ECRONICON OPEN ACCESS

EC PHARMACOLOGY AND TOXICOLOGY

Review Article

Toxicity Induced by Engineered Nanoparticles in Fresh Water Fish- a Review of Recent Developments

Suresh VS Rana*

Professor Emeritus, Department of Toxicology, Ch Charan Singh University, Meerut, India

***Corresponding Author:** Suresh VS Rana, Professor Emeritus, Department of Toxicology, Ch Charan Singh University, Meerut, India.

Received: May 12, 2021; **Published:** July 29, 2021

Abstract

Present review highlights current research available on the toxic behaviour of a few widely used nanoparticles in several fresh water fish inhabiting different niche and aquatic habitats. Information collected from suitable databases on carbon nanotubes, fullerenes, quantum dots and nanoparticles of silver, gold, titanium, zinc, copper, cerium shows their ubiquitous presence in water bodies. Anthropogenic activities discharge them into near- by aquatic systems where they tend to be residual. These particles, in fish are absorbed through gills and mouth. Trophic relationships also facilitate their intake through food chain. Depending upon their physico-chemical properties and water quality, their bioaccumulation occurs in soft tissues viz. gills, liver, kidney, brain and gonads of the fish.

Frequently used fish models for their toxicity assessment include zebra fish (*Danio rerio*); fresh water carp (*Cyprinus carpio*); rainbow trout (*Oncorhynchus mykiss*) and tilapia (*Oreochromis niloticus*). A few workers have used medaka fish and cat fish also. *In vitro* studies mainly deal with embryos of zebra fish, RTG-2 cells of rainbow trout and gonad cell lines. These reports describe their effects on gills, liver, kidney, brain, gonads and immune system. Dose dependent effects of NPs in different species have been assessed employing a variety of parameters viz. lipid peroxidation, oxidative stress, antioxidant enzymes and histopathology. Factors responsible for inflammation and apoptosis have also been studied in a few species.

Nonetheless, several endpoint mechanisms i.e. mitochondrial homeostasis, endoplasmic reticulum stress, reductive stress and metallothionein induction need to be studied further. In nut shell, our understanding on nanoparticle toxicity in fish so far, remains incomplete and warrants further research on molecular mechanisms of their toxicity.

***Keywords**: Fish; Nanoparticles; Oxidative Stress; Apoptosis and Organ Toxicity*

Abbreviations

ENP: Engineered Nanoparticle; CNT: Carbon Nanotube; QDs: Quantum Dots; AgNPs: Silver Nanoparticles; AuNPs: Gold Nanoparticles; TiO2NPs: Titanium Dioxide Nanoparticles; ZnONPs: Zinc Oxide Nanoparticles; CuNPs: Copper Nanoparticles; CeO2NPs: Cerium Oxide Nanoparticles; SWCNT: Single Walled Carbon Nanotubes; DWCNT: Double Walled Carbon Nanotubes; MWCNT: Multiwalled Carbon Nanotubes; GSH: Reduced Glutathione; TBARS: Thiobarbituric Acid Reactive Substances; SOD: Super Oxide Dismutase; GPx: Glutathione Peroxidase; GR: Glutathione Reductase; CAT: Catalase; LPO: Lipid Peroxidation; ROS: Reactive Oxygen Species

Introduction

The word nano has been derived from a Greek term "nanos" meaning-dwarf. Nanotechnology has been briefly defined as designing, characterization, production and application of structures/devices whose shape and size are restricted to nanometer (nm) level [1]. Nanoparticles (NPs) possess multiple applications in several commercial products viz. electronics, cosmetics, drug delivery, medicine, agriculture, industry and environmental remediation. According to an estimate there exist about 1015 consumer products made from nanomaterials [2].

Engineered nanoparticles (ENPs) have emerged as a new class of environmental pollutants that may contaminate atmosphere, hydrosphere and terrestrial ecosystems [3]. ENPs can alter soil chemistry, migrate to surface or ground water and affect the aquatic life. Waste water effluents, rain water wash offs and accidental spillage further increase their load in the ecosystem. Physical processes like aggregation, dissolution, absorption and surface transformation determine their behaviour in aquatic ecology. Prevalent environmental conditions i.e. temperature, pH, salinity, and hardness influence their entry into biogeochemical cycles. Additionally, presence of natural organic matter (NOM) can also influence the interaction between ENPs and aquatic biota altering their bioavailability, electrostatic interactions and steric repulsion (Figure 1).

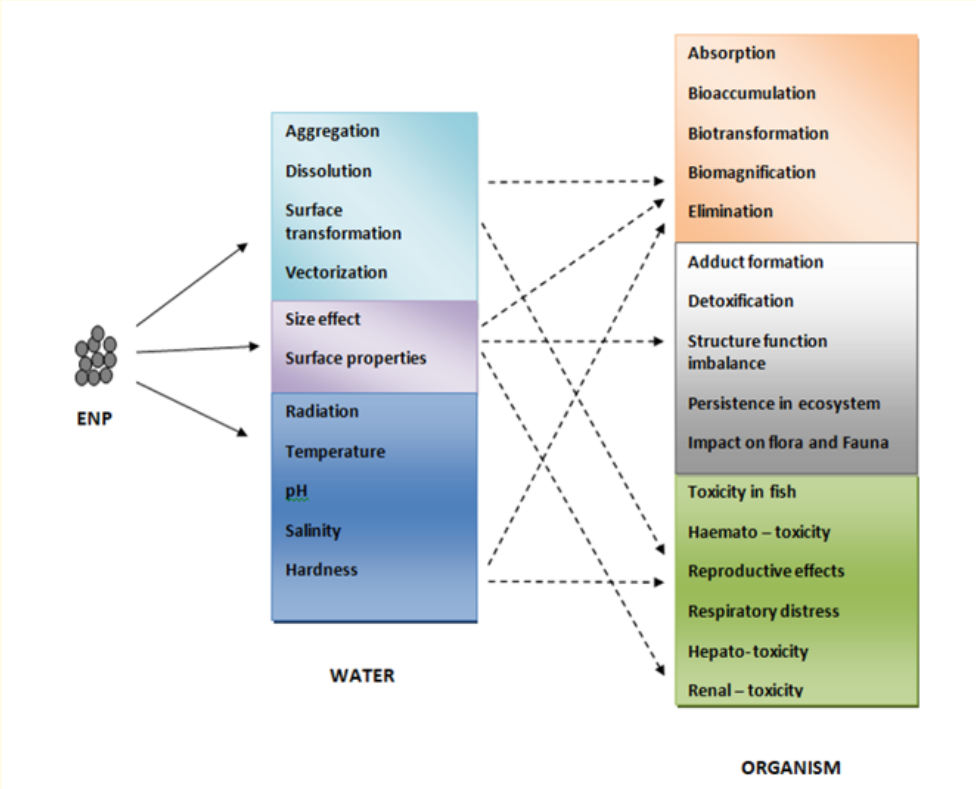

Figure 1: *Determinants involved in the toxicity of ENPs in a fish.*

Theoretically, NPs can be produced from any chemical, however, most of them are made from transition metals i.e. silicon, carbon (carbon nano tubes and fullerenes) and metal oxides [4]. In a water body, they can bind with other pollutants through vectorization [5]. Very high surface to volume ratio facilitates their binding with inorganic and organic substances [6]. Furthermore, ENPs can be bio-transformed by aquatic microorganisms and biomagnified through food chain. Therefore, understanding the toxic manifestations of ENPs in aquatic organisms appears to be much more difficult than other experimental animals.

Purpose of the Study

The purpose of present review is to highlight the current trends in toxicology of ENPs particularly in fresh water fish. The available information collected from suitable databases on the toxicity of carbon nanotubes, fullerenes, quantum dots, silver nano-particles, titanium dioxide nanoparticles along with NPs of cerium, copper and gold in fresh water fish is described in this overview.

Methods

Available information on the toxicity of ENPs in fresh water fish was assessed exploring various bibliometric databases viz. www.googlescholar.com., www.pubmed/gov and www.researchgate.com. Research on marine fish was excluded from this review. The basic results of this survey are exhibited by table 1 and 2.

