

Effects of nanochemical particles on some histological parameters of fish (Review)**Mahdi Yousefian, Banafsheh Payam***Department of Fisheries, Islamic Azad University, Qaemshahr Branch, Qaemshahr, Iran*

Mahdi Yousefian, Banafsheh Payam; Effects of nanochemical particles on some histological parameters of fish (Review)

ABSTRACT

Nanotechnology is the unique properties and behavior of matter at the nanoscale. The impacts are related to the smaller size of the particles, their increased reactivity as a result of greater surface area per particle, or the greater number of particles in a dose. With this respect that, the wall of cells, blood–brain barrier and blood–eyes barrier can not be effective to prevent xenobiotics, the particles also can enter the brain and the blood stream easily. Recently studying toxicity and distribution of nanoparticles, lead us to know that particles are distributed in the brain, heart, yolk and blood of fish embryos. Since one of the healthy food for humans is fish, knowing the fact that how engineered nanoparticle can affect fish and its body is becoming a hot topic, From this point of view we collect the information about the histological effects of nanoparticles sedimentation in a group of fish both in adults and embryos .

Key words: nanomaterial, histological, rainbow trout, common carp, zebrafish**Introduction**

For biological test and the effect of biomaterial such as nanotoxicity and hormone therapy the aquarium fish e.g. cichlid, Barbus, Guppy and zebrafish are used. Among them Zebrafish were selected for most experiment because of their widespread use in ecotoxicology, including the assessment of nanoparticle toxicity, however Cichlid are used in effect of different hormones. Zebrafish is a small fish that can grow and reach in sexual maturity in laboratory conditions and produce a large number of transparent eggs. The embryonic development is quick and easily observable. Cichlid fish are also an aquarium fish that reproduce very fast and the small fish are good for genetically and genome manipulation such as sex-reversal. Some other commercial fish such as common carp and rainbow trout are also investigated to test the effect nanotoxicity in fishes.

Nanotechnology is generally defined as the design, production, and application of structures, devices, and systems through control of the size and shape of the material at the 10^{-9} of a meter scale. Nanotechnology is truly an interdisciplinary field that stretches across a whole spectrum of science including physics, chemistry, and biology as well as engineering including micro-fabrication techniques [1]. Nanomaterials, have wide applications in almost all the fields of technology such as chemical, manufacturing, medical, agricultural sector and it shows several properties include transparency,

hydrophobicity, photoluminescence, toughness and hardness, chemical sensing and bioavailability. Products produced from these materials exhibit unique properties and have a wide range of high value commercial applications in rapidly expanding markets. The key characteristics demanded of nanoparticles to capture high value markets include: small particle size, narrow size distribution, low levels of agglomeration and high dispersibility [2]. The nanotechnology had explosion in the last decade due to its application for human and their activities, mostly in pharmaceutical industry, medicine, cosmetics, antibacterial and antifungal utilities. There is a doubt using nanotechnology because together with the development of nanomaterial applications, some concerns about the potential risks to human and environment health have been recognized. Therefore in animal sciences several experiment are tested to understand the effect of nanotechnology in physiology and health of animal. Some fish as we noted previously are tested in this respect. Toxicological studies on ultra fine particles have served as a repository for understanding possible risks of nanoparticles. The studies relating the effects of nanoparticles on microorganisms are more extensive than for plants. The behaviour of nanoparticles in living organisms, soil and water is poorly understood. A study on the exposure of nanoparticles to aquatic animals has revealed possible associated ecological and food chain risk [3]. There are four types of engineered nanoparticles: Carbon based nanomaterials (fullerene, single

Corresponding Author

Mahdi Yousefian, Department of Fisheries, Islamic Azad University, Qaemshahr Branch, Qaemshahr, Iran
E-mail: yousefianeco@yahoo.com

walled- and multi walled carbon nanotubes (SWCNT and MWCNT)), metal based (quantum dots, nanogold, nanozinc, nanoaluminium and metal oxides such as TiO₂, ZnO, Al₂O₃), dendrimers (nanosized polymers) and composites (combination of nanoparticles with other nanoparticles) [4].

