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# Comparison of Toxicity Responses by Water Exposure to Silver Nanoparticles and Silver Salt in Common Carp (*Cyprinus carpio*)

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**Abstract:** Toxicity of nanoparticles depends on many factors including size, shape, chemical composition, surface area and surface charge. In this research, we compared the toxicity of different sized-silver nanoparticles (AgNPs) which are being widely used with Iranian consumers due to its unique antimicrobial activity. Ecotoxic assessments of silver nanoparticles (AgNPs) from two Iranian companies of nanotechnology (Nanocid® and Nanosil®) and silver salt ($AgSO_4$) were conducted on the freshwater fish *Cyprinus carpio*. $LC_{50}$ was determined with probit analysis. The 96h toxicity tests for Nanocid®, Nanosil® and silver salt were 0.43±0.90, 73.8±0.38 and 0.33±0.3 respectively. As there can found $LC_{50}$ of silver in nanosil® was higher than other products, however in contrast to others, it was at commercial dose. Increased mortality was concomitantly observed with AgNPs-exposed, which suggests AgNPs-induced mortality might provoke higher-level consequences. The results of the comparative toxicities of AgNPs and Ag ions suggested that AgNPs are slightly more toxic than Ag ions. Overall, these results reported that AgNPs is toxic toward common carp, which may contribute to the knowledge relating to the aquatic toxicity of AgNPs on aquatic ecosystems, for which little data are available.

**Key words:** Fish · $LC_{50}$, Nanotechnology · Nanocid® · Nanosil® · Toxicity Test

## INTRODUCTION

Silver nanoparticles (AgNPs) have the most commonly used in our planet, including spectrally selective coatings for solar energy absorption, chemical catalysts and especially antimicrobial sterilization, which has many applications made them one of nonmaterial's [1-3]. Widely used nanoparticles, such as silver nanoparticles, will most likely enter the ecosystem and may produce a physiological response in many animals, possibly altering their fitness and might ultimately change their densities or community populations. Open access literature regarding the toxicity of nanoparticles (NPs) is still emerging and gaps still exist in our knowledge of this area [3].

Despite the dramatic increase in the use of these NPs, little data is available on their potential harmful effects on the ecosystems. Most researches on the toxicity of NPs come from mammalian studies that have focused on respiratory exposure, or from *in vitro* assays using mammalian cells [4]. Toxicological researches on nanoparticles are more limited, with only a few studies on the acute toxic effects of Nps on aquatic animals [4-7].

Acute toxicity data can help identify the mode of toxic action of a substance and may provide information on doses associated with target-organ toxicity and lethality that can be used in setting dose levels for repeated-dose studies. This information may also be extrapolated for use in the diagnosis and treatment of toxic reactions in humans. The results from acute toxicity tests can provide information for comparison of toxicity and dose-response among members of chemical classes and help in the selection of candidate materials for further work [8].

In fish researches, few ecotoxicological studies on aquatic organisms have been performed, so in current study conventional median lethal concentration tests were conducted on the Common carp, as they may provide insights to the potential toxic effects of AgNPs on aquatic environments and introduce most toxicants material from Iranian common companies. Given the importance of *C. carpio* in freshwater ecosystems, information concerning the ecotoxicity of widely used nanomaterials on these species could be valuable in relation to aquatic nanoecotoxicology. To compare the toxicity of AgNPs to that of silver ions, the toxicity of silver ions was also examined in *C. carpio* using the same toxic endpoints as used in the AgNPs toxicity assay.

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## MATERIALS AND METHODS

In this study the effective doses of AgNPs and Ag salt were compared, so two nano products from Iranian nanotechnology companies (Nanocid® and Nanosil®) were compared with silver sulphate salt.

Nanocid®: A water soluble form of colloidal, brown, silver nanoparticles called Nanocid® - L2000 was used. It was concentrated at 4000 mg/l (stock solution) with an average nanoparticle size of 18 nm. This was a P-series powder product that was made by Nano Nasb Pars Company, Tehran, Iran for antimicrobial purposes. We used effective dose of Nanocid® with injection of 4000 ppm stock solution.

Nanosil®: A water soluble form of Ag ions size <100 nm, made by Kimiafaam Company, Tehran, Iran were homogenously dispersed in deionized water by sonication. As we did not had exact effective dose of Nanosil®, during the toxicity test it was used as commercial dose.

Ag salt (Silver sulphate): To compare the toxicities of AgNPs and Ag ions, $AgSO_4$ salt (Merck- Germany) aqueous in deionized water was used.