Species	Number of publications (2011 - 2020)
Marine fish	200
Zebra fish	708
Fresh water carp	80
Rainbow trout	98
Cat fish	73
Cold water fish	07
Murrels	01

Table 1: *Summary of reports available on NP toxicity in different species of fish. Source: www.pubmed.gov (assessed in Oct. 2020).*

Species	Number of publications (2011 - 2020)
Caenorahabditis elegans	272
Crustaceans	548
Aquatic insects	03
Earthworms	176
Molluscs	244
Echinoderms	40
Frog	109
Xenopus laevis	55
Salamander	07

Table 2: *Summary of publications available on NP toxicity in other aquatic organisms. Source: www.pubmed.gov (assessed in Oct. 2020).*

Exposure and toxicity of carbon nanotubes (CNTs) in fish

CNTs were first described by Iijima [7]. Cylindrical in structure, they are composed solely of carbon atoms. They exhibit unique properties like strength, hardness, thermal conductivity, microwave absorption, electrical and catalytic properties [8]. They can easily be tailored for specific applications viz. electronic devices, waste water treatment and drug delivery systems [9,10]. An estimated 33 tons of CNTs were expected to enter water bodies annually [11]. Possible release pathways include industrial discharge, land fill leachates, accidental spill or marine dumping of contaminated mud/soil.

CNTs can further be classified into single walled (SWCNT), double walled (DWCNT) and multi walled (MWCNTs) CNTs. Increased production and subsequent release into the environment raises the possibilities of their entry into the aquatic food chain. In fish, like other xenobiotics main portals of entry of NPs are mouth and gills. Gill filaments are their functional units responsible for transfer of ions and water as well as the exchange of oxygen, carbon dioxide, acids and ammonia. Each lamellum consists of a central sheet of pillar cells with concave sides that form blood spaces. These cells are covered by a thin epithelium (Figure 2). Fish gills were found to be sensitive to NPs [12]. Can NPs like oxygen diffuse from water across epithelial cells? This issue was addressed by Bjorkland and colleagues [13] who showed that little or no absorption of NPs occurs through gut but gill epithelium is the main route of their absorption. It does not occur by diffusion but through endocytosis [14]. Only five reports on the toxicity of CNTs in fish were published between 2011 - 2020 (www.pubmed.gov.). These studies revealed species differences in the effects of different types of CNTs. While no acute toxicity of SWCNTs was recorded in medaka fish (*Oryzias latipes*) [15], DWCNTs inhibited the growth of another medaka fish, *Oryzias melastigma* [16]. Another study made in two different species of fish i.e. *Danio rerio* and *Astyanax altiparanae* demonstrated no genotoxic effects of CNTs [17]. Nonetheless, oxidative injury in respiratory organs of rainbow trout (*Oncorhynchus mykiss*) exposed to SWCNTs indicated their toxic properties [18]. Environmental implications of CNTs with a classical water pollutant, lead were studied by Martinez and colleagues [19]. Nile tilapia (*Oreochromis tilapia*) was exposed to different concentrations of lead mixed with toxic concentration of HNO_3-MWCNT (1.0 mg/L) for 24, 48, 72, 96 hr). They observed five times increase in lead toxicity. Histopathological studies made by the same group also indicated their toxicity in Nile tilapia [20]. NP - carbofuran interaction was also studied in Nile tilapia in the same laboratory [21]. This report suggested that CNTs not only potentiated carbofuran toxicity but served as pesticide carriers. Intriguingly, lead toxicity was decreased in *Daphnia magna* after exposure to positively charged MWCNTs. Negatively charged MWCNTs expressed a slight effect [22]. Thus, toxicity of CNTs in fish still remains inconclusive.

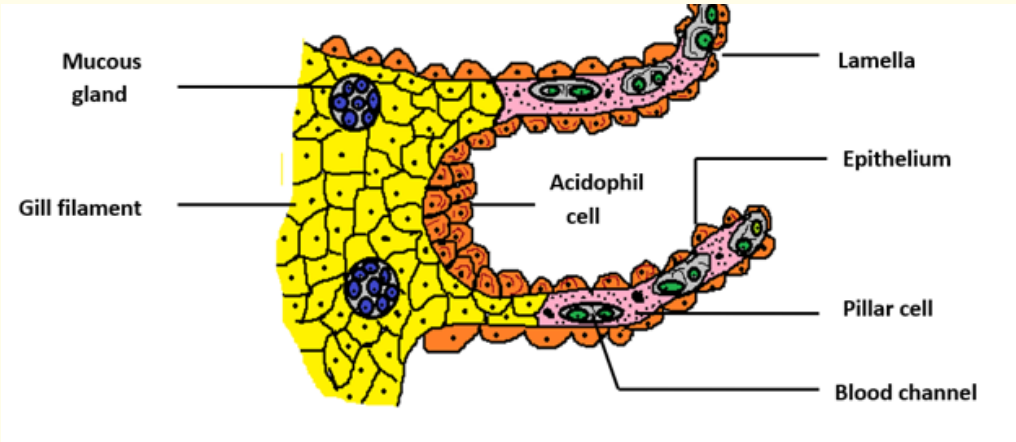

Figure 2: *Schematic presentation of the structure of a gill filament.*

Mechanisms of toxicity

Mechanisms of CNT toxicity in fish need to be studied further.

Fullerene toxicity in fish

Amongst nanomaterials, fullerenes are the molecules with 60 or 70 atoms of carbon denoted as C_{60} or C_{70}. It was first discovered by Kroto and colleagues [23]. They are composed of closed spherical shells resembling a soccer ball. They possess a three dimensional structure with unique physical and chemical properties [24]. They are lipophilic in nature and can cross membrane and blood brain barrier [25].

Due to their specific properties they have been used in a variety of biological and medical applications [26,27]. Given that, usage of C_{60} fullerenes in various industries is projected to increase dramatically in future, concerns for environmental safety have been raised by a few workers [28,29].

Only eight articles dealing with their toxicity in fish are available in the literature (www.pubmed.gov, assessed in Oct 2020). An ambitious project entitled, "ecotoxicology of underivatized fullerenes (C_{60}) in fish", was undertaken by Henry and colleagues at University of Tennessee at Knoxyville (USA) under the support of EPA. This group demonstrated that aquatic toxicity of C_{60} in fish was a consequence of its solvent (tetrahydrofuran) rather than the fullerene in zebra fish [30]. The same group suggested that absorption of NPs onto the gill surface involves mucous membrane and its microenvironment. Uptake of NPs occurs through endocytosis rather than diffusion [31]. In another report, juvenile rainbow trout (*Oncorhynchus mykiss*), fed on a diet supplemented with 500 mg/SWCNT/Kg or 500 mg C_{60}/kg for six weeks did not result in overt toxicity [32].

Therefore, toxicity of fullerenes in fish remained a debatable issue for many years. Oberdorster [33] demonstrated that 0.5 mg/L of C_{60} could induce oxidative stress in the brain of *Micropterus salmoides* after 48hr of exposure. However, pro-oxidant nature of fullerenes still remains to be established. Decrease in GSH values but increase in catalase activity in liver was observed in fish exposed to fullerene [34]. Antioxidative role of fullerene as suggested by Gharbi and colleagues [35] also need support from other workers. Nevertheless, protective role of C_{60} against arsenic toxicity was studied in zebra fish [36]. Co-exposure of arsenic and C_{60} reduced cell injury in fish hepatocytes. This conclusion was drawn through observations on GSH, TBARS and total antioxidant activity. Contrarily, recent observations suggest that exposure of fish *Anabas testudineus* to C_{60} (5 mg/L and 10 mg/L for 24, 48, 72, 96 hr induced oxidative stress in its gonads [37]. Exposure of zebra fish to C_{70} at 1.5ppm also increased ROS level in gills, muscle and brain [38]. Fullerenes can be photoactivated and induce oxidative stress in cell culture systems [39,40].

Mechanisms of toxicity

On the basis of these studies, it was concluded that C_{60} and C_{70} could induce toxicity through oxidative stress in fish.

Toxicity of quantum dots (QDs) in fish

Spherical nanocrystals having a diameter of 1 to 10 nm are known as quantum dots. They were first discovered by Ekimov and Onushchenko [41]. Reed and colleagues [42] used this term while referring to heterogenous structures with quantum confinement to zero dimensions. Their application in biological imaging started in 1998 [43]. QDs possess unique optical, electronic, magnetic and catalytic properties [44]. These ENPs are applied in molecular biology, medicine as well as information technology [45,46]. No data is available on their concentration in aquatic systems, however, global production was estimated to vary 0.6 to 55 t/year [43]. Behaviour and ecotoxicity of QDs in aquatic systems have been recently reviewed [47].