In the present study we have collected some nano-metal experiment and their effect and potential in use of aquarium fish and farm fish as animal model for investigation of the different nanoparticles.

Exposure to silver nanoparticle:

Silver nanoparticles have extremely used in industries due to antibacterial and antifungi activities. Because of its physical and chemical properties gives them unique biological activity or toxicity. The following is some sample of experimental studies of using this nano-particle.

Zebrafish:

Exposure of zebrafish embryos to small silver nanoparticles lead to their uptake and distribution in the embryo [5]. In consequence, they induced mortality and delayed hatching at 120 mg/L and higher [6]. In adult fish accumulation of nanoparticles was observed mainly in gills and intestine, but also in other tissues such as blood, liver and brain [7]. Acute toxicity occurs at nanoparticle concentrations in the high mg/L range [8]. In zebrafish early life stages, mortality of silver nanoparticles occurred at LC₅₀ values of 25–50 mg/L with severe morphological alterations at higher concentrations [6]. silver nanoparticles is consider as toxic metal. A concentration of 0.25mM silver nanoparticles was more toxic than the same concentration of gold nanoparticles in zebrafish embryos [9].

Medaka:

In investigation of silver nanoparticles on medaka it had show acute toxicity of AgNPs on this fish. In the 48 h acute toxicity test in adult fish, 100% mortality was observed in the 2.0 mg/L AgNPs group, and no deaths occurred in the 0.5 mg/L AgNPs treatment [10]. During the embryonic exposure experiment, various deformities in morphogenesis developed in AgNPs-treated medaka larvae from stage 10 to 2 dph, which were dependent on the exposure dose [10].

Another study used biomarkers based analysis to distinguish effects of nanometal exposure. Six biomarker genes were used to study the effect of silver nanoparticles on Japanese Medaka fish (*Oryzias latipes*). Gene expression patterns were compared between those exposed to silver nanoparticles and silver nitrate. It was found that

nanosilver exposure resulted in a higher overall stress response compared to soluble silver ions [11].

Rainbow trout:

Uninfluenced by changes in size and agglomeration the nanoparticles had adverse effects on rainbow trout hepatocytes by causing cytotoxicity (Ag nanoparticles) and formation of reactive oxygen species (Au nanoparticles) already at low mg/L concentrations. Silver nanoparticles, coated with citrate or PVP, accumulate in gill epithelial cells and cause silver transport through epithelial layers, which is depended on the epithelial tightness. Cytotoxic effects in terms of membrane stability were found for silver nanoparticles and ionic silver exposure [12].

Crucian carp (Perca fluviatilis):

Effect of silver nanoparticles on exposures to 45gL⁻¹ suspension suppress the olfactory response in both Crucian carp and perch. The lower suspension concentration of 0.45 gL⁻¹ nanosilver was not significant, however a clear trend showed an increased EOG signal in Crucian carp. The olfactory epithelium rapidly hyperpolarized upon exposure to nanosilver suspensions, but not when exposed to ionic silver solution [13]. Releasing toxic ions, nanosilver may also interfere with receptors in the epithelia or interfere with detection of odours by forming non-stimulating nanosilver odour complexes that prevent binding of odours to the olfactory receptors [14].

Fathead minnow:

The effects of silver nanoparticles on fathead minnow (*Pimephales promelas*) embryos LC₅₀ values for NanoAmor and Sigma Ag NPs were 9.4 and 10.6 mg/L for stirred and 1.25 and 1.36 mg/L for sonicated NPs, respectively. Uptake of Ag NPs into the embryos was observed after 24 h using Transmission Electron Microscopy and Ag NPs induced a concentration-dependent increase in larval abnormalities, mostly edema. Dissolved Ag released from Ag NPs was measured using Inductively Coupled-Mass Spectrometry and the effects tested were found to be three times less toxic when compared to Ag nitrate [15].