Acute toxicity tests were conducted on common carp (~18 g & 12 cm). *C. carpio* were obtained in commercial fish farms, Gorgan-Iran and maintained in fiberglass tanks in Veniro laboratory. Only healthy fish, as indicated by their activity and external appearance, were maintained alive on board in a fiberglass tank. Samples transferred to a 400-L aerated tank equipped with aeration with 200 L of test medium.

All samples were acclimated for one week in a 15 aerated fiberglass tank at 25°C under a constant 12:12 L: D photoperiod. Acclimatized fish were fed daily with a formulated feed. Dead fish were immediately removed with special plastic forceps to avoid possible deterioration of the water quality [9].

Silver tested concentrations were 0, 2, 5, 10, 20 and 40 ppm pure oil, groups of seven fish were exposed to 96h in fiberglass tank. Test medium was not renewed during the assay and no food was provided to the animals. Values of mortalities were measured at time 0, 24, 48, 72 and 96h [10].

Acute toxicity tests were carried out in order to calculate the 96h-$LC_{50}$ for silver. Mortality was recorded after 24, 48, 72 and 96h and $LC_{50}$ values and its confidence limits (95%) were calculated by Boudou and Ribeyre [11]. Percentages of fish mortality were calculated for each silver concentration at 24, 48, 72 and 96h of exposure.

Also $LC_{50}$ values were calculated from the data obtained in acute toxicity bioassays, by Finney's [12] method of ''probit analysis'' and with SPSS computer statistical software. In Finney's 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 [12].

The 95% confidence limits of the $LC_{50}$ values obtained by Finney's method were calculated with the formula of Mohapatra and Rengarajan [13]. 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 [14].

The $LC_{1,10,30,50,70,90,99}$ 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 by using the formula $LC_{50}$ (95% CL) = $LC_{50}$±1.96 [SE ($LC_{50}$)]. The SE of $LC_{50}$ is calculated from the formula: $SE(LC50) = 1/b\sqrt{pnw}$

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 LOEC and NOEC were determined for each endpoint measured. In addition, the maximum acceptable toxicant concentration (MATC) was estimated for the endpoint with the lowest NOEC and LOEC [15].

## RESULTS

The mortality of silver doses for Nanocid® 0, 0.01, 0.1, 0.5, 1, 2.5 and 5 ppm, Nanosil® 0, 0.2, 2, 20, 50, 100 and 200 ppm and silver sulphate 0.001, 0.01, 0.1, 0.5, 1 and 2 ppm were examined during the exposure times at 24, 48, 72 and 96h (Tables 1-3). Fishes exposed during the period 24-96h had significantly increased number of dead individual with increasing concentration. There were significant differences in number of dead fish between the duration 24-96 in each. There were 100% mortality at 1 ppm of Nanocid® ans silver sulphate and 200 ppm of nanosil® concentration within the 96h after exposure for all fishes and no mortality at 0.1, 20 and 0.01 ppm within the exposure times for nanocid®, nanosil® and silver sulphate respectively.

Table 1: Cumulative mortality of common carp during acute exposure to Nanocid® (n=21, effective dose).

| | No. of died fishes | | | |
|---|---|---|---|---|
| Concentration (ppm) | 24h | 48h | 72h | 96h |
| Control | 0 | 0 | 0 | 0 |
| 0.01 | 0 | 0 | 0 | 0 |
| 0.1 | 0 | 0 | 0 | 0 |
| 0.5 | 2 | 5 | 9 | 16 |
| 1 | 11 | 18 | 21 | 21 |
| 2.5 | 16 | 21 | 21 | 21 |
| 5 | 21 | 21 | 21 | 21 |

Table 2: Cumulative mortality of common carp during acute exposure to Nanosil® (n=21, commercial dose)

| | No. of died fishes | | | |
|---|---|---|---|---|
| Concentration (ppm) | 24h | 48h | 72h | 96h |
| Control | 0 | 0 | 0 | 0 |
| 0.2 | 0 | 0 | 0 | 0 |
| 2 | 0 | 0 | 0 | 0 |
| 20 | 0 | 0 | 0 | 0 |
| 50 | 1 | 1 | 3 | 7 |
| 100 | 6 | 7 | 11 | 16 |
| 200 | 19 | 21 | 21 | 21 |

