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Toxicity of silver nanoparticles in aquatic organisms

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Abstract
Despite numerous published studies which focused on the toxicity of different chemicals on aquatic organisms. Among the various aquatics, amphibians are good candidates for aquatic Nano toxicology since they have the potential of being bio indicators and also are of great importance for investigation of the pollution transference between terrestrial and aquatic ecosystems. In different studies difference in toxicity was also seen due to change in the size time, age and condition of test organisms. Zebra fish is extensively studied model in the Ag-NPs toxicological studies. The toxicity indicators may include, drop in heart rate, hatching delay and higher mortality rate. Amphibians play both the role of prey and top predators in trophic chains based on their habitat and life stage.

Keywords: Amphibians, Nanotechnology, Carp, Zebra

1. Introduction
The increasing commercial application of nanomaterials (NMs), with at least one dimension of ≤100 nm, is currently showing inventory listings of 1317 nanotechnology- based consumer products in 30 countries (Woodrow Wilson Database, 2011) [38], while the production of engineered nanoparticles is expected to reach approximately 60 000 tons in 2011 (Jovanovic´ *et al*., 2011) [14]. The most common nanomaterial mentioned in consumer product inventories is silver, with 313 products (Woodrow Wilson Database, 2011) [38]. In addition, to their importance as antimicrobial agents (Cho *et al*., 2005; Mohan *et al*., 2007; Shahverdi *et al*., 2007; Zheng *et al*., 2008) [5, 20, 29, 40], silver nanoparticles (AgNPs) are also widely utilized in material science, chemistry and physics fields due to their particular magnetic, optical, electronic, and catalytic properties.

1.1 Nanotechnology
Nanotechnology is of promising technologies that has found extensive applications in many scientific and industrial fields. Based on the statistics obtained from thirty countries of the world, the number of consuming products by human being in which the nanomaterials are used, has increased from 54 in 2005 to 1317 in 2011 and it is predicted that this number would be rapidly increasing in future years too (Woodrow Wilson Database, 2011) [38]. According to these statistics, the more frequent nanomaterials used in consumer products are silver, carbon, titanium, silicon, zinc and gold, respectively. Increasing development of nanotechnology and the extension of the applications of nanomaterials in human life has caused many concerns on possible dangers of the release of these materials into the environment. Aquatic ecosystems are one of the final destinations of the released nanomaterials in the environment. These materials may have harmful effects on the aquatic organisms and so, the study of these effects is of great importance. Nanotoxicology is the assessment of toxic effects of nanomaterials on living organisms and hence, aquatic nanotoxicology investigates the toxic effects of nanomaterials on aquatic organisms including aquatic bacteria, unicellular and multicellular algae, zooplanktons, mollusks, crustaceans, amphibians, fish, and etc (Woodrow Wilson Database, 2011) [38].

1.2 Two methods for production of nanomaterials
In general, there are two methods for production of nanomaterials. In bottom-up approaches, molecules are joined together during very special processes to generate larger structures with nano dimensions; Vice versa in top-down approaches, large dimension materials are changed to nano dimension structures by physical methods (Rodgers, 2006) [25]. AgNPs may be also manufactured by both physical and chemical methods. In top-down approach, a large volume of silver metal is first ablated by mechanical method and then the manufactured AgNPs are fixed by adding colloids protectants (Gaffet *et al*., 1996; Amulyavichus *et al*., 1998) [7, 1]. Bottom-up approaches include chemical reduction of silver ions, electrochemical methods and sonochemical processes (Prabhu & Poulose, 2012) [22]. Different chemical precursors which are used in bottom-up approaches for reducing silver ion to AgNPs, may cause secondary effects and sometimes have toxic impacts on organisms. In contrast, no use of chemicals in top-down approaches may reduce these secondary effects. To test this hypothesis, acute toxic effects of two types of AgNPs produced by physical and chemical methods were investigated and compared on the survival of marsh frog tadpoles.

1.3 Toxicity of different chemicals
Despite numerous published studies which focused on the toxicity of different chemicals on aquatic organisms (Imanpour Namin *et al*., 2011; Khodabakhsh *et al*., 2014; Ramzanpour *et al*., 2014; Shirdel and Kalbassi, 2014) [12, 18, 31], the field of aquatic nanotoxicology is relatively new field (Kalbassi *et al*., 2011; Salari Joo *et al*., 2012, 2013; Hosseini *et al*., 2014; Johari, 2014; Johari *et al*., 2015; Tavana *et al*., 2014; Sohn *et al*., 2015a, b) [16, 27, 28, 35, 32]. Among the various aquatics, amphibians are good candidates for aquatic nanotoxicology since they have the potential of being bioindicators and also are of great importance for investigation

of the pollution transference between terrestrial and aquatic ecosystems (Sparling *et al*., 2000) although much less attention have been paid to them in ecotoxicology studies compared other vertebrates.