Exposure to Titanium dioxide nanoparticle:

Many investigations of acute toxicity showed that the TiO₂ NPs are low toxicity or safe to aquatic organisms. The following is some sample illustrated this indication.

Zebrafish:

A recent study indicated that nano-TiO₂ exposure altered expression of a number of genes

involved in ribosomal function in zebrafish [16]. This observation raises the possibility that, although not overtly toxic within the time frame studied, exposure to TiO₂ NPs may have effects on zebrafish gills that are not overt until longer exposures are performed [17].

Common Carp (Cyprinus carpio):

TiO₂-NPs can have adverse influences on carps, but they are not lethal to the carps with exposure to 10–200 mg/L TiO₂-NPs, and abnormal physiological and behavioral changes of the carps occur under the higher concentrations during the experimental period. Exposure to TiO₂-NPs resulted in the increase in the incidence of thickening, edema, fusion and hyperplasia in the gill lamellae and filaments which impaired the structure and function of gills with the increasing TiO₂-NPs concentration. The gill pathologies with our observation were similar to those reported for TiO₂-NPs in rainbow trout [18].

A study by Zhang *et al.* [19] showed a much greater uptake of cadmium in Carp in the presence of TiO₂ nanoparticles than in the presence of cadmium alone, highlighting this potential for enhanced bioavailability.

Rainbow trout (Oncorhynchus mykiss):

TiO₂ NPs cause respiratory toxicity, and disturbances to the metabolism of some trace elements like Zn and Cu. Similar to the findings in mammals, oxidative stress is also a main concern for trout during TiO₂ NP exposure. Exposure to 1 mg L⁻¹ TiO₂ NPs in rainbow trout caused oedema in the gills [20]. Addition of TiO₂ nanoparticles at concentrations of 10 and 100 mg/kg to the feed of rainbow trout of less than a year of age for 2 months did not influence their growth and hematological characteristics.

The ecotoxicity assessment of TiO₂ on rainbow trout was investigated by Federici *et al.* [20]. In a 2-week study the authors found respiratory problems and other sublethal effects in the fish that were possibly tied to metal ions, such as copper, and the presence of sodium and potassium ions.

For TiO₂ NPs, there seems to be no major disturbances to blood cell counts or plasma electrolytes in rainbow trout [20] and no evidence of lipid peroxidation (TBARS assay) in rainbow trout blood plasma following intravenous administration [21].

Exposure to Cu nanoparticle:

Exposure to copper nanoparticle is mostly in combination with TiO₂ and it has a wide range of expression based on different exposure.

Zebrafish:

The embryos were evaluated 24 hpf (hours post fertilization). In the groups exposed to the CuTiO₂ and pure TiO₂ nanosized photocatalysts very seriously injured larvae were detected, and many had abnormal notochord development, which were loss or curved in both (10 and 20 ppt) groups. Some of them lacked head development [22].

In another investigation, global gene expression analysis in gills of zebrafish demonstrated that the exposure to silver (1 mg/L), copper (0.1 mg/L), and TiO₂ (1 mg/L) nanoparticles or their soluble metals produced a distinct gene expression profile, suggesting that each exposure is producing a biological response by a different mechanism [17]. Copper and silver nanoparticles caused gill pathology and mortality in zebrafish [23].

Griffitt *et al.* (24) found that exposure to nano-Cu caused concentration-dependent damage to the lamellae characterised by proliferation of epithelial cells and oedema of primary and secondary filaments whilst an increase in gill filament width was observed in adult zebrafish exposed to nano-Cu.

Common carp (Cyprinus carpio):

The use of Cu nanoparticle have shown potential toxicity effects in juvenile of common carp. CuO NPs had no obvious acute toxicity to juvenile carp. Growth of carp was significantly inhibited by CuO NPs during the 30 day sub-acute toxicity tests. Intestine and gill of the carp contributed to most of whole body Cu amounts after CuO NPs exposure, suggesting intestine and gill were the cumulative organs to CuO NPs [25].

