ECOTOXICITY OF $CuCl_2$ AND CuO NANOPARTICLES ON SELECTED BIOINDICATORS

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Abstract

Metal oxide nanoparticles are increasingly being produced and will inevitably and up in the aquatic environment. In this work, we focus on the metal oxide nanoparticles CuO. These nanoparticles are used in many commercial products (e.g. CuO nanoparticles in catalysts, semiconductors and chemical sensors).

We focused on acute and adverse effects of $CuCl_2$ and CuO NPs <50 nm, in particular. The aim of this study was to investigate the acute toxicity of the nanoparticles to *Daphnia magna* and *Tubifex tubifex*. Selected representatives are an important part of the fish food chain. The effect of the $CuCl_2$ salt and CuO NPs was tested on the growth *D. magna* in an acute test scenario according to OECD guidelines 202. Tests with *T. tubifex* were performed according to method ASTM E1706-04. The concentrations of $CuCl_2$ and CuO NPs tested were as follows: 0.0005, 0.01, 0.05, 0.1, 0.5, 1 $mg \cdot l^{-1}$ and 0.1, 0.25, 0.5, 0.75, 1 $mg \cdot l^{-1}$ to *D. magna* and *T. tubifex*, respectively. Graphpad Prism was used for data visualization and statistics. Dose-response curves were constructed and EC50/LC50 values were calculated. In particular, changes in the behaviour of test organisms were noted. The impairment of Daphnids movement and holding at the bottom and *Tubifex tubifex* burrowing behaviour may lead to ecologically detrimental effects, such as an increase in the susceptibility of invertebrate species to predation. This could lead to possibly biomagnifying NPs up to food chain, thereby affecting the entire ecosystem.

Keywords: Nano, metal, aquatic toxicity, *Daphnia magna, Tubifex tubifex*

1. INTRODUCTION

Engineered metal nanoparticles (NPs), such as copper oxide (CuO) NPs, have unique properties because of their small size (1-100 nm) and high surface-to-volume ratio compared to their larger counterparts [1]. CuO NPs and other Cu-containing NPs have vast applications, including antifouling paint, bioactive coatings, cosmetics, electronics, health products, inks, lubricants, plastics, solar cells, and batteries [2]. Therefore, there has been a dramatic increase in the use of Cu-containing particles over the past decades. These nanoparticles are used in many commercial products (e.g. CuO nanoparticles in catalysts, semiconductors and chemical sensors). As the production of CuO NPs increases the release of these particles via wastewater and agricultural runoff, release from weathered surfaces treated with NP-coatings and antifouling paints will likely cause increased exposure in the aquatic environment [3]. Aquatic sediments have been recognized as a major compartment for metal NP accumulation. CuO accumulation in the sediment compartment may lead to concentrations in the ng to $\mu g \cdot kg^{-1}$ range [4]. Once in the aquatic environment, NPs are highly affected by their surroundings and consequently undergo transformations (e.g., agglomeration, aggregation, dissolution, sulfidation). The fate and behaviour of NPs depends both on their physical-chemical properties and on the characteristics of the receiving environment, including pH, temperature, concentration of natural organic matter, ionic strength and salinity and water hardness [5]. As a result of these dynamics, aquatic organisms are not only exposed to NPs but also to their dissolution and/or aggregation products. In aquatic sediments, arrange of transformation processes will occur, but the complex sediment matrix makes it very difficult to predict NP transformations in this compartment [3].

The freshwater crustacean *Daphnia magna* is commonly used for standardised toxicity testing of 24-48 h acute immobility and 21-day chronic effects regarding reproduction, growth and mortality. Daphnids have high ecological relevance and a relatively short life-cycle that enables chronic studies within a practical and reasonable testing timeframe of 21 days, in addition to being easily managed in the laboratory [6]. The sediment-dwelling Oligochaete, *Tubifex tubifex* was selected as model organism, because they are widely distributed in the freshwater eco-system, and have a feeding behaviour that includes ingesting large amounts of fine particles (<60 µm) and extract organic matter associated with ingested sediment. Due to their bioturbation activities, high tolerance for polluted ecosystems, intermediate position in the trophic network and the easy of breeding in the laboratory, they are widely used as a standard model organism in ecotoxicological studies [7].

