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EVALUATION OF SILVER NANOPARTICLES ECOTOXICITY IN SILVER CARP (*HYPOPHTHALMICTHYS MOLITRIX*) AND GOLDFISH (*CARASSIUS AURATUS*)

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Summary

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Recently, commercial nanometer sized silver is extensively used for its antibacterial effect; however, nanoparticles may also have ecotoxicological effects after being discharged into aquatic ecosystems. Nanometer sized silver can flow into water ecosystems, where it can exert a variety of physiologically effects in aquatic animals, including fish. The current research aimed to examine the effect of nanometer sized Iranian commercial nanosilver (Nanocid®) on the mortality response of two freshwater fish species – Silver Carp (*Hypophthalmicthys molitrix*) and Goldfish (*Carassius auratus)* and define the relationship between nanoparticle toxicity concentration and survival of these species. Fish were exposed to nanometer sized silver at concentrations of 0, 0.01, 0.1, 0.5, 1, 2.5 and 5 ppm. LC_{50} was determined with probit analysis. The LC_{50} in goldfish (0.53 ppm) was higher than that of silver carps (0.34 ppm). Increased mortality was concomitantly observed with exposure to silver nanoparticles (AgNPs), which suggests that AgNPs could accumulate in aquatic environments and seriously disturb the development of fish species.

Key words: acute test, fish, mortality, nanotechnology

INTRODUCTION

Silver nanoparticles (AgNPs) are widely used worldwide as spectrally selective coatings for solar energy absorption, chemical catalysts and especially for antimicrobial sterilisation (Boudou & Ribeyre, 1997; Pal *et al.*, 2007). After their discharge, AgNPs will most likely enter the ecosystems and may produce a physiological response in many animals, possibly altering their fitness, and ultimately changing their densities or community populations. Open access literature regarding the toxicity of nanoparticles is still emerging, and gaps still exist in our knowledge of this area. Wu *et al.* (2010) studied the effects of silver nanoparticles on the development and histopathology of Japanese medaka (*Oryzias latipes*) and after observing many changes, suggested further research on toxicity of silver nanoparticle in fish species. Yeo & Kang (2008) confirmed fatal effects of nanometer sized silver materials on zebrafish embryogenesis. Also, Solatni *et al.* (2011) reported the toxic effect of nanosilver particles on hatchability of rainbow trout (*Oncorhynchus mykiss*) eggs and the survival of the produced larvae.

Toxicological researches on nanoparticles are limited, with only a few studies on their acute toxic effects on aquatic animals (Houk & Waters, 1996; Lovern & Klaper, 2006; Handy & Shaw, 2007; Lovern *et al.*, 2007). Despite the dramatic increase in the use of AgNPs, little data are available on their potential harmful effects on ecosystems (Lovern *et al.*, 2007).

LC_{50} is the biological index of 50% mortality in an exposed population. The 96-hour LC_{50} tests are conducted to measure the susceptibility and mortality potential of biota to particular toxic substances (Boudou & Ribeyre, 1997). Higher LC_{50} values are less toxic because greater concentrations are required to produce 50% mortality in exposed animals.

In the current study, conventional median lethal concentration tests were conducted on two freshwater fish species: Silver Carp (*Hypophthalmicthys molitrix*) and Goldfish (*Carassius auratus*), as they may provide insights to the potential toxic effects of AgNPs in aquatic environments and the ecological impact of Iranian nanotechnology companies. Given the importance of *H. molitrix* and *C. auratus* in freshwater ecosystems, information concerning the ecotoxicity of widely used nanomaterials on these species could be valuable in relation to aquatic nanoecotoxicology.

MATERIALS AND METHODS

Acute toxicity tests were conducted on silver carp (~45 g & 18 cm) and goldfish (~15 g & 7 cm) obtained from commercial fish farms, Gorgan, Iran. Only healthy fish, as indicated by their activity and external appearance, were maintained alive on board in a fiberglass tank. Samples were transferred to a 400 L aerated tank with 200 L of test medium.

All samples were acclimated for one week in 15 aerated fiberglass tanks at 18 ^{o}C under a constant 12:12 L:D photoperiod. Acclimatised fish were fed daily a formulated feed. Dead fish were immediately removed with special plastic forceps to avoid possible deterioration of water quality.

In this study, Nanocid® L2000, a P-series powder product for antimicrobial purposes (Nano Nasb Pars Company, Theran, Iran) was evaluated. A water soluble form of colloidal, brown, silver nanoparticles was used. It was concentrated at 4000 mg/L (stock solution) with an average nanoparticle size of 18 nm. Test concentrations were made according to the formula $M_1V_1 = M_2V_2$ where M_1 – stock solution molality, V_1 – stock solution volume, M_2 – tank molality and V_2 – tank volume.

Groups of 21 fish from each species were exposed for 96 h in fiberglass tanks to each tested AgNPs concentration. The test medium was not renewed during the assay and no food was provided to the animals.

Acute toxicity tests were carried out in order to calculate the 96-hour LC_{50} for silver. Mortality was recorded after 24, 48, 72 and 96 h. The LC_{50} values were determined (Boudou & Ribeyre, 1997). Cumulative mortality percentages were calculated for each AgNPs concentration after 24, 48, 72 and 96 h of exposure.

