere **93**: 359-365

Asharani PV, Lian Wu Y, Gong Z, Valiyaveettil S 2008: Toxicity of silver nanoparticles in zebrafish models. Nanotechnology **19**: 1-7

Ates M, Arslan Z, Demir V, Daniels J, Farah IO 2015: Accumulation and toxicity of CuO and ZnO nanoparticles through waterborne and dietary exposure of goldfish (*Carassius auratus*). Environ Toxicol **30**: 119-128

Azizi-Lalabadi M, Ehsani A, Divband B, Alizadeh-Sani M 2019: Antimicrobial activity of titanium dioxide and zinc oxide nanoparticles supported in 4A zeolite and evaluation the morphological characteristic. Sci Rep **9**: 17439

Bar-Ilan O, Albrecht RM, Fako VE, Furgeson DY 2009: Toxicity assessments of multisized gold and silver nanoparticles in zebrafish embryos. Small **5**: 1897-1910

Barreto A, Luis LG, Pinto E, Almeida A, Paíga P, Santos LHMLM, Delerue-Matos C, Trindade T, Soares AMVM, Hylland K, Loureiro S, Oliveira M 2019: Genotoxicity of gold nanoparticles in the gilthead seabream (*Sparus aurata*) after single exposure and combined with the pharmaceutical gemfibrozil. Chemosphere **220**: 11-19

Batley GE, Kirby JK, McLaughlin MJ 2013: Fate and risks of nanomaterials in aquatic and terrestrial environments. Acc Chem Res **46**: 854-862

Bilberg K, Hovgaard MB, Besenbacher F, Baatrup E 2012: *In vivo* toxicity of silver nanoparticles and silver ions in zebrafish (*Danio rerio*). J Toxicol **2012**: 1-9

Binnig G, Rohrer H, Gerber C, Weibel E 1982: Surface studies by scanning tunneling microscopy. Phys Rev Lett **49**: 57-61

Botha TL, Brand SJ, Ikenaka Y, Nakayama SMM, Ishizuka M, Wepener V 2019: How toxic is a non-toxic nanomaterial: Behaviour as an indicator of effect in *Danio rerio* exposed to nanogold. Aquat Toxicol **215**: 1-10

Caloudova H, Hodkovicova N, Sehonova P, Blahova J, Marsalek B, Panacek A, Svobodova Z 2018: The effect of silver nanoparticles and silver ions on zebrafish embryos (*Danio rerio*). Neuro Endocrinol Lett **39**: 299-304

Carmo TLL, Siqueira PR, Azevedo VC, Tavares D, Pesenti EC, Cestari MM, Martinez CBR, Fernandes MN 2019: Overview of the toxic effects of titanium dioxide nanoparticles in blood, liver, muscles, and brain of a Neotropical detritivorous fish. Environ Toxicol **34**: 457-468

Chandra P, Singh J, Srivastava A, Goyal RN, Shim YB 2013: Gold nanoparticles and nanocomposites in clinical diagnostics using electrochemical methods. J Nanoparticles **2013**: 1-12

Chen J, Li H, Han X, Wei X 2015: Transmission and accumulation of nano-TiO_2 in a 2-step food chain (*Scenedesmus obliquus* to *Daphnia magna*). Bull Environ Contam Toxicol **95**: 145-149

Chen T-H, Lin C-Y, Tseng M-C 2011: Behavioral effects of titanium dioxide nanoparticles on larval zebrafish (*Danio rerio*). Mar Pollut Bull **63**: 303-308

Christen V, Capelle M, Fent K 2013: Silver nanoparticles induce endoplasmatic reticulum stress response in zebrafish. Toxicol Appl Pharmacol **272**: 519-528

Chupani L, Barta J, Zuskova E 2019. Effects of food-borne ZnO nanoparticles on intestinal microbiota of common carp (*Cyprinus carpio* L.). Environ Sci Pollut Res **26**: 25869-25873