Table 3: Cumulative mortality of common carp during acute exposure to $AgSO_4$ (n=21, each concentration)

| | No. of died fishes | | | |
|---|---|---|---|---|
| Concentration (ppm) | 24h | 48h | 72h | 96h |
| Control | 0 | 0 | 0 | 0 |
| 0.001 | 0 | 0 | 0 | 0 |
| 0.01 | 0 | 0 | 0 | 0 |
| 0.1 | 4 | 6 | 6 | 9 |
| 0.5 | 4 | 7 | 13 | 15 |
| 1 | 3 | 8 | 14 | 21 |
| 2 | 16 | 21 | 21 | 21 |

Table 4: Lethal Concentrations ($LC_{1-99}$) of Nanocid® (mean±SE) depending on time (24-96h) for common carp

| | Concentration (ppm-effective dose) (95 % of confidence limits) | | | |
|---|---|---|---|---|
| Point | 24h | 48h | 72h | 96h |
| $LC_1$ | 0.01±0.23 | 0.10±0.69 | 0.05±0.91 | 0.01±0.90 |
| $LC_{10}$ | 0.46±0.23 | 0.37±0.69 | 0.27±0.91 | 0.17±0.90 |
| $LC_{30}$ | 1.12±0.23 | 0.56±0.69 | 0.43±0.91 | 0.33±0.90 |
| $LC_{50}$ | 1.57±0.23 | 0.70±0.69 | 0.54±0.91 | 0.43±0.90 |
| $LC_{70}$ | 2.03±0.23 | 0.83±0.69 | 0.66±0.91 | 0.54±0.90 |
| $LC_{90}$ | 2.68±0.23 | 1.03±0.69 | 0.82±0.91 | 0.70±0.90 |
| $LC_{99}$ | 3.59±0.23 | 1.29±0.69 | 1.04±0.91 | 0.91±0.90 |

Table 5: Lethal Concentrations ($LC_{1-99}$) of Nanosil® (mean±SE) depending on time (24-96h) for common carp

| | Concentration (ppm-commercial dose) (95 % of confidence limits) | | | |
|---|---|---|---|---|
| Point | 24h | 48h | 72h | 96h |
| $LC_1$ | 22.43±0.40 | 35.55±0.69 | 16.92±0.48 | 6.14±0.38 |
| $LC_{10}$ | 71.93±0.40 | 69.83±0.69 | 52.08±0.48 | 36.5±0.38 |
| $LC_{30}$ | 107.8±0.40 | 94.67±0.69 | 77.56±0.48 | 58.5±0.38 |
| $LC_{50}$ | 132.6±0.40 | 111.8±0.69 | 95.21±0.48 | 73.8±0.38 |
| $LC_{70}$ | 157.5±0.40 | 129.0±0.69 | 112.8±0.48 | 89.0±0.38 |
| $LC_{90}$ | 193.3±0.40 | 153.9±0.69 | 138.3±0.48 | 111±0.38 |
| $LC_{99}$ | 242.8±0.40 | 188.2±0.69 | 173.5±0.48 | 141±0.38 |

Table 6: Lethal Concentrations ($LC_{1-99}$) of $AgSO_4$ (mean±SE) depending on time (24-96h) for common carp

| | Concentration (ppm-effective dose) (95 % of confidence limits) | | | |
|---|---|---|---|---|
| Point | 24h | 48h | 72h | 96h |
| $LC_1$ | 0.01±0.2 | 0.01±0.2 | 0.01±0.3 | 0.01±0.3 |
| $LC_{10}$ | 0.40±0.2 | 0.16±0.2 | 0.06±0.3 | 0.05±0.3 |
| $LC_{30}$ | 1.04±0.2 | 0.61±0.2 | 0.39±0.3 | 0.21±0.3 |
| $LC_{50}$ | 1.49±0.2 | 0.92±0.2 | 0.63±0.3 | 0.33±0.3 |
| $LC_{70}$ | 1.94±0.2 | 1.22±0.2 | 0.86±0.3 | 0.44±0.3 |
| $LC_{90}$ | 2.59±0.2 | 1.67±0.2 | 1.20±0.3 | 0.61±0.3 |
| $LC_{99}$ | 3.48±0.2 | 2.28±0.2 | 1.66±0.3 | 0.84±0.3 |

Median lethal concentrations of 1%, 10%, 30%, 50%, 70%, 90% and 99% tests were shown in Tables 4-6. Because mortality (or survival) data were collected for each exposure concentration in a toxicity test at various exposure durations (24, 48, 72, or 96 hours), data can be plotted in other ways; the straight line of best fit is then drawn through the points. These were time-mortality lines. As there can found $LC_{50}$ of silver in nanosil® was higher than others, however silver sulphate had the lowest one.