Eco-toxicological information on quantum dots in micro-organisms, algae, protozoa, fungi, crustaceans, annelids, molluscs and fish has been described in several reports [48-52]. However, three species of fish viz. the zebra fish (*Danio rerio*), rainbow trout (*Oncorhynchus mykiss*) and fresh water carp (*Carassius auratus gibelio*) have been mainly used as models to assess their toxicity using suitable parameters of cytotoxicity, embryotoxicity, immunotoxicity and genotoxicity. QDs and their degradation products can enter the fish through mouth or gills. They can be distributed and accumulated in different organs such as liver, intestine and muscles. Cellular uptake is facilitated by endocytosis [13]. Zebra fish was found to absorb little amount of Cd/Se and ZnS QDs through trophic structure due to the absence of stomach in this fish [53]. However, Si/SiO_2 QDs accumulated in muscles and liver of fresh water carp after intraperitoneal injection (5 nm; 2 mg/kg; 7d) [54].

Mechanism of toxicity

Limited studies have been carried out to delineate MoA of QDs in fish. Available data show that QDs promote lipid peroxidation, protein oxidation and oxidative stress in target organs of fish. They affect the activities of detoxication enzymes i.e. superoxide dismutase (SOD), glutathione peroxidise (GPx), glutathione reductase (GR) and catalase (Cat) and induce gene expression changes [55,56]. Experiments made in rainbow trout confirmed their immunotoxic effects [57]. Immuno-competence was noticed after exposure of rainbow trout to Cds/CdTe QDs. Significant reductions in leukocyte count, viability and phagocytic activity were observed. They were poor inducers of metallothionein and CYP2K1 gene expression. 25 genes specific to QDs and related to toxic end points viz. inflammation, biotransformation and endocrine system were affected [58]. Gao and coworkers [59] identified 545 differentially expressed genes in the liver cells of zebra fish exposed to nanomaterial loaded cadmium and nano manganese dioxide or n-hydroxyapatite adsorbed $CdCl_2$ composites. 33 of these genes were common in these two groups that could be classified as hydrolases, metabolic enzymes, intermediate filaments and biological binding. These biomarkers might be helpful in understanding the molecular toxicity of quantum dots.

Effects of silver nanoparticles AgNPs) on fish

To date, silver nanoparticles (AgNPs) are widely used is cosmetics, textiles, surgical prostheses and bactericidal activities. It has been estimated that 435 consumer products contain AgNPs. United States alone produces 2.8 - 20 tons of AgNPs per annum. It is speculated that with increasing usage, large quantities of AgNPs may end up in aquatic systems posing a threat to ecosystem health.

Information on their release into the environment, bioavailability, presence in trophic structure and toxicity in aquatic organisms is poorly known [60]. Approximate concentration of silver in riparine waters is expected to vary between 0.01 to 100 ng/L. Much of this element is complexed with negatively charged ligands present in surface waters [61]. Factors like their size, coating and synthetic methods directly affect agglomeration, uptake, bioaccumulation and toxicodynamics [62].

Gill lamellum, the functional unit of the gill is the main route of their absorption [63]. The release of Ag^+ from AgNPs into water depletes oxygen creating anoxic conditions that may cause respiratory failure in fish [64]. Bioaccumulation of AgNPs in epithelial cells of lamellum manifests into electrolyte imbalance, inhibition of Na^{2+} and K^{2+} dependent ATPase activity and oxidative stress [65,66]. Different species of fish viz. zebra fish (*Danio rerio*), rainbow trout (*Oncorhynchus mykiss*), tilapia (*Oreochromis niloticus*) have been used in the past as suitable models to test their toxicity. Zebra fish alone was used as model to test the toxicity of AgNPs by 126 authors between 2011 - 2020 (www.pubmed.gov, assessed in Oct 2020). Briefly, AgNPs induced developmental toxicity [66]; hepatotoxicity [67]; renal and cardiotoxicity [68] and neurotoxicity [69]. Toxic manifestations were largely attributed to oxidative and endoplasmic reticulum stress and associated mechanisms [70]. Moreover, a few reports acclaim other mechanisms of their toxicity. In rainbow trout, exposure to AgNPs disturbed hormone regulated cell signalling pathway in hepatocytes [71]. Exposure of Mosambique tilapia (*Oreochromis mossambicus*) to AgNPs generated free radicals in gills and liver [72]. Variables like concentration and exposure period influenced the bioaccumulation of AgNPs in the liver of *Prochilodus lineatus*. Exposure of this fish for 15 days to 25 µg/L increased glycogen and antioxidant enzymes in liver. Major

changes occurred after subchronic exposure [73]. Experiments made to determine their neurotoxicity also showed time and dose dependent effects. Two different species i.e. *Oreochromis niloticus* and *Tilapia zillii* showed no significant changes in oxidative stress parameters after exposure to 2 mg/L AgNPs. However, fish exposed to 4 mg/L AgNPs exhibited a decline in GSH, SOD, GPx, CAT and GR values.

Finally histopathological and histochemical observations confirmed that the worst affected organs by AgNPs are gills. Gills of the African cat fish, *Clarius garepinus* showed several pathological lesions viz. hyperplasia, hypertrophy and epithelial necrosis after exposure to 10 µg/L and 100 µg/L AgNPs [75].

Mechanism of toxicity

Toxicity of AgNPs has been extensively studied in fish. Most of these reports confirm that oxidative stress and related mechanisms contribute in their toxicity.

Toxicity of gold nanoparticles (AuNPs) in fish

Potential health hazards posed by AuNPs in man and animals are poorly known. However, they are now being increasingly used in drug delivery, cellular labelling, imaging and diagnosis of diseases like cancer, diabetes and Alzheimer. Therefore, it becomes necessary to determine health risk(s) posed by environmental or occupational exposure to AuNPs. Toxicity of AuNPs alike other ENPs can be influenced by its physiochemical properties (shape, size and surface charge), methods used in their synthesis, dose, route of administration and the selected model [75,76].

Several workers in recent past, have employed zebrafish (*Danio rerio*) as suitable experimental model to test the toxicity of AuNPs. Fifty four reports were published between 2011 - 2020 on its toxicity in zebrafish alone (www.pubmed.gov, assessed in Oct 2020). Exposure of this fish to AuNPs caused embryonic lethality, immunotoxicity [77] and neurotoxicity [78]. AuNPs induced less severe toxicity in zebra fish embryos than the AgNPs [79]. Further, different shapes (sphere, rod and star) of NPs determined their biodistribution in different organs of zebra fish [80]. Molecular responses like inflammation and immune response were influenced by surface charge [81]. Factors like size, concentration and exposure time also contributed to mitochondrial malfunction in the brain and muscles of zebra fish [82].

Species other than zebra fish were also employed by a few workers to assess the toxicity of AuNPs. Toxicogenomic studies in a marine fish, sea bream (*Sparus aurata*) at two concentrations (0.5 and 50 ug/L) were performed [83]. Low concentration and short term exposure to AuNPs could modulate gene expression in liver and affect several biochemical and genetic functions in fish. Gold nanoparticles and ionic gold expressed differential effects in a number of species viz. zebra fish (*Danio rerio*); guppy (*Poecilia reticulata*); small mouth yellow fish (*Labeobarbus aeneus*); southern mouth brooder (*Pseudocrenilabrus philander*); banned tilapia (*Tilapia sparrmanii*) and Mozambique tilapia (*Oreochromis mossambicus*). These studies were made using species sensitivity distribution approach [84].

Mechanism of toxicity

Molecular and biochemical mechanisms responsible for AuNPs toxicity are not known at present. Role of mitochondria has been suggested by a few workers.

Toxicity of titanium dioxide (TiO_2) nanoparticles in fish

TiO_2 stands at 5th amongst NPs in terms of their use in consumer products over the globe [85]. It is known to exist in three crystalline forms e.g. *anatase, rutile* and *brookite*. First two of these are widely used in personal, household, food, industrial and commercial products while brookite is rarely used [86]. Environment Protection Agency allowed its addition in water treatment plants to remove arsenic [87]. According to an estimate, 165,050,000 metric tonnes of TiO2 were produced worldwide between 1916-2011 [88]. Therefore, its release

into aquatic systems appears to be no surprise. Several workers have detected it in surface waters [89], waste water [90], urban runoff and raw sewage [91].

Ecotoxicological issues related to TiO_2 have been addressed by a few authors [92-94]. Mechanisms of its absorption, bioaccumulation, bioconcentration and biomagnification in a few species of fish have also been elucidated in a few laboratories. Seventy four authors employed zebra fish to test the toxicity of TiO_2NPs between 2011-2020 (www.pubmed.gov). Nano TiO_2 was detected in juvenile and adult zebra fish with a bioconcentration factor (BCF,181) [95]. Another study calculated its BCF in the range of 600 - 700 and biomagnification factor < 1 [96]. It is internalized in the fish following absorption through gills, skin and intestine [13,95]. Lethality of TiO_2NPs in zebra fish has been attributed to its shape, size, dose and exposure period [81]. Chronic exposure for six months caused low toxicity but led to its accumulation in gills, liver, heart and brain. TiO_2NP can cross the blood brain barrier and enter the brain of zebra fish [97].