Chupani L, Niksirat H, Velíšek J, Stará A, Hradilová Š, Kolařík J, Panáček A, Zusková E 2018a: Chronic dietary toxicity of zinc oxide nanoparticles in common carp (*Cyprinus carpio* L.): Tissue accumulation and physiological responses. Ecotoxicol Environ Saf **147**: 110-116

Chupani L, Niksirat H, Lünsmann V, Haange SB, Von Bergen M, Jehmlich N, Zuskova E 2018b: Insight into the modulation of intestinal proteome of juvenile common carp (*Cyprinus carpio* L.) after dietary exposure to ZnO nanoparticles. Sci Total Environ **613-614**: 62-71

Chupani L, Zusková E, Niksirat H, Panáček A, Lünsmann V, Haange S-B, von Bergen M, Jehmlich N 2017: Effects of chronic dietary exposure of zinc oxide nanoparticles on the serum protein profile of juvenile common carp (*Cyprinus carpio* L.). Sci Total Environ **579**: 1504-1511

Clemente Z, Castro VL, Feitosa LO, Lima R, Jonsson CM, Maia AHN, Fraceto LF 2013: Fish exposure to nano-TiO_2 under different experimental conditions: Methodological aspects for nanoecotoxicology investigations. Sci Total Environ **463-464**: 647-656

Coello WF, Khan MAQ 1996: Protection against heavy metal toxicity by mucus and scales in fish. Arch Environ Contam Toxicol **30**: 319-326

Dedeh A, Ciutat A, Treguer-Delapierre M, Bourdineaud J-P 2014: Impact of gold nanoparticles on zebrafish exposed to a spiked sediment. Nanotoxicol **9**: 71-80

Deng R, Lin D, Zhu L, Majumdar S, White JC, Gardea-Torresde JL, Xing B 2017: Nanoparticle interactions with co-existing contaminants: joint toxicity, bioaccumulation and risk. Nanotoxicol **11**: 591-612

Devi GP, Ahmed KBA, Varsha MKNS, Shrijha BS, Lal KKS, Anbazhagan V, Thiagarajan R 2015: Sulfidation of silver nanoparticle reduces its toxicity in zebrafish. Aquat Toxicol **158**: 149-156

Diniz MS, De Matos APA, Lourenço J, Castro L, Peres I, Mendonça E, Picado A 2013: Liver alterations in two freshwater fish species (*Carassius auratus* and *Danio rerio*) following exposure to different TiO_2 nanoparticle concentrations. Microsc Microanal **19**: 1131-1140

European Commission: Opininon on nanosilver: safety, health and environmental effects and role in antimicrobial resistance. Publications Office of the EU. Available at: https://op.europa.eu/en/publication-detail/-/publication/0dde6746-59a2-419d-91d8-fe2d14a2fec0/language-en. Last modified June 11, 2014. Accessed December 1, 2020.

Fard JK, Jafari S, Eghbal MA 2015: A review of molecular mechanisms involved in toxicity of nanoparticles. Adv Pharm Bull **5**: 447-454

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

Fu PP, Xia Q, Hwang H-M, Ray PC, Yu H 2014: Mechanisms of nanotoxicity: Generation of reactive oxygen species. J Food Drug Anal **22**: 64-75

Gaiser BK, Fernandes TF, Jepson MA, Lead JR, Tyler CR, Baalousha M, Biswas A, Britton GJ, Cole PA, Johnston BD, Ju-Nam Y, Rosenkranz P, Scown TM, Stone V 2011: Interspecies comparisons on the uptake and toxicity of silver and cerium dioxide nanoparticles. Environ Toxicol Chem **31**: 144-154

García-Cambero JP, Núñez García M, Lopéz GD, Herranz AL, Cuevas L, Pérez-Pastrana E, Cuadal JS, Castelltort MR, Calvo AC 2013: Converging hazard assesment of gold nanoparticles to aquatic organisms. Chemposphere **93**: 1194-1200

Garcia-Reyero N, Thornton C, Hawkins AD, Escalon L, Kennedy AJ, Steevens JA, Willett KL 2015: Assessing the exposure to nanosilver and silver nitrate on fathead minnow gill gene expression and mucus production. Environ Nanotechnol Monit Manag **4**: 58-66

Geertsma RE, Park MVDZ, Puts CF, Roszek B, Van der Stijl R, De Jong WH 2015: Nanotechnologies in medical devices. National Institute for Public Health and the Environment, Bilthoven, 83 p.