Statistical results of 96h exposure for observed mortality, expected response and prob during probit analysis of studied parameters are in Figs. 1-3.

Toxicity Testing Statistical Endpoints showed that Lowest Observed Effect Concentration (LOEC) were 0.5, 50 and 0.1 ppm and NOEC (No Observed Effect Concentration) were 0.1, 20 and 0.01 for nanocid®, nanosil® and silver sulphate respectively, however $LC_{50}$ (the median Lethal Concentration) had significant different between parameters. The Maximum Acceptable Toxicant Concentration (MATC) for nanocid®, nanosil® and silver sulphate were 0.04, 7.3 and 0.03 ppm respectively.

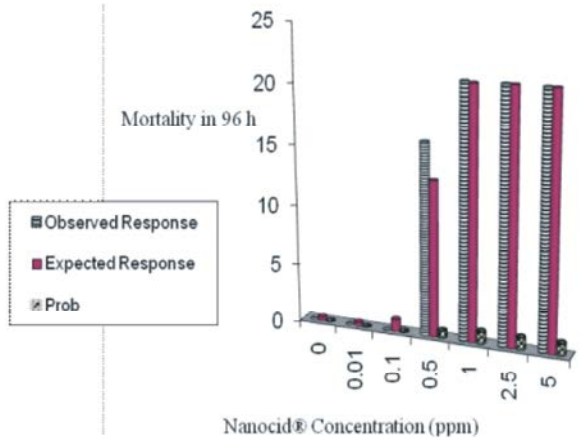


Fig. 1: Nanocid® response curve of common carp exposed to 96h acute toxicity test.

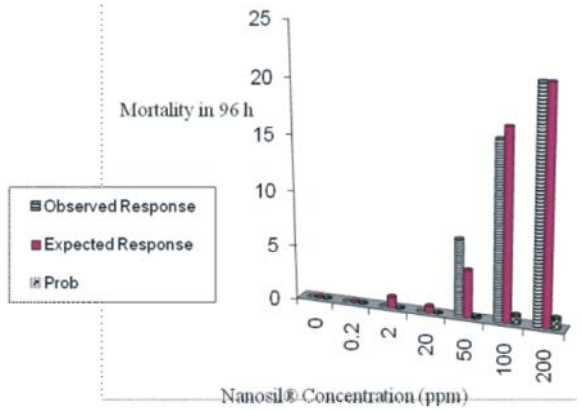


Fig. 2: Nanosil® response curve of silver carp exposed to 96h acute toxicity test.

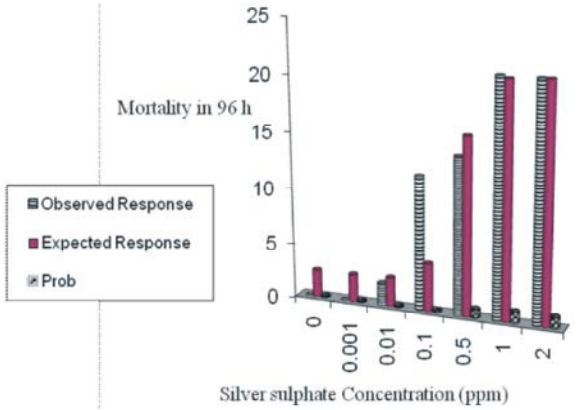


Fig. 3: $AgSO_4$ response curve of roach exposed to 96h acute toxicity test.

## DISCUSSION

Aquatic toxicity tests may provide insights to the relative sensitivity of *C. carpio* to AgNPs, which may also provide suitable data 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 *C. carpio* exposed to 0.5 and 50 mg/l of nanocid® and nanosil® AgNPs; whereas, no significant alteration was observed in 0.1 and 20 mg/l.

It seemed Ag ion exposure leads to a slight increase in mortality. As mortality is the most obvious sign of progression of serious pollutant at the organism level, the impairment role of survival due to AgNPs exposure may be considered a consequence of a serious progression of population mortality.

In recent years, silver and silver nanoparticles (AgNPs) are widely being applied to medical and agricultural uses [16-18]. With the increased applications, animal and eventually human exposure to AgNPs has been increased. It has been confirmed that AgNPs are translated into blood circulation and accumulated in some organs to cause organotoxicity and eventually death [19]. Regarding the AgNPs toxicities, the researches are rapidly increased.