Mechanisms of toxicity

It is known to inhibit Na^2, K^2 ATPase activity in the brain [98,99]. Inhibition of neurotransmitters, neurone apoptosis and alterations in gene expression profiles were also observed [100]. Genes like *pink 1, parkin, alpha syn and uchl 1* related to Lewy bodies were also activated. It has been suggested that exposure to TiO_2NP may be a risk factor for the development of Parkinson's disease [101]. Respiratory distress induced by TiO_2 was also recorded in rainbow trout [11].

Molecular and biochemical mechanisms of TiO_2 toxicity in the liver of a fresh water carp, *Cyprinus carpio* [94] and blood, liver, muscles and brain of two neotropical fish, *Prochilodus lineatus* and *Hoplius intermedius* have also been determined [102]. These workers showed elevation in LPO and inhibition of antioxidant enzymes suggesting oxidative stress to be responsible for their toxicity. Gene expression studies in the liver of tilapia (*Oreochromis niloticus*) on catalase, glutathione transferase and superoxide dismutase supported these results [103].

Several reports show that ENPs intermingle with other water pollutants in sediments and water phases and form agglomerates. These agglomerates might be more toxic than individual xenobiotics. TiO_2NPs and inorganic lead together expressed different genotoxic effects in gills, kidney and brain of a neotropical fish, *Hoplius intermediius* [104]. Variations in antioxidative status of these tissues were also observed. *In vitro* studies on catfish hepatocytes and human HepG2 cells showed that these cells were more sensitive to TiO_2NPs than catfish primary hepatocytes. The toxicity was attributed to ROS and mitochondrial membrane damage [105].

Toxicity of zinc oxide nanoparticles (ZnONPs) in fish

Amongst ENPs, ZnONPs are considered to be highly toxic. They are used in a wide range of commercial and industrial products e.g. cosmetics, ceramics, glass, rubber, paints, plastics, cement, pigments, batteries, fire retardants and food items. These applications have increased the probability of their release into different eco-compartments and consequent eco-toxicological threats to respective flora and fauna [106]. ZnONPs exhibit several peculiar characters viz. high isoelectric point, transparency, solubility, photocatalytic efficiency and biocompatibility [107]. They can permeate, agglomerate, accumulate and persist within organisms [108]. ZnONPs could easily accumulate in aquatic food chain [109] and caused harmful effects in amphibians and fish [110].

Forty one reports are available in literature on its toxicity in zebra fish only (www.pubmed,gov. assessed in Oct 2020). Reproductive and developmental toxicity of ZnONPs in zebra fish in terms of fertility, fecundity, pre and post larval development have been described by a good number of authors. These reports confirmed inhibitory effect of ZnONPs on hatching of embryos [111-112]. Further, toxicity was found to be shape dependent as nanosticks of ZnONPs were more toxic than nano -spheres [113]. NPs coated with chitosan (ZnO-CTS) or polyethylene glycol (ZnO-PEG) caused greater embryonic mortality than uncoated particles [114]. Whether Zn^{2+} ions released

from ZnONPs contribute to their toxicity remains a debatable issue. A group of workers suggest major role of Zn^{2+} in their toxicity [115]. Contrarily, other researchers suggest their partial role only [116].

The main target organs of ZnONPs in fish are gills, liver, kidney and brain. The nanoparticles accumulate in these tissues and manifest toxicity. Histopathological changes in gills of common carp, *Cyprinus carpio* and tilapia, *Oreochromis mossambicus* have been reported on exposure to ZnONPs [117,118].

Mechanisms of toxicity

Attempts have been made to delineate the biochemical and molecular mechanisms of their toxicity in different fish models. These results show that ZnONPs affect serum enzymes in common carp [119], induce oxidative stress in zebra fish [120] and stimulate enzymatic antioxidant system in the liver of tilapia [118]. Their potential to impair immune system in fish was demonstrated through observations on leucocyte viability, phagocytic activity, LPO and DNA strand breaks in the gills of fat head minnows [58].

Toxicogenomic profile obtained from the embryos of zebra fish after exposure to 0.01; 0.1; 1.0 and 10 mg/L ZnONPs and $ZnSO_4$ for 96 hrs post fertilization yielded interesting results [121]. Amongst 689 affected genes, 498 were up -regulated while 191 were down-regulated. The effect was found to be similar between NP and bulk zinc treated fish except six genes i.e. *aicda, cyb5d1, edar, intl2, ogfrl2 and tnfsf13b* that were associated with inflammation and immune system. These observations explain the mechanism of ZnONPs induced embryotoxicity in zebra fish.

Effects of copper nanoparticles (CuNPs) on fish

Copper nanoparticles (CuNPs) are known to act as antibiotic, antimicrobial and antifungal agents when added to plastics, coatings and textiles. Due to great catalytic property, it is used in biosensors and electrochemical sensors. Further research is in progress to discover their dielectric, magnetic, electrical, optical, imaging and biomedical applications. It is widely used in boat antifouling paints that can pollute aquatic systems.

Lethality of CuNPs in different partners of aquatic food chain has been discussed in the past [122-124]. Thirty nine studies account for its toxicity in zebra fish alone (www.pubmed.gov, assessed in Oct 2020). Cumulative mortalities at LC_{50} values of 1.5 mg/L and 3 mg/L in zebra fish have been reported by a few workers [125,126]. Embryos of zebra fish developed anamolies on exposure to CuNPs [111]. These changes were attributed to oxidative stress and apoptosis [127]. Reports on its neurotoxicity and behavioural changes in fish are also available. Exposure to CuNPs reduced the number of lateral line neuromast cells [128].

Gills are considered the target organs of NP toxicity. Intriguingly, the effects of CuNPs in the gills varied in different species. A detailed study in rainbow trout (*Oreochromis mykiss*); fat head minnow (*Pinephales promelas*) and zebra fish (*Danio rerio*) demonstrated species specific variations on the effects of CuNPs in the gills. It was concluded that physiological differences amongst fish contribute to these variations [129]. Similar studies made in two Amazon ornamental fish i.e. dwarf cichlid (*Apistogramma agassizii*) and cardinal tetra *(Paracheirodon axelvodi*) exposed to 50% of LC_{50} of CuNP for 24, 48, 72 and 96 hr, on gill morphology, osmo-regulatory physiology, mitochondrial function and oxidative stress showed significant variations in its toxicity [130].

In addition, hepatic and renal effects of CuNPs in fish have also been observed. Structural changes were recorded in the liver, kidney and gills of juvenile common carp after exposure to two different doses (20 and 100 µg/L) [131]. They attributed these effects to enhanced oxidative stress. Further, proteomic analysis indicated down regulation of ferritin heavy chain, rhoguanine nucleotide exchange factor 17 and up -regulation of diphosphomevalonate decarboxylase, selenide and water dikinase-1.

CuNPs affected the reproductive organs also. An *in vitro* and *in vivo* study made in cat fish (*Clarius batrachus*) demonstrated histological and ultrastructural changes in the testis. It was concluded that exposure to Cu/CuNP could be detrimental to cat fish testicular recrudescence vis –a-vis reproduction [132]. Exposure of tilapia (*Tilapia mossambicus*) to CuNPs for 14 days could bring several haematological and biochemical changes in gills, liver and muscles [133].

Tributylin and dibutylin are frequently used as antifouling and molluscicides to protect ships, boats and fish nets and gears. These organotin compounds also pose a potential threat to aquatic species. A combined study on the effects of dibutylin and CuNPs was made in tilapia by Ghais and colleagues [134]. The study on fish gills, liver and brain found CuNPs to be more deleterious than dibutylin.

Mechanism of toxicity

These reports show that CuNPs are a serious threat to fish and other aquatic organisms. Given that, whether toxicity is manifested by released Cu^{2+} ions or by CuNPs themselves still remains a debatable issue [135,136].

Effect of cerium oxide nanoparticles (CeO_2NPs) in fish

Cerium is one of the most abundant rare earth elements. Cerium oxide nanoparticles (CeO_2NPs) are widely used as additives of diesel fuel. It causes water pollution through landfills from leachates of electronic waste, sludge and waste water discharge from ceramic plants. It is one of the prioritized nanomaterials listed by Organization for Economic Cooperation and Development (OECD).