Geffroy B, Ladhar C, Cambier S, Treguer-Delapierre M, Brèthes D, Bourdineaud J-P 2011: Impact of dietary gold nanoparticles in zebrafish at very low contamination pressure: The role of size, concentration and exposure time. Nanotoxicol **6**: 144-160

Global Industry Analysts, Inc. 2020: Metal Nanoparticles - Global Market Trajectory & Analytics. Global Industry Analysts, Inc, Dublin, 187 p.

Gottschalk F, Lassen C, Kjoelholt J, Christensen F, Nowack B 2015: Modeling flows and concentrations of nine engineered nanomaterials in the Danish environment. International Int J Environ Res Public Health **12**: 5581-5602

Gottschalk F, Sonderer T, Scholz RW, Nowack B 2009: Modelled environmental concentrations of engineered nanomaterials (TiO_2, ZnO, Ag, CNT, fullerenes) for different regions. Environ Sci Technol **43**: 9216-9222

Grand View Research, Inc. 2020: Nanomaterials Market Size, Share & Trends Analysis Report By Product (Carbon Nanotubes, Titanium Dioxide), By Application (Medical, Electronics, Paints & Coatings), By Region, And Segment Forecasts, 2020 - 2027. Grand View Research, Inc., San Francisco, 113 p.

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

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

Gurunathan S 2015: Biologically synthesized silver nanoparticles enhances antibiotic activity against Gram-negative bacteria. J Ind Eng Chem **29**: 217-226

Handy RD, Henry TB, Scown TM, Johnston BD, Tyler CR 2008: Manufactured nanoparticles: their uptake and effects on fish – a mechanistic analysis. Ecotoxicology **17**: 396-409

Hawkins AD, Thornton C, Kennedy AJ, Bu K, Cizdziel J, Jones BW, Steevens JA, Willett KL 2015: Gill histopathologies following exposure to nanosilver or silver nitrate. J Toxicol Environ Health A **78**: 301-315