Carbon nanomaterials:

In exposure to carbon nanomaterial fish showed signs of gill irritation and mucus secretion during exposure to SWCNT, which was not observed in the solvent or water only control. Also fish exposed to low or high SWCNT concentration shows different appearance related to dosage of carbon treatment.

Zebra fish:

The size and shape of carbon nanomaterials can affect the potential for exposure and toxicity in organisms. Cheng *et al.* [26] reported that zebrafish embryos appear to be protected from aggregates of CNTs, because aggregates are larger and unable to pass through nanometer-size pores in the chorion. Fish gill surfaces may be more susceptible to irritation by NPs of a defined size range, or specific sizes or shapes; and it may be more difficult for fish to dislodge some shapes or sizes of carbon nanomaterials from the cell surface. Such experiments on size/shape effects remain to be

conducted in fish. Single wall carbon nanotubes delayed the hatching of zebrafish but did not influence further development of larvae [26]. Exposure to high concentrations greater than 120 mg/L of single wall nanotubes (SWNT) delayed hatching of zebrafish embryos, whereas carbon black had no effect [26,27]. Toxicity in zebrafish embryos exposed to SWCNTs has been attributed to the presence of Co and Ni catalysts used in the preparation of the SWCNTs [26].

Rainbow trout:

The fish exposed to SWCNT showed elevated ventilation rates compared to the controls, but no mortalities had occurred. Fish from the 0.25 mg l⁻¹ SWCNT treatment showed changes to nuclear morphology with condensed nuclear bodies [28]. Results on oxidative injury in rainbow trout exposed to SWCNTs varied between tissues, with intestine and brain showing no effect, but elevation of glutathione in the gills and liver at some SWCNT concentrations [28]. Carbon NTs accumulated on gill surfaces leading to irritation and lesions leading to conclude that CNTs acted as a respiratory toxicant in rainbow trout. Dose dependent respiratory pathologies and increase in aggression of rainbow trout were observed after supplementing water with carbon nanotubes at a concentration of 0.1 to 0.5 mg/l [28].

Fathead minnow (Pimephales promelas):

In experiments with the large mouth bass (*Micropterus salmoides*) and fathead minnow (*Pimephales promelas*), addition of fullerenes C₆₀ caused biochemical changes in the brain, gills, and liver, suggesting a negative effect on the fish organism [29,30].

Titanium dioxide nanoparticles:

Rainbow trout:

In intravenously injected at large doses (100 µg), accumulated in the kidneys of rainbow trout and remained detectable for 90 days after injection. Insignificant amounts were also found in the liver. However, the nanoparticles were undetectable in the spleen, blood, brain, and gills [21].

Titanium dioxide nanoparticles at a concentration of up to 1 mg/l did not cause acute toxicity in rainbow trout; however, sublethal effects were detected, including pathology of internal organs and biochemical and respiratory abnormalities [20].

Common carp:

Nonetheless, accumulation of titanium in carp (*Cyprinus carpio*) muscles and liver has been

demonstrated when keeping them in the water containing titanium dioxide nanoparticles [31].

Exposure to Sn nanoparticle:

Guppy:

Tin was detected in the gills, gut, and spleen of the guppy (*Poecilia reticulata*) kept in water supplemented with a suspension of hydrated tin dioxide (SnO₂) nanoparticles [32]. The nanoparticles of hydrated tin dioxide (SnO₂) did not cause any acute toxicity and genotoxicity in guppies [32]. Contradictory results have been noted in recent studies, suggesting the possibility for a size dependence of toxicity, distinct from adverse effects associated with the presence of dissolved ions [33].

Exposure to Fe nanoparticle:

Medaka:

Oxidative stress was implicated in a study exposing Japanese medaka to nano-Fe [34]. During the latter study both embryos and adult medaka experienced concentration-dependent decreases in the enzyme; superoxide dismutase (SOD).