Environmental exposure(s) of aquatic organisms to CeO_2NPs makes them susceptible to their toxicity. Trophic structure allows their translocation from lower organisms to invertebrates and vertebrates. *Chironomus riparius* and larvae of *Xenopus laevis* were found to be affected by CeO_2NPs [137]. A few workers have tried to establish their toxicity in fish through *in vitro* and *in vivo* experiments. Rosenkranz., *et al*. [138] demonstrated its cytotoxicity in RTG-2 rainbow trout gonadal cell line using MTT assay. Size, concentration and time dependent effects were observed after 24 and 72 hr exposure. Genotoxicity was reported in *Oncorhynchus mykiss* after an acute exposure (96hr) to three different concentrations viz. 0.25, 5 and 25 mg/L [139].

Mechanisms of toxicity

Combined exposure to CeO_2NPs and UV radiation induced LPO in the gills of Amazon cardinal tetra [140]. Biomarkers of immunotoxicity i.e. viability of immune cells and phagocytic activity were also observed in rainbow trout [141]. Further studies are needed to determine CeO_2NP toxicity in fish.

Conclusion and Perspectives

In an aquatic ecosystem, fish constitute a group of ecological dominant species. They play a significant role in maintaining ecological homeostasis. Nonetheless, they are the prime targets of water pollution. ENPs reach the fish through food chain and bioaccumulate in soft organs like gills, liver, kidney, gonads, brain and muscles. The information reviewed in this article shows that physical and chemical characters of NPs, water quality and biology of the fish determine their bioaccumulation and toxicokinetics. These mechanisms can be influenced by the presence of other pollutants viz. metals or pesticides. Further, toxic endpoints for different ENPs in different species have been determined through selected parameters mainly in gills, liver, gonads and brain. These results suggest their reproductive, respiratory and neurotoxicity in fish. Majority of studies confirm the pro-oxidant nature of ENPs. They induce LPO, oxidative stress and inhibit the activity of detoxification enzymes i.e. SOD, GPx, CAT and GR in gills and liver of fish. In certain cases, toxicogenomic and proteomic studies have also been made to support their pro-oxidative effects (Figure 3).

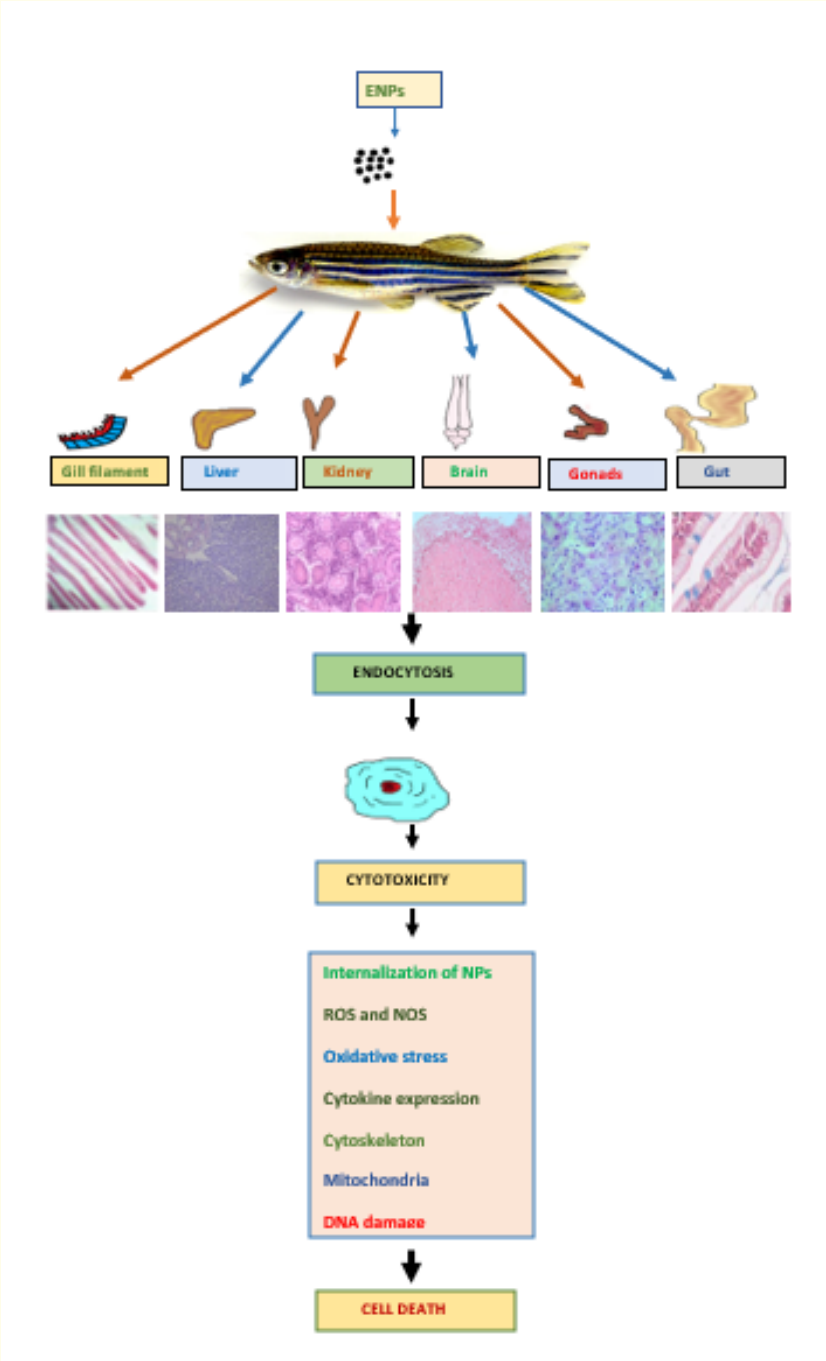

Figure 3: *Schematic presentation of possible effects of ENPs in a Fish.*

However, much research is still needed to delineate ENPs toxicity especially in fish. The effects of NPs on marine fish have been least studied. Metallothionein induction and its role in ENP toxicity in fish and other aquatic species warrant further studies. Antagonistic or synergistic behaviour of ENPs with co-pollutants has been least studied. Their effects on cytoskeleton, mitochondrial homeostasis, endoplasmic reticulum stress and reductive stress in target cells e.g. gill epithelium or hepatocytes also need to be investigated. System biology awaits more studies on the effects of ENPs. Development of specific biomarkers of their toxicity seems to be important for environment health/ ecosystem health/ and risk assessment strategies.

Acknowledgements

The author is thankful to Indian Science Congress Association for awarding him Sir Asutosh Mookerjee Fellowship.

Conflict of Interest

Author declares no conflict of interest in the preparation of this article.

Bibliography

1. Whatmore RW. "Nanotechnology-what is it? Should we be worried?" *Occupational Medicine, Oxford* 56 (2006): 295-299.

2. Fabrega J., *et al*. "Silver nanoparticles: behaviour and effects in the aquatic environment". *Environment International* 37 (2011): 517-531.

3. Colvin VL. "The potential environmental impact of engineered nanomaterials". *Nature Biotechnology* 21 (2003): 1166-1170.

4. Murray CB., *et al*. "Synthesis and characterization of monodisperse nanocrystals and dose packed nanocrystal assemblies". *Annual Review of Materials Research* 30 (2000): 545-610.

5. Gagnon C and Turcotte P. "Effects of a major municipal effluent on the St Lawrence River. A case study". *Water Science and Technology* 20 (2007): 275-285.

6. Cheng X., *et al*. "Naphthalene adsorption and desorption by aqueous C60 fullerene*". Journal of Chemical and Engineering Data* 49 (2004): 675-683.

7. Iijima S. "Helical microtubules of graphic carbon". *Nature* 354 (1991): 56.

8. Woodrow Wilson Database. Nanotechnology consumer product inventory (2013).

9. Lacerda L., *et al*. "Carbon nanotubes as nanomedicine: from toxicology and pharmacology". *Advanced Drug Delivery Reviews* 58 (2006): 1460-1470.

10. Lee J., *et al*. "Nanomaterials in the construction industry: a review of their applications and environmental health safety considerations". *ACS Nano* 4 (2010): 3580-3590.

11. Keller AA., *et al*. "Global life cycle releases of engineered nanomaterials". *Journal of Nanoparticle Research* 15 (2013).

12. Federici G., *et al*. "Toxicity of titanium dioxide nanoparticles to rain bow trout (Onchorhynchus mykiss) gill injury, oxidative stress and other physiological effects". *Aquatic Toxicology* 84 (2007): 415.

13. Bjorkland R., *et al*. "Increasing evidence indicateslow accumulation of carbon nanotubes". *Environmental Science: Nano* 4 (2017).

14. Handy RD., *et al*. "Manufactured nanoparticles: their uptake and effects on fish- a mechanistic analysis". *Ecotoxicology* 17 (2008): 396-405.

