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A comparative analysis on the in vivo toxicity of copper nanoparticles in three species of freshwater fish

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HIGHLIGHTS

- Comparative in vivo toxicity of nano-copper was studied in three fish species.
- Temperature can significantly affect the fate and toxicity of nano-copper in water.
- Copper ions were the main driver for the toxic effect of nano-copper.
- Nano-copper can cause damage to gill filaments and gill pavement cells.
- Physiological differences affect the sensitivity of fish species to nano-copper.

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ABSTRACT

Copper nanoparticles (CuNPs) are used extensively in a wide range of products and the potential for toxicological impacts in the aquatic environment is of high concern. In this study, the fate and the acute toxicity of spherical 50 nm copper nanoparticles was assessed in juvenile rainbow trout (*Oncorhynchus mykiss*), fathead minnow (*Pimephales promelas*) and zebrafish (*Danio rerio*) for in vivo aqueous exposures following standardized OECD 203 guideline tests. The fate of the CuNPs in the aqueous media was temperature dependent. At the higher study temperature (26 ± 1 °C), there was both an enhanced particle aggregation and higher rate of dissolution compared with that at the lower study temperature (15 ± 1 °C). 96 h LC_{50}s of the CuNPs were 0.68 ± 0.15, 0.28 ± 0.04 and 0.22 ± 0.08 mg Cu/L for rainbow trout, fathead minnow and zebrafish, respectively. The 96 h lowest-observed-effect concentration (LOEC) for the CuNPs were 0.17, 0.023 and <0.023 mg/L for rainbow trout, fathead minnow, and zebrafish respectively, and are below the predicted environmental concentration of CuNPs for some aquatic environments suggesting a possible ecotoxicological risk to fish. Soluble copper was one of main drivers for the acute toxicity of the copper nanoparticles suspensions. Both CuNPs suspension and copper nitrate caused damage to gill filaments and gill pavement cells, with differences in sensitivity for these effects between the fish species studied. We show therefore common toxicological effects of CuNPs in different fish species but with differences in sensitivity with implications for hazard extrapolation between fish species.

1. Introduction

The potential toxicological impacts of nanoparticles (NPs) are of high concern due to the widespread application and high reactivity of NPs. One type of nanoparticle of particular concern is copper nanoparticles (CuNPs). CuNPs are applied widely in various products, including in electronics, metallic inks and textiles, because of their optical, electrical, and catalytic properties (Han et al., 2011; Hatamie et al., 2014; Kida et al., 2007; Kim et al., 2011; Kubota et al., 2014; Lee et al., 2014; Li et al., 2014). The predicted environmental concentration of CuNPs in some receiving waters is 0.06 mg Cu/L, with a 95% confidence interval of 0.01–0.92 mg Cu/L (Chio et al., 2012) and this has raised concern on their potential for adverse effects on aquatic organisms (Chen et al., 2014; Ganesh et al., 2010).

Some metallic NPs readily undergo dissolution and aggregation in the aquatic environment (Lowry et al., 2012). Dissolution of

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CuNPs produces copper ions, which are highly toxic to fish (Black et al., 2015; Kong et al., 2013). The aggregates of CuNPs may also cause adverse effect to fish because they can remain in a nanostructured form and thus more highly reactive (Griffitt et al., 2009; Zhao et al., 2011). Studies have shown that rates of dissolution and aggregation of metallic NPs can vary considerably under different environment conditions (Handy et al., 2008). For instance, lowering pH can significantly increase the amount of ion release from metallic NPs (Baalousha et al., 2008) and the presence of organic matter may affect the aggregation size of metallic NPs (Grillo et al., 2015). This illustrates the importance of considering the fate of metallic NPs in studies investigating their toxicity.

Studies conducted to date have reported adverse effects of CuNPs in diverse fish species at sub-lethal levels. For example, exposure to CuNPs via aqueous has been shown to affect the gill filament structure in rainbow trout (*Oncorhynchus mykiss*; 80 nm particles exposed at 0.25 mg/L; Griffitt et al., 2007), and induce tissues oxidative stress in the liver, gills and muscles of juvenile *Epinephelus coioides* after 25 days exposure to CuNPs via aqueous with a mean primary particle diameter of 85 ± 29 nm (Wang et al., 2014). A further study on juvenile rainbow trout found that CuNPs (mean primary particle size of 87 ± 27 nm) induced similar types of pathologies in gill, gut, liver, kidney, brain and muscle as occurs for copper ions (Al-Bairuty et al., 2013). Little is known however on the comparative sensitivity of different fish species to the effects of CuNPs. Making comparisons between species for effects across different studies is complicated by the fact that the studies published have used different particles, exposure conditions, and effect measures. Furthermore, as the dissolution profile of CuNPs were not investigated in many of the studies reported in the literature (Al-Bairuty et al., 2013; Wang et al., 2014), it is still unclear whether the toxicity of CuNPs suspensions in fish species was attributed to the particles in the CuNPs suspensions or the copper ions released from CuNPs.