Hlavkova D, Caloudova H, Palikova P, Kopel P, Plhalova L, Beklova M, Havelkova B 2020: Effect of gold nanoparticles and ions exposure on the aquatic organisms. Bull Environ Contam Toxicol **105**: 530-537
Hogstrand C, Wood CM 1998: Toward a better understanding of the bioavailability, physiology, and toxicity of silver in fish: Implications for water quality criteria. Environ Toxicol Chem **17**: 547-561
Horikoshi S, Serpone N 2013: Microwaves in nanoparticle synthesis: fundamentals and applications. Wiley-VCH Verlag GmbH and Co, Weinheim, 352 p.
Hou WC, Stuart B, Howes R, Zepp RG 2013: Sunlight-driven reduction of silver ions by natural organic matter: formation and transformation of silver nanoparticles. Environ Sci Technol **47**: 7713-7721
Hua J, Vijver MG, Richardson MK, Ahmad F, Peijnenburg WJGM 2014: Particle-specific toxic effects of differently shaped zinc oxide nanoparticles to zebrafish embryos (*Danio rerio*). Environ Toxicol Chem **33**: 2859-2868
Impellitteri CA, Harmon S, Silva RG, Miller BW, Scheckel KG, Luxton TP, Schupp D, Panguluri S 2013: Transformation of silver nanoparticles in fresh, aged, and incinerated biosolids. Water Res **47**: 3878-3886
International Organization for Standardization 2011: Nanotechnologies–Vocabulary–Part 4: Nanostructured materials. International (ISO Standard 80004-4). ISO copyright office, Geneva, 7 p.
Iswarya V, Manivannan J, De A, Subhabrata P, Rajdeep R, Johnson JB, Kundu R, Chandrasekaran N, Mukherjee A, Mukherjee A 2016: Surface capping and size-dependent toxicity of gold nanoparticles on different trophic levels. Environ Sci Pollut Res **23**: 4844-4858
Ivask A, Kurvet I, Kasemets K, Blinova I, Aruoja V, Suppi S, Vija H, Käkinen A, Titma T, Heinlaan M, Visnapuu M, Koller D, Kisand V, Kahru A, Quigg A 2014: Size-dependent toxicity of silver nanoparticles to bacteria, yeast, algae, crustaceans and mammalian cells *in vitro*. PLoS One **9**: 102-108
Jemec Kokalj A, Novak S, Talaber I, Kononenko V, Bizjak Mali L, Vodovnik M, Žegura B, Eleršek T, Kalčikova G, Žgajnar Gotvajn A, Kralj S, Makovec D, Caloudova H, Drobne D 2019: The first comprehensive safety study of Magnéli phase titanium suboxides reveals no acute environmental hazard. Environ SciNano **6**: 1131-1139
Jovanović B, Whitley EM, Kimura K, Crumpton A, Palić D 2015: Titanium dioxide nanoparticles enhance mortality of fish exposed to bacterial pathogens. Environ Pollut **203**: 153-164
Kaegi R, Voegelin A, Ort C 2013: Fate and transformation of silver nanoparticles in urban wastewater systems. Water Res **47**: 3866-3877
Khan MS, Jabeen F, Qureshi NA, Asghar MS, Shakeel M, Noureen A 2015: Toxicity of silver nanoparticles in fish: a critical review. JBES **6**: 211-227
Khanna P, Kaur A, Goyal D 2019: Algae-based metallic nanoparticles: Synthesis, characterization and applications. J Microbiol Methods. **163**: 105656
Khot LR, Sankaran S, Maja JM, Ehsani R, Schuster EW 2012: Applications of nanomaterials in agricultural production and crop protection: A review. J Crop Prot **35**: 64-70
Klaine SJ, Alvarez PJJ, Batley GE, Fernandes TF, Handy RD, Lyon DY, Mahendra S, McLaughlin MJ, Lead JR 2008: Nanomaterials in the environment: Behavior, fate, bioavailability, and effects. Environ Toxicol Chem **27**: 1825-1851
Kovářík L, Dvořák P, Beňová K, Doležalová J, Tomko M, Špalková M 2020: Interaction of radionuclide 131I and cadmium chloride in an alternative bioassay with *Artemia franciscana* evaluated by a digital record. Acta Vet Brno **89**: 413-420
Kwak JI, An YJ 2016: The current state of the art in research on engineered nanomaterials and terrestrial environments: Different-scale approaches. Environ Res **151**: 368-382
Lapresta-Fernández A, Fernández A, Blasco J 2012: Nanoecotoxicity effects of engineered silver and gold nanoparticles in aquatic organisms. Trends Analyt Chem **32**: 40-59
Lee KJ, Nallathamby PD, Browning LM, Osgood CJ, Xu X-HN 2007: *In vivo* imaging of transport and biocompatibility of single silver nanoparticles in early development of zebrafish embryos. ACS Nano **1**: 133-143
Li L, Sillanpää M, Risto M 2016: Influences of water properties on the aggregation and deposition of engineered titanium dioxide nanoparticles in natural waters. Environ Pollut **219**: 132-138
Liu Y, Majetich SA, Tilton RD, Sholl DS, Lowry GV 2005: TCE dechlorination rates, pathways, and efficiency of nanoscale iron particles with different properties. Environ Sci Technol **39**: 1338-1345
Ma S, Lin D 2013: The biophysicochemical interactions at the interfaces between nanoparticles and aquatic organisms: adsorption and internalization. Environ Sci Processes Impacts **15**: 145-160
Mahapatra I, Sun TY, Clark JRA, Dobson PJ, Hungerbuehler K, Owen R, Nowack B, Lead J 2015: Probabilistic modelling of prospective environmental concentrations of gold nanoparticles from medical applications as a basis for risk assessment. J Nanobiotechnology **13**: 1-14
Manke A, Wang L, Rojanasakul Y 2013: Mechanisms of nanoparticle-induced oxidative stress and toxicity. BioMed Res Int **2013**: 1-15
Massarsky A, Dupuis L, Taylor J, Eisa-Beygi S, Strek L, Trudeau VL, Moon TW 2013: Assessment of nanosilver toxicity during zebrafish (*Danio rerio*) development. Chemosphere **92**: 59-66
Minghetti M, Schirmer K 2016: Effect of media composition on bioavailability and toxicity of silver and silver nanoparticles in fish intestinal cells (RTgutGC). Nanotoxicol **10**: 1526-1534