15. Sohn EK., *et al*. "Acute toxicity comparison of single walled carbon nanotubes in various fresh water organisms". *BioMed Research International* 1-7 (2015).

16. Kwok KWH., *et al*. "Chronic toxicity of double walled carbon nanotubes to three marine organisms: influence of different dispersion methods". *Nanomed Future Medicine* 5 (2010): 951-996.

17. Cimbaluk GV., *et al*. "Evaluation of multiwalled carbon nanotubes toxicity in two fish species". *Ecotoxicology and Environmental Safety* 150 (2018): 215-223.

18. Smith CJ., *et al*. "Toxicity of single walled carbon nanotubes in rainbow trout (Onchorhynchus mykiss): respiratory toxicity, organ pathologies and other physiological effects". *Aquatic Toxicology* 82 (2007): 94-109.

19. Martinez DST., *et al*. "Carbon nanotube enhanced lead toxicity in fresh water fish". *Journal of Physics Conference Series* 429 (2013).

20. Barbieri E., *et al*. "Histopathological effects on gills of Nile tilapia (Onchorhynchus niloticus) exposed to lead and carbon nanotubes". *Microscopy and Microanalysis* 22 (2016): 1162-1169.

21. Campos-Garcia J., *et al*. "Ecotoxicological effects of carbofuran and oxidized multiwalled carbon nanotubes in fresh water fish Nile tilapia: nanotubes enhance pesticide toxicity". *Ecotoxicology and Environmental Safety* 111 (2015): 131-137.

22. Jang MH and Hwang YS. "Effect of functionalized and multiwalled carbon nanotubes on toxicity and bioaccumulation of lead in Daphnia magna". *PLOS ONE* 13 (2018): e0194935.

23. Kroto HW., *et al*. "C60 Buckminster fullerene". *Nature* 318 (1985): 162.

24. Usenko CY., *et al*. "Fullerene C60 exposure elicits an oxidative stress response in embryonic zebra fish". *Toxicology and Applied Pharmacology* 229 (2008): 44-55.

25. Porter AE., *et al*. "Visualizing the uptake of C60 in the cytoplasm and nucleus of human monocyte derived macrophage cells using energy filtered transmission electron microscopy". *Environmental Science and Technology* 41 (2007): 3012-3017.

26. Das Ros T., *et al*. "Biological applications of fullerene derivatives. Brief over view". *Croatica Chemica Acta* 74 (2001): 743-755.

27. Hollister P., *et al*. "Fullerenes. Technology White Papers". Cientifica, Amsterdam (2003):1-12.

28. Oberdorster G., *et al*. "Toxicology of nanoparticles: Historical perspective". *Nanotoxicology* 1 (2007): 2-25.

29. Kohru A and Dubourguier HC. "From ecotoxicology to nanotoxicology". *Toxicology* 269 (2010): 105-119.

30. Henry TB., *et al*. "Attributing the toxicity of aqueous C60 nanoaggregates to tetrahydrofuran composition products in larval zebra fish by assessment of gene expression". *Environmental Health Perspectives* 115 (2007): 1059-1065.

31. Handy RD., *et al*. "The ecotoxicology of nanoparticles and nanomaterials: current status, knowledge gaps, challenges and future needs". *Ecotoxicology* 17 (2008): 315-325.

32. Fraser TW., *et al*. "Dietary toxicity of single walled carbon nanotubes and fullerenes (C60) in rainbow trout (Onchorhynchus mykiss)". *Nanotoxicology* 5 (2011): 98-108.

33. Oberdorster G., *et al*. "Manufactured nanomaterials (fullerenes, C60) induce oxidative stress in the brain of juvenile large mouth bass". *Environmental Health Perspectives* 112 (2004): 1058-1062.

34. Zhu X., *et al*. "Oxidative stress and growth inhibition in fresh water fish, Carassius auratus induced by chronic exposure to sub -lethal fullerene aggregates". *Environmental Toxicology and Chemistry* 27 (2008): 1979-1865.

35. Gharbi N., *et al*. "Fullerene is a powerful antioxidant in vivo with no acute or subacute toxicity". *Nano Letters* 5 (2005): 2578-2585.

36. Azevedo Costa CL., *et al*. "In vitro evaluation of coexposure to arsenium and organic nanomaterial (fullerene (C60) in zebra fish hepatocytes". *Comparative Biochemistry and Physiology Part C* 155 (): 206-212.

37. Sumi N and Chitra KC. "Fullerene C60 nanomaterial induced oxidative imbalance in gonads of fresh water fish, Anabus trestiduneus (Bloch, 1792)". *Aquatic Toxicology* 210 (2019): 196-206.

38. Sarasamma S., *et al*. "Behavioural impairments and oxidative stress in brain, muscles and gill caused by chronic exposure to C70 nanoparticles in adult zebra fish". *International Journal of Molecular Sciences* (2019).

39. Usenko CY., *et al*. "In vitro evaluation of carbon fullerene toxicity using embryonic zebra fish". *Toxicology and Applied Pharmacology* 229 (2007): 44-55.

40. Shinohara N., *et al*. "In vitro and in vivo genotoxicity tests on fullerene C60 nanoparticles". *Toxicology Letters* 191 (2009): 289-296.

41. Esmikov AI and Onushchenko AA. "Quantum size effect in the optical-spectra of semiconductor microcrystals". *Soviet Physics: Semiconductors* 16 (1982): 775-778.

42. Reed MA., *et al*. "Observations on discrete electronic states in a zero dimensional semiconductor nanostructure". *Physical Review Letters* 60 (1988): 535-537.

43. Piccinno F., *et al*. "Industrial production quantities and uses of ten engineered nanomaterials in Europe and the worlds". *Journal of Nanoparticle Research* 14 (2012). doi.org/10.1007/s 11051-012-1109-9.

44. Wang M., *et al*. "Water soluble quantum dots parsivated by a multidentate di block copolymer". *Macromolecules* 40 (2007): 6377-6384.

45. Gao X and Nile S. "Quantum dot encoded mesosporous beads with high brightness and uniformity: rapid readout using flow cytometry". *Analytical Chemistry* 76 (2004): 2406-2410.

46. Chen Z., *et al*. "Biodistribtion and metabolic paths of silica coade CdSeS quantum dots". *Toxicology and Applied Pharmacology* 230 (2008): 364-371.

47. Rocha CL., *et al*. "Environmental behaviour and ecotoxicity of quantum dots at various trophic levels: A review". *Environment International* 98 (2017): 1-17.

48. Dwarkanath S., *et al*. "Antibody quantum dots conjugates exhibit enhanced antibacterial effect vs inconjugated quantum dots". *Folia Microbiologica* 52 (2007): 31-34.

49. Morelli E., *et al*. "The response of Phaeodactylum trocormutum to quantum dot exposure: elimination and changes in protein expression". *Marine Environmental Research* 111 (2015): 149-157.

50. Han X., *et al*. "Toxicity of CdTe quantum dots on yeast Saccharomyces crevisae". *Small* 8 (2012): 2680-2689.

51. Feswick A., *et al*. "Uptake, retention and internalization of quantum dots in Daphnia is influenced by particle surface functionalization". *Aquatic Toxicology* 130131 (2013): 210-218.

52. Rocha TL., *et al*. "Ecotoxicological effects of engineered nanomaterials in bivalve molluscs, an over view". *Marine Environmental Research* 111 (2015): 74-88.

53. Lewinski A., *et al*. "Trophic transfer of amphiphilic polymer coated CdSe/ZnS quantum dots to Denio rerio". *Nanoscale* 3 (2011): 3080-3083.

54. Stanca L., *et al*. "Interaction of silicon based quantum dots with gibel carp liver: oxidative and structural modifications". *Nanoscale Research Letters* 8 (2013): 254.

55. Stancea L., *et al*. "Impact of silicon based quantum dots on the antioxidative system in white muscles of Carassius auratus gilbello". *Fish Physiology and Biochemistry* 38 (2012): 963-975.

56. Tang S., *et al*. "Cadmium sulphate and CdTe quantum dots alter DNA repair in zebra fish (Danio rerio) liver cells". *Toxicology and Applied Pharmacology* 272 (2013a): 443-452.

57. Brunea A., *et al*. "In vitro immunotoxicology of quantum dots and comparison with cadmium and tellurium". *Environmental Toxicology* 30 (2015): 19-25.

58. Gagne F., *et al*. "Immuno competance and alterations in hepatic gene expression in rainbow trout exposed to CdS/CdTe quantum dots". *Journal of Environmental Monitoring* 12 (2010): 556-565.