AgNPs significantly increase cell death through oxidative stress-related mechanisms that cause DNA damage in animal cells [20].

AgNPs also showed cytotoxicity and harmful effects on fish cell lines, embryos, larvae and adult fishes. For example medaka (*Oryzias latipes*) cell line was used to investigate the cytotoxicity and genotoxicity of 30nm diameter silver nanospheres, that serious cytotoxicity was shown [21]. Also in this fish species at early-life stages as experimental models, the developmental toxicity of silver nanoparticles was investigated following exposure to AgNPs at high concentrations (≥400µg/l) [22].

In recent years there are a few published toxicological studies related to the nano silvers, for examples a study on the sized effect of AgNPs using rainbow trout (*Oncorhynchus mykiss*) has been published [18]. In the study, rainbow trout were exposed via the water to commercial silver particles of three nominal sizes: 10nm [N (10)], 35nm [N (35)] and 600–1600nm [N (Bulk)] for 10 days. When the uptake of AgNPs from the water medium into the tissues of exposed fish was measured, the uptake level was low. of the silver particles tested, N (10) was found to be the most highly concentrated within gill tissues. In this research, four different sized-AgNPs were prepared and repeated-dose toxicity was evaluated after administration to rat. Furthermore, inflammatory responses were evaluated to investigate the hazardous effects of AgNPs.

Silver particles especially for the nanomaterials concerned, in spite of increase in the use of nanomaterials and their ubiquitous distribution in aquatic environments, little knowledge is available on their potential toxicity on aquatic animals. Considering the potential of *C. carpio* as a bioindicator species and the importance of the toxicity of nanoparticles in ecotoxicity monitoring, the measurement of the mortality response in these species after exposure to nanoparticles could provide useful data for aquatic monitoring.

There have been discussions regarding the comparative toxicity of AgNPs and Ag ions [23]. Our study comparing the toxicity of AgNPs and Ag ions, suggested that AgNPs were slightly more toxic than Ag ions in terms of their effect on mortality potential and it also appeared that different mechanisms exerted the toxicity of AgNPs and with Ag ions [24].

In current research, the toxicities of AgNPs on *C. carpio* were evaluated. The results suggested that AgNPs may have toxic potential toward *C. carpio* and AgNPs-induced mortality might provoke higher-level consequences, which could comprise a contribution to the knowledge on the aquatic toxicity of AgNPs on aquatic ecosystems, for which little data are available. However, further research on the mechanism behind AgNPs-induced damage and mortality are needed to better explain the ecotoxicity of AgNPs in *C. carpio*.

Based on the obtained results of this study, it is suggested that small-sized nanosilvers are more active to exert toxicological or biological responses and they induce mortality responses by repeated water exposure.