Exposure to Zn nanoparticle:

Zebrafish:

Kept in water with zinc oxide nanoparticles did not accumulate zinc in the gills, liver, brain, and kidney, whereas when the fish was exposed to cerium dioxide nanoparticles, the dioxide was detected in the liver [35]. Assessment of the toxicities of zinc, aluminum, and titanium oxides for zebrafish embryos and larvae demonstrated that only zinc oxide was toxic for embryos [36]. In their work they obtained the lethal toxicity in this fish. In addition to lethal toxicity test, the molecular biomarker responses were essential to determine the sublethal toxicities of the test chemicals to zebrafish and provide insights into toxic mechanisms of NPs. By monitoring several biomarkers, including SOD, CAT, GSH, MDA and protein carbonyl contents, the disturbances of the oxidative defense system caused by exposure to TiO₂ NPs, ZnO NPs and their bulk particle suspensions were observed in the gill, gut and liver tissue of zebrafish [37]. It should be notice that crucial factor that may affect the toxicity of metal oxide nanoparticles is the release of metal ions. It has been shown previously that the toxicity of nZnO was mainly attributed to the dissolved Zn ions [38,39].

Discussion:

ENPs are small scale substances with various sizes which are usually less than 100 nm. They have

been formed by molecular_level engineering in order to gain unique properties such as optical, magnetic etc. To be used in a large variety of application, being produced increasingly and using in various processes, they are likely to release in to the environment. Toxicity to copper nanoparticles [24], nickel nanoparticles [40], metal oxide nanoparticles [36], carbon nanotubes [26], and fullerenes [41] was assessed. Furthermore, toxicity to other nanoparticles including dendrimers (highly branched polymers with low polydispersity and high functionality) [42] was reported in fish early life stages. Consequently, concerns have arisen with respect of potential health effect on humans, animal and the environment. The toxicological. and ecotoxicological risks connected with nanoparticles are partially known. Several studies demonstrated toxic effects of ENPs such as inflammation [43], cytoskeletal and membrane changes [44], oxidative stress [45], apoptosis [46], changes in gene expression [47] and etc. In spite of numerous study in fish but more studies are required illustrate the effect of nano particles in body of fish and consequently in body of other animals and human as well.