The aim of this study was to investigate the acute toxicity and adverse effects of the nanoparticles to important representatives of the aquatic environment *Daphnia magna* and *Tubifex tubifex*. Since benthic invertebrates represent a major food source for fish, it is hypothesized that CuO NPs may be further transferred along the food chain.

2. METHODS

The effect of the $CuCl_2$ salt and CuO NPs (<50 nm) was tested on the mortality and growth of *Daphnia magna* in an acute test scenario according to OECD guidelines 202 [8]. Tests with *Tubifex tubifex* were performed according to method ASTM E1706-04 [9].

The CuO NPs were added to pure water to obtain a stock suspension of 1 $g \cdot l^{-1}$, which was sonicated for 5-6 h in a sonication bath. Test solutions were prepared by diluting the initial dispersion of $CuCl_2$/CuNPs in culture medium to the desired concentration. The concentrations of $CuCl_2$ and CuO NPs tested were as follows: 0.0005, 0.01, 0.05, 0.1, 0.5, 1 $mg \cdot l^{-1}$ and 0.1, 0.25, 0.5, 0.75, 1 $mg \cdot l^{-1}$ to *D. magna* and *T. tubifex*, respectively. These concentrations were chosen on the basis of preliminary study. Both $CuCl_2$ and CuO were purchased from Sigma-Aldrich Co. and were used without any purification. The particle sizes were indicated by the manufacturer as <50 nm for CuO. For statistical analysis, the EC50/LC50 values (the median effect concentration/the median lethal concentration), as well as their associated 95% confidence intervals (95% CI), were determined by Graphpad Prism version 6.05 software (San Diego, CA, USA). Nonlinear regression analysis was performed on dose–response immobilization/mortality and a curve with variable slope was placed on each of the data sets.

Test organism (*Daphnia magna*) originated in cultures from the Ecotoxicological laboratory of the University of Veterinary Sciences Brno (Czech Republic). Acute immobilization tests were conducted with 48 h continuous exposure in accordance with OECD Guideline 202. Immobility was recorded after 24 and 48 h. The test include control and six exposure concentrations with four replicates, each comprised of ten neonates (<24 h old) in 50 ml test suspension contained in 100 ml beakers. After 24/48 hour exposure to the test solutions at 20°C with a photoperiod of 16 h light and 8 h darkness, immobilized neonates were counted. Neonates that were not able to swim within 15 s were considered to be immobilized after gentle agitation of the vessel. The validity criteria were met for all acute tests, with no control mortalities and an oxygen content >8 $mg \cdot l^{-1}$ [8].

Test organism (*Tubifex tubifex*) originated in cultures from the Ecotoxicological laboratory of the University of Veterinary Sciences Brno (Czech Republic). The effect of the $CuCl_2$ salt and CuO NPs (<50 nm) was tested on the mortality and growth of *Tubifex tubifex.* Acute tests were conducted for 96 h into an incubator at 22±1 °C, in the dark, and without water renewal or feeding. The number of dead worms in each vessel was counted at least once every 24 h. A worm was considered to be dead when there was no response in the 10 s after receiving a slight disturbance with a bar [10]. After 5 days of exposure to CuO/CuCl2, surviving worms were transferred to beakers containing 2 cm uncontaminated natural sediment and 40 ml *T. tubifex* media. Burrowing behaviour has been subjectively recorded at 24 hour.

3. RESULTS AND DISCUSSION

Ecotoxicity of $CuCl_2$ and CuO was determined in two aquatic tests. The influence of selected matters on the observed effect in *Daphnia magna* and *Tubifex tubifex* is shown in **Figure 1**. In this study, Daphnids was more sensitive to copper than *T. tubifex*. This is confirmed in other work, where algae, molluscs, crustaceans, insects, other invertebrates and fish were included and crustaceans were the most sensitive to copper [11].