Miranda RR, Damaso da Silveira ALR, de Jesus IP, Grötzner SR, Voigt CL, Campos SX, Garcia JRE, Randi MAF, de Oliveira Ribeiro CA, Filipak Neto F 2016: Effects of realistic concentrations of TiO_2 and ZnO nanoparticles in *Prochilodus lineatus* juvenile fish. Environ Sci Pollut Res **23**: 5179-5188

Monteiro-Riviere NA, Tran CL 2007: Nanotoxicology characterization, dosing and health effects. Informa Healthcare, New York, 425 p.

Morgan IJ, Henry RP, Wood CM 1997: The mechanism of acute silver nitrate toxicity in freshwater rainbow trout (*Oncorhynchus mykiss*) is inhibition of gill Na^+ and Cl^{-1} transport. Aquat Toxicol **38**: 145-163

Nam D-H, Lee, B-C, Eom I-C, Kim P, Yeo M-K 2014: Uptake and bioaccumulation of titanium – and silver-nanoparticles in aquatic ecosystems. Mol Cell Toxicol **10**: 9-17

Nel A, Xia T, Mädler N, Li N 2006: Toxic potential of materials at the nanolevel. Science **311**: 622-627

Nowack B, Krug HF, Height M 2011: 120 Years of nanosilver history: Implications for policy makers. Environ Sci Technol **45**: 1177-1183

Pamies R, Cifre JGH, Espín VF, Collado-González M, Baños FGD, de la Torre JG 2014: Aggregation behaviour of gold nanoparticles in saline aqueous media. J Nanopart Res, **16**: 2376

Park K, Tuttle G, Sinche F, Harper SL 2013: Stability of citrate-capped silver nanoparticles in exposure media and their effects on the development of embryonic zebrafish (*Danio rerio*). Arch Pharm Res **36**: 125-133

Patibandla S, Zhang Y, Tohari AM, Gu P, Reilly J, Chen Y, Shu X 2018: Comparative analysis of the toxicity of gold nanoparticles in zebrafish. J Appl Toxicol **38**: 1153-1162

Peters RJB, van Bemmel G, Milani NBL, den Hertog GCT, Undas AK, van der Lee M, Bouwmeester H 2018: Detection of nanoparticles in Dutch surface waters. Sci Total Environ **621**: 210-218

Piccinno F, Gottschalk F, Seeger S, Nowack B 2012: Industrial production quantities and uses of ten engineered nanomaterials in Europe and the world. J Nanopart Res **14**: 1109-1120

Pulit-Prociak J, Banach M 2016: Silver nanoparticles – a material of the future…? Open Chem J **14**: 76-91

Ray PC, Yu H, Fu PP 2009: Toxicity and environmental risks of nanomaterials: Challenges and future needs. J Environ Sci Health, Part C **27**: 1-35