References

1. Neethirajan, S. and D.S. Jayas, 2001. Nanotechnology for the Food and Bioprocessing Industries Food Bioprocess Technol., 4: 39-47.
2. Lines, M.G., 2008. Nanomaterials for practical functional uses Journal of Alloys and Compounds, 449: 242-245.
3. Moore, M.N., 2006. Do nanoparticles present ecotoxicological risks for the health of the aquatic environment? Environ Int., 32(8): 967-976.
4. US Environment Protection Agency, 2005. Nanotechnology white paper. External Review draft. Available from http://www.epa.gov/OSA/pdfs/EPA_nanotechnology_white_paper_external_review_draft.
5. Lee, K.J., P.D. Nallathamby, L.M. Browning, C.J. Osgood and X.H.N. Xu, 2007. In vivo imaging of transport and biocompatibility of single silver nanoparticles in early development of zebrafish embryos. ACS Nano, 1: 133-143.
6. Asharani, P.V., G.L.K. Mun, M.P. Hande and S. Valiyaveetil, 2009. Cytotoxicity and genotoxicity of silver nanoparticles in human cells. ACS Nano, 3: 279-290.
7. Handy, R.H., R. Owen and E. Valsami-Jones, 2008. The ecotoxicology of nanoparticles and nanomaterials: current status, knowledge gaps, challenges, and future needs. Ecotoxicology, 17: 315-325.
8. Kashiwada, S., 2006. Distribution of nanoparticles in the see-through medaka (*Oryzias latipes*). Environ. Health Perspect., 114: 1697-1702.
9. Bar-Ilan, O., R.M. Albrecht, V.E. Fako and D.J. Furgeson, 2009. Toxicity assessments of multisized gold and silver nanoparticles in zebrafish embryos. Small, 5: 1897-1910.
10. Wu, Y., Q. Zhou, H. Li, W. Liu, Th. Wang and G. Jiang, 2010. Effects of silver nanoparticles on the development and histopathology biomarkers of Japanese medaka (*Oryzias latipes*) using the partial-life test. State Key Laboratory of Environmental Chemistry and Ecotoxicology, Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences. Aquatic Toxicology, 100: 160-167.
11. Chae, Y.J., C.H. Pham, J. Lee, E. Bae, J. Yi, M.B. Gu, 2009. Evaluation of the toxic impact of silver nanoparticles on Japanese medaka (*Oryzias latipes*) Aquat Toxicol., 94(4): 320-327.
12. Farkas, J., P. Christian, J.A. Gallego Urrea, N. Roos, M. Hassellöv, K.E. Tollefsen and K.V. Thomas, 2010. Effects of silver and gold nanoparticles on rainbow trout (*Oncorhynchus mykiss*) hepatocytes. Norwegian Institute for Water Research, Gaustadalléen 21, 0349 Oslo, Norway. Aquatic Toxicology, 96: 44-52.
13. Bilberg, K., K.B. Døving, K. Beedholm and E. Baatrup, 2011. Silver nanoparticles disrupt olfaction in Crucian carp (*Carassius carassius*) and Eurasian perch (*Perca fluviatilis*). Faculty of Science, Interdisciplinary Nanoscience Center (iNANO), Aarhus University, Ny Munkegade, Bldg. 1521, Dk-8000 Aarhus C, Denmark. Aquatic Toxicology, 104: 145-152.
14. Klaprat, D.A., R.E. Evans and T.J. Hara, 1992. Environmental contaminants and chemoreception in fishes. In Fish Chemoreception (ed. T. J. Hara), pp: 321-341. London: Chapman and Hall.
15. Laban, G., L.F. Nies, R.F. Turco, J.W. Bickham and M.S. Sepulveda, 2010. The effects of silver nanoparticles on fathead minnow (*Pimephales promelas*) embryos Ecotoxicology, 19: 185-195.
16. Bermudez, E., J.B. Mangum, B. Asgharian, B.A. Wong, E.E. Reverdy, D.B. Janszen, P.M. Hext, D.B. Warheit and J.I. Everitt, 2002. Long-term pulmonary responses of three laboratory rodent species to subchronic inhalation of pigmentary titanium dioxide particles. Toxicol. Sci., 70: 86-97.
17. Griffith, R.J., K. Hyndman, N.D. Denslow and D.S. Barber, 2009. Sources fate and effects of engineered nanomaterials in the aquatic environment. Toxicol. Sci., 107: 404-412.