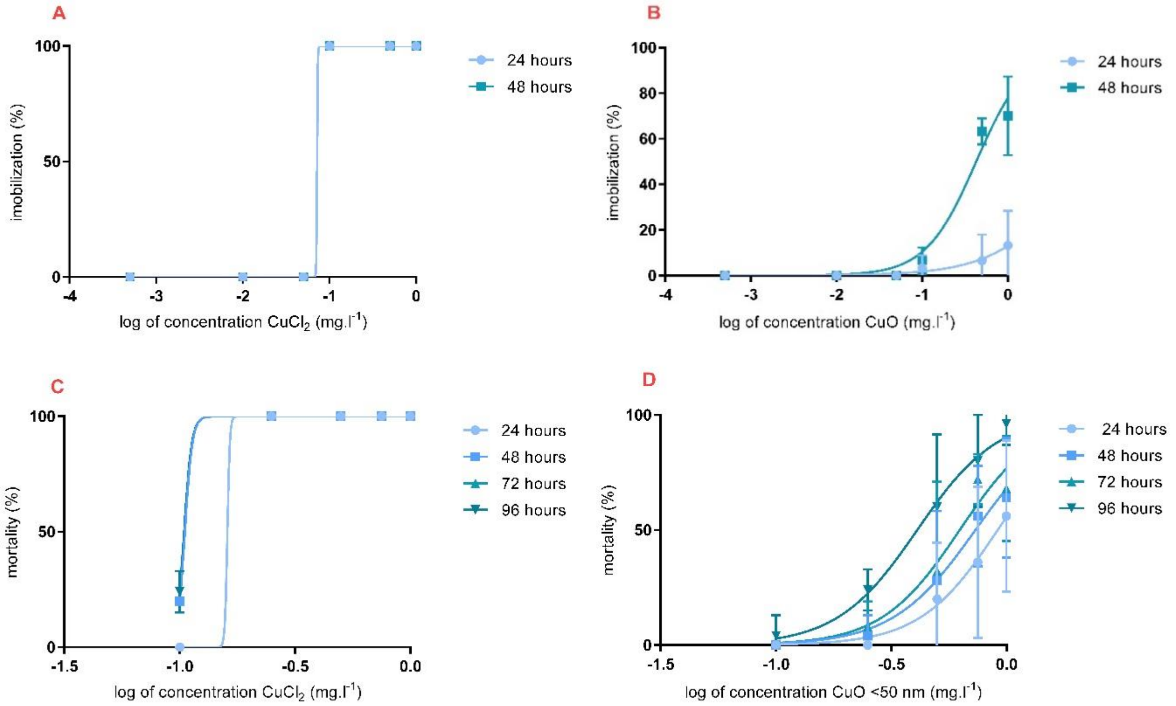

Figure 1 Dose-response curves of tested substances for *D. magna* and *T. Tubifex.* Data points are the average of four replicates. **A** the EC50 for $CuCl_2$ in *D. magna* was determined at 48 h 0.048 (CI 0.024 to 0.121) mg·l^{-1} **B** the EC50 for CuO in *D. magna* were determined at 24 and 48 h 7.89 (CI 0.21 to 297) and 0.43 (0.34 to 0.54) mg·l^{-1}, respectively **C** the LC50 for $CuCl_2$ in *T. tubifex* was determined at 96 h 0.125 (CI 0.84 to 1.60) mg·l^{-1} **D** the LC50 for CuO in *T. tubifex* were determined at 24, 48, 72 and 96 h 0.92 (CI 0.72 to 1.17), 0.73 (0.61 to 0.89), 0.63 (0.51 to 0.77) and 0.41 (0.34 to 0.50) mg·l^{-1}, respectively

We noticed nanoparticles in the intestine of *D. magna*. It is obvious that nanoparticles or nanoparticle aggregates are ingested by the Daphnids (**Figure 2 B**). This ingestion of nanoparticles aggregates is possible through the filter feeding mechanism of *D. magna*. Nanoparticle aggregates that are taken in may occur as dispersed nanoparticles or aggregates in the gut or dissolve in the gut or in the cells (e.g. after uptake by endocytosis) due to lower pH values [11]. Subsequently, the nanoparticles (or their derivates) can be either incorporated or eliminated from the body. In this study, we observed as a side effect changes in movement and holding at the bottom of the vessel was subjectively observed from the concentration of 0.1 mg·l^{-1}.

