Chemosphere 135 (2015) 166–174

Contents lists available at ScienceDirect

Chemosphere

journal homepage: www.elsevier.com/locate/chemosphere

Subchronic and chronic developmental effects of copper oxide (CuO) nanoparticles on *Xenopus laevis*

Shawna Nations [a], Monique Long [a], Mike Wages [a], Jonathan D. Maul [a], Christopher W. Theodorakis [b], George P. Cobb [c,*]

[a] Department of Environmental Toxicology, Texas Tech University, Lubbock, TX 79409, USA
[b] Department of Biological Sciences, Southern Illinois University Edwardsville, Edwardsville, IL 62026, USA
[c] Department of Environmental Science, Baylor University, Waco, TX 76712, USA

HIGHLIGHTS

- Moving from acute (4 d) to subacute (14 d) to chronic (50 d) nano-CuO exposure times increase Xenopus mortality.
- NanoCuO subacute LC50s are below LOECs for Xenopus.
- At low concentrations nano-CuO has marginally beneficial effects on Xenopus growth and development.
- At 0.3 mg L^{-1}, less than 40% of tadpoles had completed metamorphosis.

ARTICLE INFO

Article history:
Received 3 December 2014
Received in revised form 13 March 2015
Accepted 15 March 2015

Handling Editor: Frederic Leusch

Keywords:
Nanoparticles
FETAX
CuO
Development amphibians

ABSTRACT

Metal oxide nanoparticles, such as copper oxide (CuO), are mass produced for use in a variety of products like coatings and ceramics. Acute exposure to CuO nanoparticles has caused toxicity to many aquatic organisms, yet there is no information on the effect of prolonged CuO nanomaterial exposures. This study examined effects of chronic exposure to CuO nanoparticles on *Xenopus laevis* growth and development. Experiments included a 14 d subchronic exposure and a 47 d chronic exposure throughout metamorphosis. The subchronic exposure caused mortality in all tested CuO concentrations, and significant growth effects occurred after exposure to 2.5 mg L^{-1} CuO. Chronic exposure to 0.3 mg L^{-1} CuO elicited significant mortality and affected the rate of metamorphosis. Exposure to lower concentrations of CuO stimulated metamorphosis and growth, indicating that low dose exposure can have hormetic effects.

1. Introduction

Metal oxide nanoparticles (NP) are among the most widely produced nanoparticles (Kumar, 2006), and are used in many applications. Metal oxide nanoparticles can be used to remediate a wide variety of hazardous materials due to high surface area, enhanced interfacial reactivity, increased dispersibility, and facile sorption kinetics (Kumar, 2006). Some metal oxide nanoparticles, such as zinc oxide (ZnO) and CuO, have antimicrobial and antifungal properties, which make them ideal for a variety of coating applications. NanoArc® CuO antimicrobial properties are active for extended periods of time, even in harsh conditions (Society of Manufacturing Engineers, 2008; Copper Development Association, 2009). NanoArc® CuO is utilized in wood preservation, textile fibers, marine antifouling, coatings, and thermoplastics to inhibit microbial and fungal growth (Society of Manufacturing Engineers, 2008). These CuO nanoparticles are also used in optical glass polishing, additives for ceramics processing and colorants, and pigments for other materials (Nanophase Technologies Corporation, 2009).

Because commercial production capabilities of nanoparticles such as CuO are on the order of metric tons (Society of Manufacturing Engineers, 2008), it is important to investigate the toxicity of these materials, especially to aquatic organisms, because products and wastes containing CuO nanoparticles can enter aquatic ecosystems. For example, copper oxide nanoparticles have exhibited toxicity to aquatic organisms such as the algae *Pseudokirchneriella subcapitata* growth (Aruoja et al., 2009), the bacterium *Vibrio fischeri*, and the crustaceans *Daphnia magna* and *Thamnocephalus platyurus* (Heinlaan et al., 2008; Mortimer et al., 2008). Copper oxide nanoparticles were relatively more toxic than bulk CuO, and CuO NP toxicity was attributed to soluble Cu ions (Heinlaan et al., 2008; Mortimer et al., 2008).

* Corresponding author. Tel.: +1 254 710 6556; fax: +1 254 710 3409.
E-mail address: george_cobb@baylor.edu (G.P. Cobb).

http://dx.doi.org/10.1016/j.chemosphere.2015.03.078
0045-6535/

There are several reasons to evaluate CuO nanoparticle effects in *X. laevis*. First, copper salts decrease embryo hatching, survival, and body length, and increase occurrence of malformations in this species (Luo et al., 1993; Haywood et al., 2004; USEPA, 2007). Because soluble copper ions may contribute to CuO NP toxicity (Heinlaan et al., 2008; Aruoja et al., 2009), it was hypothesized that similar effects would be seen in *X. laevis* chronically exposed to CuO NP. Acute exposure of *X. laevis* tadpoles to CuO nanoparticles has also been investigated in our lab in previous studies utilizing the Frog Embryo Teratogenesis Assay-Xenopus (FETAX; 18). In that study, CuO NP did not decrease embryo hatching or survival, but did significantly decrease snout vent length at 10 mg L^{-1} and total body length at 1000 mg L^{-1}. Increased malformation incidence occurred at 1000 mg L^{-1} with 33.3 ± 8.8% malformation (Kumar, 2006). We further hypothesize that similar effects will occur at lower concentrations during chronic exposures.