18. Linhua, H., W. Zhenyu, X. Baoshan, 2009. Effect of sub-acute exposure to TiO₂ nanoparticles on oxidative stress and histopathological changes in Juvenile Carp (*Cyprinus carpio*). College of Environmental Science and Engineering, Ocean University of China, Qingdao 266100, China. E-mail: haolh@126.com. Journal of Environmental Sciences, 21: 1459-1466.
19. Zhang, L., Y. Jiang, Y. Ding, M. Povey and D. York, 2007. Investigation into the antibacterial behaviour of suspensions of ZnO nanoparticles (ZnO nanofluids). J Nanopart Res., 9: 479-89.
20. Federici, G., B. Shaw and R. Handy, 2007. Toxicity of titanium dioxide nanoparticles to rainbow trout (*Oncorhynchus mykiss*): gill injury, oxidative stress, and other physiological effects. Aquat Toxicol., 84: 415-30.
21. Scown, T.M., R. van Aerle, B.D. Johnston, *et al.*, 2009. High Doses of Intravenously Administered Titanium Dioxide Nanoparticles Accumulate in the Kidneys of Rainbow Trout but with No Observable Impairment of Renal Function. Toxicol. Sci., 109(2): 372-380.
22. Yeo, M.K. and M. Kang, 2008. Effects of CuxTiOy nanometer particles on biological toxicity during zebrafish Embryogenesis. Department of Environmental Science and Engineering, KyungHee University, Yongin, Gyeonggi 449-701, Korea Department of Chemistry, College of Science, Yeungnam University.
23. Griffith, R.J., J. Luo, J. Gao, J.C. Bonzongo and D.S. Barber, 2008. Effects of particle composition and species on toxicity of metallic nanomaterials in aquatic organisms. Environ. Toxicol. Chem., 27: 1972-1978.
24. Griffith, R.J., R. Weil, K.A. Hyndman, N.D. Denslow, K. Powers, D. Taylor and D.S. Barber, 2007. Exposure to copper nanoparticles causes gill injury and acute lethality in zebrafish (*Danio rerio*). Environ. Sci. Technol., 41: 8178-8186.
25. Zhao, J., Zh. Wang, X. Liu, X. Xie, K. Zhang, B. Xing, 2011. Distribution of CuO nanoparticles in juvenile carp (*Cyprinus carpio*) and their potential toxicity. College of Environmental Science and Engineering, Ocean University of China, Qingdao 266100, China. Journal of Hazardous Materials, 197: 304-310.
26. Cheng, J., E. Flahaut and S.H. Cheng, 2007. Effect of carbon nanotubes on development zebrafish (*Danio rerio*) embryos. Environ. Toxicol. Chem., 26: 708-716.
27. Fent, K., C.J. Weisbrod, A. Wirth-Heller and U. Pieles, 2009. Assessment of uptake and toxicity of Fluorescent silica nanoparticles in zebrafish (*Danio rerio*) early life stage. university of Applied sciences Northwestern Switzerland, Institute of Ecopreneurship, School for life sciences.
28. Smith, C.J., B.J. Shaw and R.D. Handy, 2007. Toxicity of single walled carbon nanotubes to rainbow trout (*Oncorhynchus mykiss*): respiratory toxicity, organ pathologies, and other physiological effects. Aquat. Toxicol., 82: 94-109.
29. Oberdorster, E., 2004. Manufactured Nanomaterials (Fullerenes, C60) Induce Oxidative Stress in the Brain of Juvenile Large mouth Bass, Env. Health. Perspect, 112: 1058-1062.
30. Zhu, Y., T. Ran, Y. Li, *et al.*, 2006. Dependence of the Cytotoxicity of Multi Walled Carbon Nanotubes on the Culture Medium, Nanotechnology, 17: 4668-4674.
31. Sun, H., X. Zhang, Q. Niu, *et al.*, 2007. Enhanced Accumulation of Arsenate in Carp in the Presence of Titanium Dioxide Nanoparticles, Water Air Soil Pollut., 178: 245-254.
32. Krysanov, E. Yu., T.B. Demidova, L.A. Pel'gunova, *et al.*, 2009. Effect of nanoparticles of Hydrated Stannous Dioxide (SnO₂ × H₂O) on Guppi (*Poecilia reticulata* Peters, 1860), Dokl. Akad. Nauk., 426(6): 844-846.
33. Nair, S., A. Sasidharan, V.V.D. Rani, D. Menon, S. Nair, K. Manzoor, *et al.*, 2009. Role of size scale of ZnO nanoparticles and microparticles on toxicity toward bacteria and osteoblast cancer cells. J Mater Sci Mater Med., 20: 235-41.
34. Li, H., Q. Zhou, Y. Wu, J. Fu, T. Wang, G. Jiang, 2009. Effects of waterborne nano-iron on medaka (*Oryzias latipes*): antioxidant enzymatic activity, lipid peroxidation and histopathology. Ecotoxicol Environ Saf. Mar., 72(3): 684-92.
35. Johnston, B.D., T.M. Scown, J. Moger, *et al.*, 2010. Bioavailability of Nanoscale Metal Oxides TiO₂, CeO₂, and ZnO to Fish, Environ. Sci. Technol., 44(3): 1144-1151.
36. Zhu, X.S., L. Zhu, Z.H. Duan, R.Q. Qi, Y. Li and Y.P. Lang, 2008. Comparative toxicity of several metal oxide nanoparticle aqueous suspensions to Zebrafish (*Danio rerio*) early developmental stage. J. Environ. Sci. Health A Tox Hazard. Subst. Environ. Eng., 43: 278-84.
37. Xiong, D., T. Fang, L. Yu, X.F. Sima and W.T. Zhu, 2011. Effects of nano-scale TiO₂, ZnO and their bulk counterparts on zebrafish: Acute toxicity, oxidative stress and oxidative damage. Institute of Hydrobiology, Chinese Academy of Sciences.
38. Aruoja, V., H. Dubourguier K. Kasemets and A. Kahru, 2009. Toxicity of nanoparticles of CuO, ZnO and TiO₂ to microalgae *Pseudokirchneriella subcapitata*. Sci Total Environ., 407: 1461-8.
39. Franklin, N., N. Rogers, S. Apte, G. Batley, G. Gadd and P. Casey, 2007. Comparative toxicity of nanoparticulate ZnO, bulk ZnO, and ZnCl₂ to a freshwater microalga (*Pseudokirchneriella*