Ribeiro F, Gallego-Urrea JA, Jurkschat K, Crossley A, Hassellöv M, Taylor C, Soares AMVM, Loureiro S 2014: Silver nanoparticles and silver nitrate induce high toxicity to *Pseudokirchneriella subcapitata, Daphnia magna* and *Danio rerio*. Sci Total Environ **466-467**: 232-241

Sevcikova M, Modra H, Slaninova A, Svobodova Z 2011: Metals as a cause of oxidative stress in fish: a review. Vet Med **56**: 537-546

Shaalan M, Saleh M, El-Mahdy M, El-Matbouli M 2016: Recent progress in applications of nanoparticles in fish medicine: A review. Nanomedicine **12**: 701-710

Schaumann GE, Philippe A, Bundschuh M, Metreveli G, Klitzke S, Rakcheev D, Grün A, Kumahor SK, Kühn M, Baumann T, Lang F, Manz W, Schulz R, Vogel H-J 2015: Understanding the fate and biological effects of Ag- and TiO2-nanoparticles in the environment: The quest for advanced analytics and interdisciplinary concepts. Sci Total Environ **535**: 3-19.

Sharma R, Garg R, Kumari A 2020: A review on biogenic synthesis, applications and toxicity aspects of zinc oxide nanoparticles. EXCLI J **19**: 1325-1340

Shi H, Magaye R, Castranova V, Zhao J 2013: Titanium dioxide nanoparticles: a review of current toxicological data. Part Fibre Toxicol **10**: 15-48

Shih YJ, Su CC, Chen CW, Dong CD, Liu WS, Huang CP 2016: Adsorption characteristics of nano-TiO_2 onto zebrafish embryos and its impacts on egg hatching. Chemosphere **154**: 109-117

Skjolding LM, Sørensen SN, Hartmann NB, Hjorth R, Hansen SF, Baun A 2016: Aquatic ecotoxicity testing of nanoparticles – The quest to disclose nanoparticle effects. Angew Chem **55**: 15224-15239

Speshock JL, Elrod N, Sadoski DK, Maurer E, Braydich-Stolle LK, Brady J, Hussain S 2016: Differential organ toxicity in the adult zebra fish following exposure to acute sub-lethal doses of 10 nm silver nanoparticles. Front Nanosci Nanotech **2**: 114-120

Speshock JL 2018: Review of the toxicological effects of silver nanomaterials on the model aquatic organism *Danio rerio*. Front Nanosci Nanotech **4**: 1-4

Stark WJ, Stoessel PR, Wohlleben W, Hafner A 2015: Industrial applications of nanoparticles. Chem Soc Rev **44**: 5793-5805

Sun TY, Gottschalk F, Hungerbühler K, Nowack B 2014: Comprehensive probabilistic modelling of environmental emissions of engineered nanomaterials. Environ Pollut **185**: 69-76

Sun, TY, Bornhöft NA, Hungerbühler K, Nowack B 2016: Dynamic probabilistic modeling of environmental emissions of engineered nanomaterials. Environ Sci Technol **50**: 4701-4711

Sungur Ş 2020: Titanium Dioxide Nanoparticles. In: Kharissova O, Martínez L, Kharisov B (Ed.): Handbook of nanomaterials and nanocomposites for energy and environmental applications. Springer Cham, Switzerland, pp. 1-18

Svoboda M, Drápal J, Haruštiaková D, Svobodová Z 2020: A multiannual survey of cadmium content in pig tissues collected in the Czech Republic during the years 2015–2019. Acta Vet Brno **89**: 349-355

Tian J, Wong KKY, Ho C-M, Lok C-N, Yu W-Y, Che C-M, Chiu J-F, Tam PKH 2007: Topical delivery of silver nanoparticles promotes wound healing. Chem Med Chem **2**: 129-136

Uddin MN, Desai F, Asmatulu E 2020: Engineered nanomaterials in the environment: bioaccumulation, biomagnification and biotransformation. Environ Chem Lett **18**: 1073-1083