1.4 Common Carp (Cyprinus carpio)
The results of the comparative toxicities of Ag-NPs and Ag ions suggested that Ag-NPs are slightly more toxic than Ag ions (Hedayati *et al*., 2012b) [13]. Ag-NPs alter the metabolic enzymes in the organs like gills, kidney, brain and liver (Reddy *et al*., 2013) [24]. The liver was found most susceptible to change in Ag-NPs concentration among all the examined tissues (Lee *et al*., 2012). Jung *et al.* (2014) found mean concentra-tion of 5.61 mg kg-1 in the liver when exposed to 0.06±0.12 mgL-1 for 7 days. The other organs were found to have concentration of 3.32 mg kg-1 in gills, 2.93 mg kg-1 in gastrointestinal tract, 0.48 mg kg-1 in the skeletal muscle 0.48 mg kg-1 in skeletal muscle, 0.14 mg kg-1 in brain and 0.02 mg kg-1 in blood. The localized Ag-NPs badly reduce the activities of the metabolic enzymes (SOD, CAT and GST) in brain and other tissues (Lee *et al*., 2012). Silver salts (AgNO3), Nanocid and Nanosil are mostly used in the toxicological studies of Ag-NPs in the case of the juvenile common carp (Hedayati *et al*., 2012b) [13].

1.5 Different aspects of the toxicity of silver compounds
More than 50 papers have already been published on different aspects of the toxicity of silver compounds (except silver nanoparticles) in rainbow trout, plus the acute and chronic toxicity mechanisms of these compounds have also been studied in various fish species, including rainbow trout (for a more detailed description, the reader is invited to see: Hogstrand and Wood, 1998; Wood *et al*., 1999) [37]. To the end of December 2011, based on an online search of different search engines, about 137 papers have been published on the toxicity of 44 different nanomaterials in 14 different fish species, where 34 papers focused on the toxicity of silver nanoparticles in 9 different fish species, including 13 papers on zebrafish and 7 papers on rainbow trout. In summary, these papers found that silver nanoparticles could cause an increase in mortality (Asharani *et al*., 2008; Griffitt *et al*., 2008; Yeo and Yoon, 2009; Asharani *et al*., 2010) [2, 9, 6, 3].

1.6 Toxicity to silver carp (Hypophthalmicthys molitrix)
Hedayati *et al*. (2012a) [11] suggested Ag-NPs are very toxic to the silver carp than the metallic silver. The recorded LC50 value was 0.34 ppm in case of nanocid (Hedayati *et al*., 2012a) [11] and 66.4 ppm in case of nanosil (Jahanbakhsi *et al*., 2012). The mortality also increases as the time of exposure and concentration increases. There was 100% mortality seen in case of 1ppm and 96 hours of exposure (Hedayati *et al*., 2012). In different studies difference in toxicity was also seen due to change in the size time, age and condition of test organisms (Rathore and Khangarot, 2002) [23]. Shalui *et al.* (2013) [30] found 0.810 mg L-1 LC50 value for 24h explore and 0.64, 0.383, 0.202 mg L-1 for 48, 72 and 96h respectively. The Ag-NPs also decrease the RBC, hemoglobin and hematocrit level in the silver carp (Shalui *et al*., 2013) [30].

2. Amphibians
Rowe & Freda (2000) [26] stated that some species of amphibians can avoid contaminated breeding sites but according to Weir *et al.* (2010) [36], some unexpected events like runoff or leaching may contaminate the sites and so, water dependent early-life stages of amphibians which are sensitive to contaminants face contamination. Amphibians play both the role of prey and top predators in trophic chains based on their habitat and life stage (Murphy *et al*., 2000) [21]. This ability makes them even more important in ecotoxicology studies because their roles are very important in accumulation and transfer of toxic substances. Sensitivity of amphibians to contamination is more in larval than in adult stage.

2.1 Zebra fish (Danio rerio)
Zebra fish is extensively studied model in the Ag-NPs toxicological studies (Asharani *et al*., 2008; Kanan *et al*., 2011) [2]. The toxicity indicators may include, drop in heart rate, hatching delay and higher mortality rate (Asharani *et al*., 2008) [2]. The LC50 value is 250 mg L-1 in case of embryo (Choi *et al*., 2010). Bar-llan *et al*. (2009) [4] calculated the LC50 values of 3nm to 100nm of Ag-NPs. The calculated values were 93.31 μM for 3nm particle size and 137.26 μM for 100 nm. The higher value of LC50 for lager particles suggest that the toxicity increases as the particle size decreases. In some studies free Ag+ also demonstrated the almost same cytotoxicity as Ag-NPs with almost same LC50 values in zebra fish model (Kim *et al*., 2009) [6]. The Ag-NPs also accumulated in the different organs like intestine, gills, blood, liver and brain upon exposing fish to particles (Handy *et al*., 2008) [10]. The concentration of accumulated Ag in the liver tissues was found 0.29 and 2.4ng/mg liver when treated with 30 and 120mgL-1 (Choi *et al*., 2010). The accumulated Ag-NPs cause number of cellular alterations in the liver. These alterations are haptic cell cords, apoptotic changes, condensation of chromatin and pyknosis in adult (Gonzalez *et al*., 2006) [8] and circulatory and morphological abnormalities in embryo (Asharani *et al*., 2008; Bar-Ilan *et al*., 2009) [2, 4]. 2 to 4 mgL-1 exposure for 14 days causes decrease the Na(+)/K(+)ATPase activity in the gills and erythrocytes acetylcholinestrase activity (Katuli *et al*., 2014) [17]. The Ag-NPs treatment also causes oxidative damage in the hepatic cells. The DNA damage includes double strand breaks cause lesions in cells (Rothkamm and Lobrich, 2003).

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