The length of the Daphnids (mm) was monitored for the (unexposed) blank Daphnids and the ones exposed to the CuO and $CuCl_2$ during the 21-day experiment. No significant differences were observed in length between the blank and exposed Daphnids (one-way ANOVA indicated no significant differences at most time points). Here as well, no effect of the exposure (nanoparticle or metal salt) could be seen on the Daphnia length.

In this study, after 48 h of exposure to immobilization EC50 values of the copper salt $CuCl_2$ 0.048 (CI 0.024 to 0.121) mg·l^{-1} and CuO nanoparticles 0.43 (0.34 to 0.54) mg.l^{-1} were measured (**Figures 1 A, B**). The results indicate that the copper from the nanoparticles is taken less efficiently than the copper from the copper salt. Other authors [11,12,13,14] mention, that in the nanoparticle exposure, the Daphnids were mostly exposed to CuO aggregates and only to very low concentrations of dissolved copper, whereas in the metal salt exposure, the Daphnids were solely exposed to dissolved copper. Whilst some studies have reported that the toxicity of CuO on aquatic organisms were due to dissolved Cu^{2+} ions, others have reported that the observed toxicity is due to the metal oxide NPs [14].

In this study with *T. tubifex*, after 96 h of exposure to mortality LC50 values of the CuO nanoparticles 0.41 (0.34 to 0.50) $mg{\cdot}l^{-1}$ and copper salt $CuCl_2$ 0.125 (Cl 0.84 to 1.60) $mg{\cdot}l^{-1}$ were measured (**Figures 1 C, D**). The mortality of *T. tubifex* was 0 % in the control. The burrowing behavior of surviving *T. tubifex* in clean sediment was significantly affected (decreased) following exposure in the present study. Zhang et al. (2017) mention that burrowing behavior of benthic invertebrates may be a more sensitive endpoint in behavior tests, and likely of particularly importance when considering NP effects [7]. Since *T. Tubifex* plays an important role in biogeochemical processes through its burrowing and irrigation activity, the impairment of burrowing behavior may lead to ecologically detrimental effects, such as an increase in the susceptibility of sediment-dwelling species to predation [15-17].

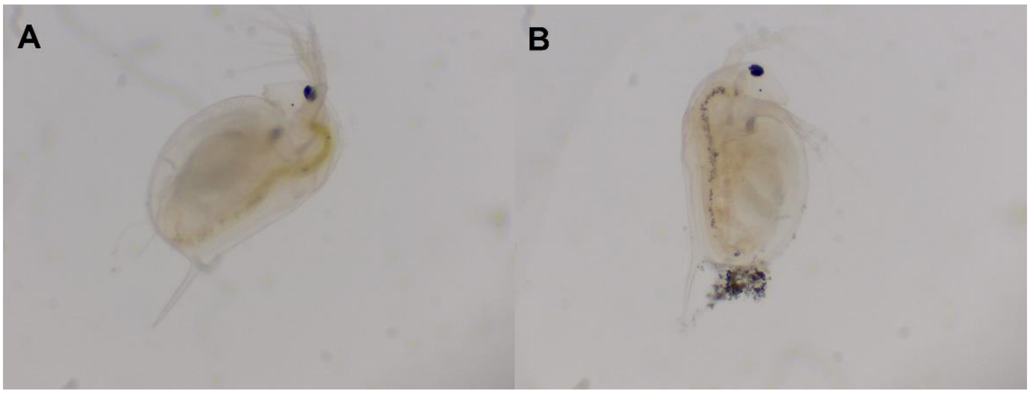

Figure 2 A *Daphnia magna* (control) **B** *Daphnia magna* (1 $mg{\cdot}l^{-1}$ CuO)

4. CONCLUSION

Metal nanoparticle bioaccumulation in benthic invertebrates in exposed aquatic ecosystem has been studied in a number of benthic invertebrates and these studies suggested that benthic invertebrates could accumulate nanoparticles like CuO NPs. The impairment of movement and holding at the bottom (*Daphnia magna*) and burrowing behavior (*Tubifex tubifex*) may lead to ecologically detrimental effects, such as an increase in the susceptibility of invertebrates species to predation. This could lead to an increased predation of contaminated Daphnids and worms by fish, possibly biomagnifying NPs up to food chain, thereby affecting the entire ecosystem.

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