Ulrich K, Jakob U 2019: The role of thiols in antioxidant systems. Free Radic Biol Med **140**: 14-27
Valério A, Sárria MP, Rodriguez-Lorenzo L, Hotza D, Espiña B, Gómez González SY 2020: Are TiO_2 nanoparticles safe for photocatalysis in aqueous media? Nanoscale Adv **2**: 4951-4960
Van Aerle R, Lange A, Moorhouse A, Paszkiewicz K, Ball K, Johnston BD, De-Bastos E, Booth T, Tyler CR, Santos EM 2013: Molecular mechanisms of toxicity of silver nanoparticles in zebrafish embryos. Environ Sci Technol **47**: 8005-8014
Wang J, Zhu X, Zhang X, Zhao Z, Liu H, George R, Wilson-Rawls J, Chang Y, Chen, Y 2011: Disruption of zebrafish (*Danio rerio*) reproduction upon chronic exposure to TiO_2 nanoparticles. Chemosphere **83**: 461-467
Warheit DB 2008: How meaningful are the results of nanotoxicity studies in the absence of adequate material characterization? Toxicol Sci **101**: 183-185
Wijnhoven SWP, Peijnenburg WJGM, Herberts CA, Hagens WI, Oomen AG, Heugens EHW, Roszek B, Bisschops J, Gosens I, Van De Meent D, Dekkers S, De Jong WH, Van Zijverden M, Sips AJAM, Geertsma RE 2009: Nano-silver – a review of available data and knowledge gaps in human and environmental risk assessment. Nanotoxicol **3**: 109-138
Xia G, Liu T, Wang Z, Hou Y, Dong L, Zhu J, Qi J 2016: The effect of silver nanoparticles on zebrafish embryonic development and toxicology. Artif Cells Nanomed Biotechnol **44**: 1116-1121
Xin Q, Rotchell JM, Cheng J, Yi J, Zhang Q 2015: Silver nanoparticles affect the neural development of zebrafish embryos. J Appl Toxicol **35**: 1481-1492
Yaqoob AA, Ahmad H, Parveen T, Ahmad A, Oves M, Ismail IMI, Quari HA, Umar K, Mohamad IMN 2020: Recent advances in metal decorated nanomaterials and their various biological applications: A review. Front Chem **8**: 341
Yeo M-K, Kang M 2008: Effects of nanometer sized silver materials on biological toxicity during zebrafish embryogenesis. Bull Korean Chem Soc **29**: 1179-1184
Yoo-iam M, Chaichana R, Satapanajaru T 2014: Toxicity, bioaccumulation and biomagnification of silver nanoparticles in green algae (*Chlorella* sp.), water flea (*Moina macrocopa*), blood worm (*Chironomus* spp.) and silver barb (*Barbonymus gonionotus*). Chem Speciat Bioavailab **26**: 257-265
Yue Y, Behra R, Sigg L, Suter M J-F, Pillai S, Schirmer K 2016: Silver nanoparticle – protein interactions in intact rainbow trout gill cells. Environ Sci Nano **3**: 1174-1185
Zhang X-F, Liu Z-G, Shen W, Gurunathan S 2016: Silver nanoparticles: Synthesis, characterization, properties, applications, and therapeutic approaches. Int J Mol Sci **17**: 1534
Zhou M, Wang B, Rozynek Z, Xie Z, Fossum JO, Yu X, Raaen S 2009: Minute synthesis of extremely stable gold nanoparticles. Nanotechnology **20**: 1-10
Zhu X, Wang J, Zhang X, Chang Y, Chen Y 2010: Trophic transfer of TiO_2 nanoparticles from daphnia to zebrafish in a simplified freshwater food chain. Chemosphere **79**: 928-933
Zhu XS, Zhou J, Cai ZH 2011: TiO_2 nanoparticles in the marine environment: Impact on the toxicity of tributyltin to abalone (*Haliotis diversicolor supertexta)* embryos. Environ Sci Technol **45**: 3753-3758