59. Gao M., *et al*. "Transcriptome analysis of the effects of Cd and nanomaterial loaded Cd in the liver of zebra fish". *Ecotoxicology and Environmental Safety* 164 (2018): 530-539.

60. Fabrega J., *et al*. "Silver nanoparticles: behaviour and effects in the aquatic environment". *Environment International* 37 (2011): 517-531.

61. Bilberg K., *et al*. "In vitro toxicity of silver nanoparticles and silver ions in zebra fish (Danio rerio)". *International Journal of Toxicology* (2012): 293784.

62. Kim KT., *et al*. "Silver nanoparticle toxicity in the embryonic zebra fish is governed by particle dispersion and ionic environment". *Nanotechnology* 24 (2013): 115101.

63. Verma Y., *et al*. "Assessment of cadmium sulphide nanoparticle toxicity in the gills of a fresh water fish". *Environmental Nanotechnology, Monitoring and Management* 13 (2019).

64. Osborne OJ., *et al*. "Organ specific and size dependent silver nanoparticle toxicity in gills and intestine of adult zebra fish". *ACS Nano* 9 (2015): 9573- 9584.

65. Katuli KK., *et al*. "Silver nanoparticle inhibit the gill Na/K ATPase and eryhthrocyte activities and induce the stress response in adult zebra fish, Danio rerio". *Ecotoxicology and Environmental Safety* 106 (2014): 173-180.

66. Christen V., *et al*. "Silver nanoparticles induce endoplasmic reticulum stress response in adult zebra fish". *Toxicology and Applied Pharmacology* 272 (2013): 519-528.

67. Xia G., *et al*. "The effect of silver nanoparticles on zebra fish embryonic development and toxicology". *Nanomedicine, and Biotechnology* 44 (2016): 1116-1121.

68. Speshock JL., *et al*. "Differential organ toxicity in the adult zebra fish following exposure to acute sub-lethal doses of 10nm silver nanoparticles". *Frontiers in Nanotechnology and Nanotachnology* 2 (2016): 114-120.

69. Colovick MB., *et al*. "Acetylcholinesterase inhibitors: Pharmacology and Toxicology". *Current Neuropharmacology* 315 (2013): 335.

70. Choi JE., *et al*. "Induction of oxidative stress and apoptosis by silver nanoparticles in the liver of adult zebra fish". *Aquatic Toxicology* 100 (2010): 151-159.

71. Massarsky A., *et al*. "Silver nanoparticles stimulate glycogenolysis in rainbow trout (Onchorhynchus mykiss) hepatocytes". *Aquatic Toxicology* 147 (2014): 68-75.

72. Govindaswamy R and Rahaman AA. "Histopathological studies and oxidative stress of synthesized silver nanoparticles in Mozambique tilapia (Oreochromis mossambicus)". *Journal of Environmental Sciences* 24 (2012): 1091-1098.

73. Ale A., *et al*. "Integrative assessment of silver nanoparticles toxicity in Prochilodus lineatus fish". *Ecological Indicators* 93 (2018): 1190-1198.

74. Sayed AH., *et al*. "Histopathological and biochemical effects of silver nanoparticles on the gills and muscles of African fish (Clarius garepinus)". *Scientific African* 7 (2020): e00230.

75. Harper SL., *et al*. "Systemic evaluation of nanomaterial toxicity: utility of standardized materials and rapid assays". *ACS Nano* 5 (2011): 4688-4697.

76. Adewale OB., *et al*. "Toxicological behaviour of gold nanoparticles on various models: influence of physicochemical properties and other factors". *International Journal of Toxicology* (2019).

77. Truong L., *et al*. "Persistent behavioural deficits result from acute embryonic exposure to gold nanoparticles". *Comparative Biochemistry and Physiology Part C* 155 (2012): 269-274.

78. Kim KT., *et al*. "Silver nanoparticle toxicity in the embryonic zebra fish is governed by particle dispersion and ionic environment". *Nanotechnology* 24 (2013): 115101.

79. Ramachandran R., *et al*. "In vivo toxicity evaluation of biologically synthesized silver and gold nanoparticles on adult zebra fish: a comparative study". *Biotechnology* (2018).

80. Sangabathumi S., *et al*. "Mapping the glycol-gold nanoparticles of different shapes, toxicity, distribution and sequestration in adult zebra fish". *Scientific Reports* 7 (2017): 4239.

81. Zhu X., *et al*. "Comparative toxicity of several metal oxide nanoparticle aqueous suspensions to zebra fish (Danio rerio) early developmental stage". *Journal of Environmental Science and Health, Part A* 43 (2008): 278-284.

82. Geoffrey B., *et al*. "Impact of dietary gold nanoparticles in zebra fish at a very low contamination pressure: the role of size, concentration and exposure time". *Nanotoxicology* 6 (2012): 144-160.

83. Teles M., *et al*. "Toxicogenomics of gold nanoparticles in a marine fish: leakage of classical biomarkers". *Frontiers in Marine Science* 6 (2019).

84. Botha TL., *et al*. "Comparative aquatic toxicity of gold nanoparticles and ionic gold using a species sensitivity distribution approach". *Journal of Nanomaterials* (2015).

85. Masciangioli T and Zhang WX. "Peer Reviewed: Environmental Technologies at the nanoscale". *Environmental Science and Technology* 37 (2003): 102 A.

86. Arami H., *et al*. "Sonochemical preparation of TiO2 nanoparticles". *Materials Letters* 61 (2007): 4559-4561.

87. EPA (Environment Protection Agency). Nanomaterial case studies: nanoscale titanium dioxide in water treatment and in tropical sunscreen". EPA/600/R-09/057F.

88. Jovanovic B., *et al*. "Titanium dioxide nanoparticles enhance mortality in fish exposed to bacterial pathogens". *Environmental Pollution* 203 (2015): 153-164.

89. Gottschalk F., *et al*. "Modeled environmental concentrations of engineered nanoparticles (TiO2,Zno, Ag, CNT, fullerenes) for different regions". *Environmental Science and Technology* 43 (2009): 9216-9222.

90. Kiser MA., *et al*. "Titanium nanomaterial removal and release from waste water treatment plants". *Environmental Science and Technology* 43 (2009): 6757-6733.

91. Kaegi R., *et al*. "Synthetic TiO2 nanoparticle emission from exterior facades into the aquatic environment". *Environmental Pollution* 156 (2008): 233-239.

92. Hund-Rinke K and Simon M. "Ecotoxic effects of photocatalytic active nanoparticles (TiO2) on algae and Daphnids". *Environmental Science and Pollution Research* 13 (2006): 225-232.

93. Heinlaan M., *et al*. "Toxicity of nanosized and bulk Zno, CuO and TiO2 to bacteria Vibrio fischeri, crustacean Daphnia magna and Thamnocephalus platyurus". *Chemosphere* 71 (2008): 1308-1216.

94. Wang HZ., *et al*. "Effect of subacute exposure to TiO2 nanoparticles on oxidative stress and histopathological changes in juvenile carp (Cyprinus carpio)". *Journal of Environmental Sciences* 21 (2009): 1459-1466.

95. Zhu X., *et al*. "Trophic transfer of TiO2 nanoparticles from Daphnia to zebra fish, Danio rerio in a simplified fresh water food chain". *Chemosphere* 79 (2010): 928-933.

96. Zhang XZ., *et al*. "Bioaccumulation of titanium dioxide nanoparticles in carp". *Huan Zing Ke Xue Chinese Journal of Environmental Science* 27 (2006): 1631-1635.

97. Chen J., *et al*. "Effect of titanium dioxide nanoparticles on growth and some histological parameters of zebra fish (Danio rerio) after a long term exposure". *Aquatic Toxicology* 101 (2011): 493-499.

98. Chakraborty C., *et al*. "Further prospects of nanoparticles on brain targeted drug delivery". *Journal of Neuroendocrinology* 93 (2009): 285-286.

99. Ramsden CS., *et al*. "Dietary expoxure to titanium dioxide nanoparticles in rainbow trout (Onchorhynchus mykiss): no effect on growth but subtle biochemical disturbances to the brain". *Ecotoxicology* 18 (2009): 939-951.

100. Sheng L., *et al*. "Mechanism of TiO2 nanoparticle induced neurotoxicity in fish (Danio rerio)". *Environmental Toxicology* 31 (2016): 163-175.

101. Hu Q., *et al*. "Effect of titanium dioxide nanoparticles exposure on parkinsonism in zebra fish larvae and PC12". *Chemosphere* 173 (2017): 373-379.