Therefore, the purpose of this study was to determine the effects of CuO nanoparticle exposure to *Xenopus laevis* tadpoles throughout metamorphosis. To assess effects of exposure on metamorphosis, endpoints of this study included mortality, growth (body measurements), and time to complete metamorphosis. The combination of results from this study, our acute (Nations et al., 2011) and other chronic studies (Nations et al., 2010), as well as other toxicological studies evaluating manufactured nanoparticles (Lovern and Klaper, 2006; Franklin et al., 2007; Heinlaan et al., 2008; Mortimer et al., 2008; Zhu et al., 2008; Aruoja et al., 2009) is important for determination of NP risks on environmental and human health.

2. Methods and materials

Copper oxide nanoparticles were purchased from Alfa Aesar (Ward Hill, MA). Average particle size (APS) and surface area of the NanoArc® CuO nanoparticles purchased for this project were 23–37 nm, 25–40 m^2 g^{-1}, respectively. All salts for making FETAX medium were obtained from VWR (West Chester, PA): NaCl (100% purity), $NaHCO_3$ (99–100% purity), KCl (100% purity), $CaCl_2$ (99–100% purity), $CaSO_4 2H_2O$ (98–100% purity), and $MgSO_4$ (99–100% purity). For chemical determinations, trace metal grade nitric acid (70%) and hydrogen peroxide (30%) were obtained from Fisher Scientific (Fisher, Waltham, MA). Human chorionic gonadotropin (HCG) and L-cysteine (⩾98%), from non-animal source, cell culture) were obtained from Sigma–Aldrich (St. Louis, MO).

2.1. Nanoparticle solutions

Nanoparticle solutions were prepared in FETAX medium – a medium used to culture *X. laevis* larvae (Bantle et al., 1989) – hereafter referred to as FETAX (625 mg NaCl, 96 mg $NaHCO_3$, 30 mg KCl, 15 mg $CaCl_2$, 60 mg $CaSO_4 2H_2O$, and 75 mg $MgSO_4$ per liter of deionized water). Test solutions were sonicated with Fisher Scientific Model 500 Sonic Dismembrator (Fisher, Waltham, MA), until stably suspended in the medium, as described in Nations et al. (2010). Before tests began, SEM showed particles ranging from 18 to 285 nm with an average of 57 nm.

For subchronic exposures, each test solution was prepared in a 208 L drum at the appropriate concentration. For the chronic CuO test solutions, a 500 mg L^{-1} stock solution was prepared, and individual test solutions were diluted to a specified concentration with FETAX medium. Exposure solutions were prepared fresh every week during the studies. Differential light scattering (Nano-ZS, Malvern) was used to determine the CuO hydrodynamic particle size in the test solutions. Test solutions for subchronic exposure included the following concentrations: control (FETAX only), 0.156, 0.313, 0.625, 1.25, and 2.5 mg L^{-1}. Concentrations utilized in the chronic exposures were control (FETAX only), 0.01875, 0.0375, 0.075, 0.150, and 0.3 mg L^{-1}. The highest subchronic exposure concentration (2.5 mg L^{-1}) was derived from the malformation EC_{10} (2.1 mg L^{-1}) in the acute study (Nations et al., 2011). Results from the subchronic study were used to determine the highest test solution concentration for the chronic study. The highest subchronic exposure without significantly greater mortality than controls (i.e. NOEC) was 0.313 mg L^{-1}, thus the highest test solution concentration of 0.3 mg L^{-1} was selected for the chronic exposure.

2.2. Breeding and embryo collection

Breeding procedures followed a modified ASTM E1439-98 method and previously established methods utilized within our lab (Nations et al., 2010, 2011). Eggs were obtained from four *X. laevis* mating pairs per test, to increase the possibility that at least one pair of frogs produced an adequate supply of viable embryos. Males were injected with 250 IU of human chorionic gonadotropin (HCG) in the dorsal lymph sac, and females were injected with 750 IU HCG in the dorsal lymph sac to induce reproduction. Embryo collection began approximately 24 h after HCG injection and was conducted as described in previous studies (Nations et al., 2010, 2011).

2.3. Exposure procedures

Each concentration was tested in triplicate with 15 tadpoles in each replicate. Small 9.5 L glass aquaria were used as exposure chambers. To begin the exposure, 6 L of test solution was placed in every tank. After the solution was added to each tank, at least 20 tadpoles were added with plastic transfer pipettes. The number of tadpoles within each tank was reduced to 15 on day 5 of the exposure.

2.4. Test chamber and preparation

Both subchronic and chronic exposures utilized a preparation similar to a previously conducted chronic ZnO nanoparticle exposure (Nations et al., 2010). Both the aquaria and the 208 L drums (from which the test solutions were dispensed) were aerated using commercially-available aquarium air pumps and air stones. A Living Stream reservoir (Fridgid Units, Toledo, OH, USA) was used as a water bath. The water in the reservoir was heated to 23 ± 2 °C using three commercially-available glass aquarium heaters. Exposure tanks were randomly arranged within the reservoir.

A flow-through design for chronic ZnO nanoparticle exposure was utilized for both subchronic and chronic CuO exposures. One drum was designated for each exposure concentration. Each drum was aerated to maintain water quality and mixing of solution. A peristaltic pump provided a flow rate of 12.5 mL min^{-1} to each tank; which pumped at approximately 18 L d^{-1} through each tank (Nations et al., 2010). Each tank had a standpipe that allowed FETAX medium to drain when the volume exceeded 6 L, so this was equivalent to 3 water exchanges per day.

Tadpoles in this exposure were fed the same diet, which contained a combination of Nutrafin®, trout chow, and Frog Brittle (Nasco, Inc., Fort Atkinson, WI, USA), according to Nations et al. (2010), Koss and Wakeford (2000). Tadpoles were fed *ad libitum* each day, beginning on day 5 post hatch.

2.5. Observations

The following water quality parameters were monitored every other day: ammonia, temperature, conductivity, salinity, dissolved