LC_{50} values were calculated from the data obtained in acute toxicity bioassays, by Finney's probit analysis (Finney, 1971) using SPSS computer statistical software. With this method, the LC_{50} value is derived by fitting a regression equation arithmetically and also by graphical interpolation by taking logarithms of the test chemical concentration on the X axis and the probit value of percentage mortality

on the Y axis (Mohapatra & Rengarajan, 1995). Probit transformation adjusts mortality data to an assumed normal population distribution that results in a straight line. Probit transformation is derived from the normal equivalent deviate (NED) approach developed by Tort, who proposed measuring the probability of responses (i.e., proportion dying) on a transformed scale based in terms of percentage of population or the standard deviations from the mean of the normal curve.

The LC_1, LC_{10}, LC_{30}, LC_{50}, LC_{70}, LC_{90}, LC_{99} values were derived using simple substitution probit of 1, 10, 30, 50, 70, 90 and 99 respectively for probit of mortality in the regression equations of probit of mortality vs. silver. The 95% confidence limits for LC_{50} were estimated as LC_{50} (95% CL) = $LC_{50} \pm 1.96$ [SE (LC_{50})]. The SE of LC_{50} is calculated from the formula $SE(LC_{50}) = 1/b\sqrt{pnw}$, where: b – the slope of the silver/probit response (regression) line; p – the number of silver used, n – the number of animals in each group, w – the average weight of the observations.

At the end of acute test, the lowest concentration with mortality and the highest concentration with no mortality, (LOEC and NOEC, respectively) were determined for each endpoint. In addition, the maximum acceptable toxicant concentration (MATC) was estimated for the endpoint dividing LC_{50} to 10 (Di Giulio & Hinton, 2008).

RESULTS

The cumulative mortality percentages after acute exposure of Silver carp and Goldfish to Nanocid® at 0, 0.01, 0.1, 0.5, 1, 2.5 and 5 ppm for 24, 48, 72 and 96 h are presented in Tables 1 and 2. Fishes exposed during the period 24–96 h exhibited significantly increased numbers of dead individuals with increasing concentration. There were significant differences in number of dead fish between the different exposure intervals. There was 100% mortality at 1 ppm of Nanocid® within the 96-hour period of exposure for silver carps and goldfish, and no mortality at 0.01 and 0.1 ppm after 96 h exposure for both silver carps and goldfish.

Median lethal concentrations of 1%, 10%, 30%, 50%, 70%, 90% and 99% test are shown in Table 3. According to time-mortality curves, LC_{50} of goldfish was higher than that of silver carp.

Toxicity testing statistical endpoints are shown in Table 4. The lowest observed effect concentration (LOEC) and

Table 1. Cumulative mortality percentages of silver carp during acute exposure to Nanocid® (n=21 for each tested concentration)

Nanocid® concentration (ppm)	Percentage of mortality			
	24 h	48 h	72 h	96 h
Control	0	0	0	0
0.01	0	0	0	0
0.1	0	0	4.7	14.3
0.5	14.2	33.3	52.4	90.5
1	66.6	76.2	100.0	100.0
2.5	100.0	100.0	100.0	100.0
5	100.0	100.0	100.0	100.0

Table 2. Cumulative mortality percentages of goldfish during acute exposure to Nanocid® (n=21 for each tested concentration)

Nanocid® concentration	Percentage of mortality			
	24 h	48 h	72 h	96 h
Control	0	0	0	0
0.01 ppm	0	0	0	0
0.1 ppm	0	0	0	0
0.5 ppm	0	9.5	47.6	47.6
1.0 ppm	19.0	61.9	90.4	100.0
2.5 ppm	52.3	80.9	85.7	100.0
5.0 ppm	100.0	100.0	100.0	100.0

Table 3. Time course of lethal concentrations (LC_1-LC_{99}) of Nanocid® (mean ± SEM) in silver carp (n=21) and goldfish (n=21)