Here we evaluate the fate CuNPs (50 nm spherical) in the test medium and their comparative acute toxicity in three fish species (rainbow trout, fathead minnow and zebrafish) exposed via water by adopting OECD 203 test guidelines for supporting risk assessment of this material. The study both compares the sensitivities of different fish species to CuNPs suspension over time and investigates the contribution of particles and ions to the toxicity of CuNPs suspension. Histopathology was undertaken to examine for effects of the CuNPs suspension and copper ions (via exposure to copper nitrate) on the gill structure in the different fish species.

2. Materials and methods

2.1. Experimental set up

50 nm CuNPs powder stored in inert gas was purchased from IoLiTec, Inc. (Germany). The CuNPs suspension was freshly prepared by dispersing the CuNPs powder in standardized synthetic freshwater and sonicated for 10 min with a probe sonicator (Cole Parmer CPX 130 ultrasonic processor). The standardized synthetic freshwater consisted of 58 mg/L $CaCl_2{\cdot}2H_2O$, 24.65 mg/L $MgSO_4{\cdot}7H_2O$, 12.95 mg/L $NaHCO_3$, 1.15 mg/L KCl and 12.5 mg/L Tropic Marin Sea Salt with a final conductivity of 350 mS and a pH of 7.2–7.4, which is within acceptance of the U.S. EPA guidelines (EPA, 1986; Paull et al., 2008).

The exposure studies were carried out according to the OECD guideline 203 (OECD, 1992). Juvenile zebrafish (length: 26.9 ± 2.1 mm, weight: 0.17 ± 0.03 g), juvenile fathead minnow (length: 56.0 ± 7.4 mm, weight: 1.80 ± 0.66 g) and juvenile rainbow trout (length: 123 ± 13 mm, weight: 20.3 ± 4.5 g) were used for the exposures. Zebrafish and fathead minnow belong to the family of Cyprinidae. Rainbow trout belongs to the family of Salmonidae. The fish species were obtained from a local supplier in the UK and tests were carried out in 30 L tanks (Rainbow trout) and 15 L tanks (Zebrafish and fathead minnow) in reconstituted fresh water that were well aerated. Six fish were randomly placed into the exposure tanks. The dosing adopted for each fish species was based on pilot experiments that determined the toxicity for CuNPs suspension in the different study species. The final exposure concentrations of the CuNPs suspension were 0, 0.05, 0.01, 0.3, 0.5 and 1 mg Cu/L for rainbow trout, and 0, 0.03, 0.05, 0.01, 0.3 and 0.5 mg Cu/L for fathead minnow and zebrafish. The final exposure concentration of $Cu(NO_3)_2$ were 0, 0.05, 0.1, 0.2, 0.4 and 0.6 mg Cu/L for the rainbow trout exposure and 0, 0.003, 0.005, 0.01, 0.03 and 0.05 mg Cu/L for fathead minnow and zebrafish exposures. The concentration–response curve of copper nitrate was used to assess to what extent the toxicity of the CuNPs could be accounted for by the copper ions that would derived from the NPs (more information can be found in the section of data analysis and statistics). Each exposure was carried out in duplicate tanks. Fish were maintained under a 12 h light: 12 h dark photoperiod. Exposures were conducted for 96 h in a semi-static system with the test media renewed thoroughly every 24 h to help maintain the stability of exposure concentrations. The system was aerated to maintain the dissolved oxygen level at a value of at least 90% of the air saturation value. The water temperature was maintained at 15 ± 1 °C for rainbow trout and 26 ± 1 °C for both zebrafish and fathead minnow. Fish mortalities were recorded at intervals of 24, 48, 72 and 96 h and any dead fish were removed from the exposure tanks at these times. Fish were not fed during the experiment.

2.2. Physico-chemical characterization

The CuNPs were characterized as dry powder using Transmission Electron Microscopy (TEM). The hydrodynamic diameter and zeta potential of the CuNPs suspension were measured for a suspension of 1 mg Cu/L immediately after preparation (0 h), and after 24 h in the tank water under the different culture conditions (15 °C and at 26 °C) by Dynamic Light Scattering (DLS) on a Zetasizer Nano-ZS instrument (Malvern, Instruments Ltd., UK). Three independent replicates were measured with each comprising of three separate measurements.

2.3. Measured exposure concentrations and ion release

The exposure concentrations were quantified using inductively coupled plasma-optical emission spectrometry (ICP-OES). Toxicity data were calculated based on measured exposure concentrations. In order to measure the ion release from the CuNPs under each culture scenario in the presence of fish, 15 mL CuNPs suspension was sampled from the middle of the water column after 24 h of incubation at temperatures of 15 °C and 26 °C. The samples were subsequently centrifuged at 30,000*g* for 20 min at 4 °C (Beckman Avanti J-25 centrifuge, UK) to remove the particles from the CuNPs suspension (Song et al., 2014). The supernatants were then acidified using 10% HNO_3 and then analyzed using ICP–OES. Copper ion release (%) was calculated as percentage of the total copper concentration.

2.4. Gill histology

After the exposure, all remaining fish were anesthetized and killed rapidly by a schedule 1 method and according to UK Home Office regulations (Office, 1996), and the total body length and weight of the fish measured. To investigate for possible effects of the CuNPs suspension and $Cu(NO_3)_2$ on the gills, two fish from each of the controls, the highest concentration of the CuNPs suspension,