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## REFERENCES

1. Zhai, H.J., D.W. Sun and H.S. Wang, 2006. Catalytic properties of silica/silver nanocomposites. J. Nanosci. Nanotechnol., 6: 1968-1972.
2. Yamamoto, S. and H. Watarai, 2006. Surface-enhanced Raman spectroscopy of dodecanethiol-bound silver nanoparticles at the liquid/liquid interface. Langmuir, 22: 6562-6569.
3. Pal, S., Y.K. Tak and J.M. Song, 2007. Does the antibacterial activity of silver nanoparticles depend on the shape of the nanoparticle? A study of the gram-negative bacterium *Escherichia coli*. Appl. Environ. Microbiol., 73: 1712-1720.
4. Lovern, S.B., J.R. Strickler and R. Klaper, 2007. Behavioral and physiological changes in *Daphnia magna* when exposed to nanoparticle suspensions (titanium dioxide, nano-C-60 and C(60)HxC (70)Hx). Environ. Sci. Technol., 41: 4465-4470.
5. Handy, R.D. and B.J. Shaw, 2007. Ecotoxicity of nanomaterials to fish: challenges for ecotoxicity testing. Integr. Environ. Assess. Manag., 3: 458-460.
6. Lovern, S.B. and R. Klaper, 2006. *Daphnia magna* mortality when exposed to titanium dioxide and fullerene (C-60) nanoparticles. Environ. Toxicol. Chem., 25: 1132-1137.
7. Houk, V.S. and M.D. Waters, 1996. Genetic toxicology and risk assessment of complex environmental mixtures. Drug Chem. Toxicol., 19: 187-219.
8. Hedayati, A., A. Safahieh, A. Savar and J. Ghofleh Marammazi, 2010a. Detection of mercury chloride acute toxicity in Yellowfin sea bream. World J. Fish and Marine Science, 2(1): 86-92.
9. Gooley, G.J., F.M. Gavine, W. Dalton, S.S. De Silva, M. Bretherton and M. Samblebe, 2000. Feasibility of aquaculture in dairy manufacturing wastewater to enhance environmental performance and offset costs. Final Report DRDC Project No. MAF001. Marine and Freshwater Resources Institute, Snobs Creek, pp: 84.
10. Hedayati, A., A. Safahieh, A. Savar Ghofleh and J. Marammazi, 2010b. Assessment of aminotransferase enzymes in Yellowfin sea bream under experimental condition as biomarkers of mercury pollution. World J. Fish and Marine Science, 2(3): 186-192.
11. Boudou, A. and F. Ribeyre, 1997. Aquatic ecotoxicology: from the ecosystem to the cellular and molecular levels. Environ. Health Perspect. 105(Suppl. 1): 21-35.
12. Finney, D.J., 1971. Probit Analysis. Univ. Press, Cambridge, pp: 333.
13. Mohapatra, B.C. and K. Rengarajan, 1995. A Manual of Bioassays in the Laboratory and Their Techniques. CMFRI Spec. Pub. 64, CMFRI, Cochin, India, pp: 75.
14. Di Giulio, R.T. and D.E. Hinton, 2008. The Toxicology of Fishes. Taylor & Francis Group, pp: 1101.
15. Hedayati, A. and A. Safahieh, 2011. Serum hormone and biochemical activity as biomarkers of mercury pollution in the Yellowfin seabream *Acanthopagrus latus*. Toxicology and Industrial Health. DOI: 10.1177/0748233711410916.

16. Hotos, G.N. and N. Vlahos, 1998. Salinity tolerance of *Mugil cephalus* and *Chelon labrosus*, Pisces: Mugilidae/fry in experimental conditions. Aquaculture, 167: 329-338.
17. Edwards-Jones, V., 2009. The benefits of silver in hygiene, personal care and healthcare. Lett. Appl. Microbiol., 49(2): 147-152.
18. Scown, T.M., E.M. Santos, B.D. Johnston, B. Gaiser, M. Baalousha, S. Mitov, J.R. Lead, V. Stone, T.F. Fernandes, M. Jepson, R. Van Aerle and C.R. Tyler, 2010. Effects of aqueous exposure to silver nanoparticles of different sizes in rainbow trout.Toxicol. Sci., 115(2): 521–534.
19. Tang, J., L. Xiong, S. Wang, J. Wang, L. Liu, J. Li, F. Yuan and T. Xi, 2009. Distribution, translocation and accumulation of silver nanoparticles in rats. J. Nanosci. Nanotechnol. 9 (8), 4924–4932.
20. Hsin, Y.H., C.F. Chen, S. Huang, T.S.Shih, P.S. Lai and P.J. Chueh, 2008. The apoptotic effect of nanosilver is mediated by a ROS- and JNK-dependent mechanism involving the mitochondrial pathway in NIH3T3 cells. Toxicol. Lett., 179(3): 130-139.
21. Wise Sr., J.P., B.C. Goodale, S.S. Wise, G.A. Craig, A.F. Pongan, R.B. Walter, W.D. Thompson, A.K. Ng, A.M. Aboueissa, H. Mitani, M.J. Spalding and M.D. Mason, 2010. Silver nanospheres are cytotoxic and genotoxic to fish cells. Aquat. Toxicol., 97(1): 34-41.
22. Wu, Y., Q. Zhou, H. Li, W. Liu, T. Wang and G. Jiang, 2009. Effects of silver nanoparticles on the development and histopathology biomarkers of Japanese medaka (*Oryzias latipes*) using the partial-life test. Aquat. Toxicol., Epub ahead of print (PMID: 20034681).
23. Ji, J.H., J.H. Jung and S.S. Kim, 2007. Twenty-eight-day inhalation toxicity study of silver nanoparticles in Sprague-Dawley rats. Inhal. Toxicol., 19: 857-871.
24. Roh, J.Y., S.J. Sim and J. Yi, 2009. Ecotoxicity of Silver Nanoparticles on the Soil Nematode *Caenorhabditis elegans* Using Functional Ecotoxicogenomics. Environ. Sci. Technol., 43: 3933-3940.