Point	Fish Species	Nanocid® Concentration (ppm)			
		24 h	48 h	72 h	96 h
LC_1	Silver carp	0.15 ± 0.68^{a}	0.01 ± 0.55^{a}	0.01 ± 0.89^{a}	0.01 ± 0.88^{a}
	Goldfish	0.10 ± 0.34^{a}	0.01 ± 0.23^{a}	0.01 ± 0.20^{a}	0.03 ± 0.91^{a}
LC_{10}	Silver carp	0.47 ± 0.68^{ab}	0.33 ± 0.55^{a}	0.22 ± 0.89^{ab}	0.09 ± 0.88^{a}
	Goldfish	1.09 ± 0.34^{ab}	0.39 ± 0.23^{a}	0.01 ± 0.20^{a}	0.26 ± 0.91^{b}
LC_{30}	Silver carp	0.69 ± 0.68^{ab}	0.56 ± 0.55^{a}	0.38 ± 0.89^{ab}	0.23 ± 0.88^{b}
	Goldfish	1.81 ± 0.34^{b}	1.00 ± 0.23^{b}	0.55 ± 0.20^{ab}	0.42 ± 0.91^{b}
LC_{50}	Silver carp	0.85 ± 0.68^{ab}	0.72 ± 0.55^{a}	0.48 ± 0.89^{ab}	0.34 ± 0.88^{b}
	Goldfish	2.31 ± 0.34^{b}	1.42 ± 0.23^{b}	0.95 ± 0.20^{ab}	0.53 ± 0.91^{b}
LC_{70}	Silver carp	1.01 ± 0.68^{ab}	0.88 ± 0.55^{ab}	0.59 ± 0.89^{ab}	0.44 ± 0.88^{b}
	Goldfish	2.81 ± 0.34^{b}	1.84 ± 0.23^{b}	1.35 ± 0.20^{b}	0.64 ± 0.91^{b}
LC_{90}	Silver carp	1.23 ± 0.68^{b}	1.11 ± 0.55^{b}	0.75 ± 0.89^{ab}	0.59 ± 0.88^{b}
	Goldfish	3.53 ± 0.34^{c}	2.45 ± 0.23^{c}	1.92 ± 0.20^{b}	0.80 ± 0.91^{b}
LC_{99}	Silver carp	1.55 ± 0.68^{b}	1.43 ± 0.55^{b}	0.97 ± 0.89^{ab}	0.80 ± 0.88^{b}
	Goldfish	4.52 ± 0.34^{c}	3.29 ± 0.23^{c}	2.71 ± 0.20^{b}	1.02 ± 0.91^{b}

Different letters indicate statistically significant differences at $P<0.05$.

no observed effect concentration (NOEC) were the same for studied fishes (0.1 and 0.01 ppm respectively). LC_{50} differed significantly between species ($p<0.05$). The maximum acceptable toxicant concentrations (MATC) of Nanocid® for silver carp and goldfish were 0.03 and 0.05 ppm, respectively.

DISCUSSION

In determining the toxicity of a new chemical to fish, an acute toxicity test is first conducted to estimate the median lethal concentration (LC_{50}) of the chemical in water to which organisms are exposed (Di Giulio & Hinton, 2008). The relationship between the degree of res-

Table 4. Acute toxicity testing statistical endpoints of silver nanoparticles: NOEC = no observed effect concentration; LOEC = lowest observed effect concentration; MATC = maximum acceptable toxicant concentration

Point (ppm)	Fish Species	
NOEC	Silver carp	0.01
	Goldfish	0.01
LOEC	Silver carp	0.10
	Goldfish	0.10
LC_{50}	Silver carp	0.34
	Goldfish	0.53
MATC	Silver carp	0.03
	Goldfish	0.05

ponse of test organisms and the quantity of exposure to the chemical almost always assumes a concentration–response form (Di Giulio & Hinton, 2008). The cumulative responses to silver concentrations yield a sigmoid (S-shaped) curve.

The individual variability in acute toxicity even within a species and with the same toxicant depends on the size, age, and condition of the tested organism as well as on experimental factors. The differences in acute toxicity could even be due to changes in water quality and test species (Rathore & Khangarot, 2002). In the present study, LC_{50} values indicated that silver was toxic to the studied fish, especially for silver carps. Compared to corresponding values published in the literature for other species of fish, the present lower LC_{50} values confirmed the sensitivity of aquaculture species to low silver doses. It should be remembered that although the LC_{50} under a defined set of environmental conditions can provide useful information, the numeric value can be different in other conditions.

AgNPs have shown to be cytotoxic and to exert harmful effects in fish species. In medakas (*Oryzias latipes*) used at early-life stages as experimental models, the developmental toxicity of silver nanoparticles was investigated following exposure to AgNPs at high concentrations (≥ 400 μg/L) (Wu *et al.*, 2010). In recent years, few toxicological studies related to nanosilver were published. A study on the size effect of AgNPs using rainbow trout (*Oncorhynchus mykiss*) was conduced by Scown *et al.* (2010). In this study, rainbow trouts were exposed via the water to commercial silver particles of three nominal sizes: 10 nm, 35 nm and 600–1600 nm for 10 days. When the uptake of AgNPs from the water medium into the tissues of exposed fish was measured, it was low. Of the silver particles tested, those sized 10 nm were found to be the most highly concentrated within gill tissues.

In our research, the results suggested that AgNPs may have a toxic potential toward silver carps and goldfish and that the investigation on AgNPs-induced mortality could contribute to the knowledge on their toxicity in aquatic ecosystems, for which little data are available. However, further research on the mechanism of AgNPs-induced damage and mortality are needed to better explain the ecotoxicity of AgNPs in freshwater fish.

Based on the results of this study, it is suggested that small-sized nanosilver particles induce active toxicological or biological responses and mortality after repeated exposure in water. Aquatic toxicity tests may provide insights to the relative sensitivity of silver carp and goldfish to AgNPs, and information on the impact of nanoparticles on water environment, as these species hold important positions in aquatic ecosystems. A significant increase in mortality was observed in silver carp and goldfish exposed to acute dose of Nanocid®, however this acute effect was higher in silver carp.

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