102. Carmo TL., *et al*. "Overview of the toxic effects of titanium dioxide nanoparticles in blood, liver, muscles and brain of a neotropical detrivorous fish". *Environmental Toxicology* 34 (2019): 457-468.

103. Varela-Valencia R., *et al*. "The effect of titanium dioxide nanoparticles on antioxidant gene expression in tilapia (Oreochromis tilapia)". *Journal of Nanoparticle Research* 16 (2014).

104. Vicari T., *et al*. "Coexposure to titanium dioxide nanoparticles (NpTiO2) and lead at environmentally relevant concentrations in neotropical fish species (Hoplius intermedius)". *Toxicology Reports* 5 (2018): 1032-1043.

105. Wang J., *et al*. "Disruption of zebra fish (Danio rerio) reproduction upon chronic exposure to TiO2 nanoparticles". *Chemosphere* 83 (2011): 461-467.

106. Chen TH., *et al*. "Zinc oxide nanoparticles alter hatching and locomotion activity in the zebra fish (Danio rerio)". *The Journal of Hazardous Materials* 277 (2014): 120-140.

107. Mirzaei H and Darroudi M. "Zinc oxide nanoparticles: biological synthesis and biomedical applications". *Ceramics International* 43 (2017): 907-914.

108. Zhao X., *et al*. "Comparative toxicity of several metal oxide nanoparticles aqueous suspensions to zebra fish (Danio rerio) early developmental stage". *Journal of Environmental Science and Health* 43 (2008): 278-288.

109. Heinlaan M., *et al*. "Toxicity of nanosized and bulk ZnO,CuO and TiO2 to bacteria, Vibrio fischeri and crustacean, Daphnia magna and Thamnocephelus platyurus". *Chemosphere* 71 (2008): 1308-1316.

110. Zhu X., *et al*. "The impact of ZnO nanoparticle aggregates on the embryonic development of zebra fish, Danio rerio". *Nanotechnology* 20 (2019).

111. Bai W., *et al*. "Toxicity of zinc oxide nanoparticles to zebra fish embryo: a physicochemical study of toxic mechanisms". *Journal of Nanoparticle Research* 12 (2010): 1645-1654.

112. Kteeba SM., *et al*. "Zinc oxide nanoparticle toxicity in embryonic zebra fish: mitigation with different organic natural matter". *Environmental Pollution* 230 (2017): 1125-1140.

113. Hua J., *et al*. "Toxicity of different sized copper nano and submicron particles and their shed copper ions to zebra fish embryos". *Environmental Toxicology and Chemistry* 33 (2014): 17774-1782.

114. Zhou Z., *et al*. "Influence of surface chemical properties on the toxicity of engineered zinc oxide nanoparticles to embryonic zebra fish". *Beilstein Journal of Nanotechnology* 6 (2015): 1565-1579.

115. Brun NR., *et al*. "Comparative effects of zinc oxide nanoparticles and dissolved zinc ion on zebra fish embryos and eleuthero embryos: importance of zinc ions". *Science of the Total Environment* 476-477 (2014): 657-666.

116. Kim RO., *et al*. "Comparative analysis of transcriptional profile changes in larval zebra fish exposed to zinc oxide nanoparticles and zinc sulphate". *The Bulletin of Environmental Contamination and Toxicology* 98 (2017): 183-189.

117. Subashkumar S and Selvanayagam M. "First report on: acute toxicity and gill histopathology of fresh water fish Cyprinus carpio exposed to zinc oxide (ZnO) nanoparticles". *International Journal of Scientific and Research Publication* 4 (2014): 1-4.

118. Shahzad K., *et al*. "Toxicity of zinc oxide nanoparticles (ZnONP) in Indian Tilapia (Oreochromis mossambicus): tissue accumulation, oxidative stress, histopathology and gene toxicity". *International Journal of Environmental Science and Technology* 16 (2018): 1973-1984.

119. Chupani L., *et al*. "Chronic dietary toxicity of zinc oxide nanoparticles in common carp (Cyprinus carpio): tissue accumulation and physiological responses". *Ecotoxicology and Environmental Safety* 147 (2018): 110-116.

120. Xiong Z., *et al*. "Toxicity of zinc oxide nanoparticles to zebra fish embryo: a physicochemical study of toxicity mechanism". *Journal of Nanoparticle Research* 12 (2010): 1645-1654.

121. Choi JS., *et al*. "Induction of oxidative stress and apoptosis by silver nanoparticles in the liver of adult zebra fish (Danio rerio): A transcriptomic analysis". *PLOS ONE* 11 (2018): 151-159.

122. Rispoli F., *et al*. "Understanding the toxicity of aggregated zero valent copper nanoparticles against Escherichia coli". *The Journal of Hazardous Materials* 180 (2010): 212-216.

123. Pradhan A., *et al*. "Copper oxide nanoparticles can induce lethality to fresh water shredder Allogamus ligonifer". *Chemosphere* 89 (2012): 1142-1150.

124. Wang T., *et al*. "The potential toxicity of copper nanoparticles and copper sulphate on juvenile Epinephelus coioides". *Aquatic Toxicology* 152 (2014): 195-204.

125. Griffitt RJ., *et al*. "Comparison of molecular and histological changes in zebra fish gills exposed to metallic nanoparticles". *Toxicological Sciences* 107 (2009): 404-415.

126. Kovriznych JA., *et al*. "Acute toxicity of 31 different nanoparticles to zebra fish (Danio rerio) tested in adulthood and early life stages-comparative study". *Interdisciplinary Toxicology* 6 (2013): 67-73.

127. Ganesan S., *et al*. "Acute and sublethal exposure to copper oxide nanoparticles causes oxidative stress and teratogenicity in zebra fish embryos". *Journal of Applied Toxicology* 36 (2016): 554-567.

128. McNeil PL., *et al*. "Effects of metal nanoparticles on the lateral line system and behaviour in early life stages of zebra fish (Danio rerio)". *Aquatic Toxicology* 152 (2014): 318-323.

129. Song L., *et al*. "A comparative analysis on the in vivo toxicity of copper nanoparticles in three species of fresh water fish". *Chemosphere* 139 (2015): 181-189.

130. Braz-Mota S., *et al*. "Mechanisms of toxic action of copper and copper nanoparticles in two Amazon species: dwarf child (Apostogramma agassizzi) and cardinal tetra (Paracheirodon axelradi)". *Science of the Total Environment* 630 (2018): 1168-1180.

131. Gupta YR., *et al*. "Effect of copper nanoparticles exposure on the physiology of common carp (Cyprinus carpio): biochemical, histological and proteomic analysis". *Aquacult and Fisheries* 1 (2016): 15-23.

132. Murugananthkumar R., *et al*. "Copper nanoparticles differentially target testis of cat fish, Clarius batrachus, in vivo and in vitro study". *Frontiers in Environmental Science* 4 (2016).

133. Siddiqui SA and Noorjahan CM. "Toxicity of copper nanoparticles on haematology and biochemistry of tilapia (Tilapia mossambica)". *International Research Journal of Pharmacy* (2018).

134. Ghais SA., *et al*. "Effect of copper nanoparticles and organo-metallic compounds (dibutylin) on tilapia fish". *The Journal of Basic and Applied Zoology* 80 (2019).

135. Lin S., *et al*. "High content screening in zebra fish speeds up hazard ranking of transition metal oxide nanoparticles". *ACS Nano* 5 (2011): 7284-7295.

136. Vicara-Pares U., *et al*. "Cellular and molecular responses of adult zebra fish after exposure to CuO nanoparticles or ionic copper". *Ecotoxicology* 27 (2018): 89-101.

137. Bour A., *et al*. "Toxicity of CeO2 nanoparticles at different trophic levels- effects on diatoms, chromonids and amphibians". *Chemosphere* 120 (2014): 230-236.

138. Rosenkrankranz P., *et al*. "Impact of cerium oxide nanoparticles shape on their in vitro cellular toxicity". *Toxicology in vitro* 26 (2012): 888-896.

139. Nunes AC., *et al*. "Effect of cerium oxide nanoparticles in Onchorhynchus mykiss liver after an acute exposure: assessment of oxidative stress, genotoxicity and histological alterations". XV European Congress of Ichthyology. Porto, Portugal (2015).

140. Zhang Y., *et al*. "UV induced cytotoxicity of cerium oxide nanoparticles (CeO2NPs) and the protective properties of natural organic material (NOM) from the Rio Negro Amazon river". *Environmental Science: Nano* 5 (2018): 476-486.

141. Gagnon C and Turcotte P. "Effect of major principal effluent on the St Lawrence River. A case study". *Water Science and Technology* 20 (2007): 275-285.

Volume 9 Issue 8 August 2021