- subcapitata*): the importance of particle solubility. Environ Sci Technol., 41: 8484-90.
40. Ipsas, C., D. Andreescu, A. Patel, D.V. Goia, S. Andreescu and K.N. Wallace, 2009. Toxicity and developmental defects of different sizes and shape nickel nanoparticles in zebrafish. Environ. Sci. Technol., 43: 6349-6356.
 41. Zhu, X., L. Zhu, Y. Li, *et al.*, 2007. Developmental Toxicity in Zebrafish (*Danio rerio*) Embryos after Exposure to Manufactured Nanomaterials Buckminster fullerene Aggregates (NC60) and Fullerol, Env. Toxicol. Chem., 26(5): 976-979.
 42. King Heiden, T.C., E. Dengler, W.J. Kao, W. Heideman and R.E. Peterson, 2007. Developmental toxicity of low generation PAMAM dendrimers in zebrafish. Toxicol. Appl. Pharmacol., 225: 70-79.
 43. Grassian, V.H., P.T., O'Shaughnessy, A. Adamcakova-Dodd, J.M. Pettibone and P.S. Thorne, 2007. Inhalation exposure study of titanium dioxide nanoparticles with a primary particle size of 2 to 5 nm. Environ. Health Perspect., 115: 397-402.
 44. Shvedova, A., V. Castranova, E. Kisin, D. Berry-Schwegler, A. Murria, V. Gandelsman, A. Maynard, P. Baron, 2003. Exposure of carbon nanotube cytotoxicity using human keratinocyte cells. J Toxicol Environ Health Part A 66(20): 1909-1926.
 45. Limbach, K., P. Wick, P. Manser, R.N. Grass, A. Bruinink and W.J. Stark, 2007. Exposure of engineered nanoparticles to human lung epithelial cells: influence of chemical composition and catalytic activity on oxidative stress. Environ. Sci. Technol., 41: 4158-4163.
 46. Park, E., J. Yi, K. Chung, D. Ryu, J. Choi and K. Park, 2008. Oxidative stress and apoptosis induced by titanium dioxide nanoparticles in cultured BEAS-2B cells. Toxicol. Lett., 180: 222-229.
 47. Fujita, K., Y. Morimoto, A. Ogami, T. Myoiyo, I. Tanaka, M. Shimada, *et al.*, 2009. Gene expression profiles in rat lung after inhalation exposure to C60 fullerene particles. Toxicology, 258: 47-55.