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Short Communication: Magnitude of Acute Toxicity of Marine Sediments Amended with Conventional Copper and Nanocopper

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Abstract

Copper is a well-known toxic metal to marine organisms. In this investigation, we measured and compared the acute toxicity of several forms of copper (including nanocopper) amended into a marine sediment with mysids and amphipods. For all the forms of copper tested, toxicity, measured as the LC50, ranged from 708 to > 2400 mg Cu/kg (dry sediment) for mysids and 258 to 1070 mg Cu/kg (dry sediment) for amphipods.

Keywords

Copper nanomaterials; toxicity; ionic copper; sediments; bioavailability

INTRODUCTION

Copper is one of the transition cationic metals known to be toxic to aquatic organisms including marine species (Flemming and Trevors 1989, Brix et al. 2005, U.S. EPA 2016a). Several forms of copper can enter the marine environment via commercial products. Not coincidentally, many of these copper-containing products are used as pesticides to reduce damage to agricultural resources and structures adversely affected by fungi and bacteria. These products include direct application formulations like CuPro 2005 T/N/O[®] and Kocide 3000[®] used as fungicides/bactericides in agricultural applications (Tree Geek 2012, Certis USA 2018), which may enter the marine environment via surface run-off. In addition, there are forms of copper used in the pressure-treating of lumber for terrestrial and aquatic

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DATA ACCESSIBILITY

Robert Burgess (burgess.robert@epa.gov) and Ashley Parks (ashleyp@sccwrp.org) should be contacted to access the data.

wooden constructions. Specific forms of copper used in lumber include alkaline copper quaternary (ACQ) and micronized copper azole (MCA) (Parks et al. 2018a). Over the time of their application in treated lumber, copper is released to the environment either through run-off in terrestrial building applications (e.g., deck) or directly in submerged applications (e.g., a dock). The MCA form of copper contains copper carbonate (CuCO_3) in the nano- to micro-material size range (i.e., 1 to 1000 nm) (Platten et al. 2015). Recent investigations found that MCA-treated lumber released ionic copper (Cu^{2+}) into estuarine and marine waters (Parks et al. 2018a, b).

Once released into marine systems from runoff or directly from pressure treated lumber, the copper will distribute between dissolved, colloidal, and particulate phases (Forstner and Wittman 1981). Because of the affinity of copper to organic matter, much of the metal will likely accumulate in sediments as the natural particles containing organic matter sequester ionic copper, aggregate into larger organic matter enriched particles, and precipitate into the sedimentary environment (Forstner and Wittman 1981, Flemming and Trevors 1989, Kester 1986). Once in the sediments, the copper will partition into the interstitial waters (as dissolved or colloidal copper) and particulate phase. The understanding of copper speciation and bioavailability indicates the ionic form (Cu^{2+}) is what causes adverse effects to aquatic organisms (Sunda and Guillard 1976, Campbell 1995). Consequently, the amount of copper in the ionic form will dictate the magnitude of sediment toxicity.

In this investigation, the magnitude of acute toxicity associated with several forms of parent copper materials, including nanocopper, amended into a reference sediment was compared to assess the relative toxicity to two representative marine organisms (i.e., the mysid *Americamysis bahia* and the amphipod *Ampelisca abdita*). Sediment toxicity data for marine organisms for these types of copper products is very limited. This information will be useful for ranking the relative toxicity of several forms of copper found in marine systems due to normal applications, inappropriate usage, and improper disposal of copper-containing materials.

MATERIALS AND METHODS

Materials

The parent forms of copper and nanocopper materials for comparative toxicity testing with the epibenthic mysid *A. bahia* and infaunal amphipod *A. abdita* included copper sulfate (CuSO_4) (forms ionic copper (Cu^{2+})), reagent forms of nanoparticulate copper carbonate (CuCO_3), ACQ, MCA, and the commercially-available copper-based pesticides CuPro 2005 T/N/O (U.S. EPA registration # 6769037) and Kocide 3000 (U.S. EPA registration # 352–662), which were provided by several sources (Table 1). Except for the MCA, the chemical form of copper present in the products was conventional copper salts intended to dissolve when exposed to aqueous solutions. In contrast, the MCA consisted of a copper salt (i.e., copper carbonate) grounded to nano-sized particles. Characterizations of the MCA treatment solution have reported 68% of the particles being considered nanoparticles with a range of 10 to 700 nm (McIntyre 2010, Platten et al. 2015).

Sediment amendment with parent copper nanomaterials

For comparative toxicity testing of the parent forms of copper and nanocopper, the materials were amended into Long Island Sound, USA (LIS) reference sediment to provide a stable dosing medium during the exposures. Sediment was collected using a Smith MacIntyre grab sampler (0.1 m²) in September 2010 at coordinates 41° 7.95'N 72° 52.7'W. Geochemical characteristics of the LIS sediment include approximately 2% organic carbon, a composition of 90% silt-clay and less than 10% sand, and copper of approximately 50 mg/Kg (dry) (Lake et al. 1988). Reference sediment amendment with parent copper materials was performed as discussed in Burgess et al. (2000). Briefly, crystalline CuSO₄ was dissolved in a small volume of reconstituted seawater (RS), followed by addition of wet LIS sediment. The RS was prepared by combining 100‰ natural brine and deionized water to achieve a 30‰ solution. The other forms of copper were added to wet LIS as powders (i.e., CuPro, Kocide) or aqueous solutions (i.e., CuCO₃, ACQ, MCA) in pre-determined quantities calculated to arrive at the desired exposure concentrations. The various copper substances and sediment were then mixed manually with a plastic spatula for approximately three minutes. Long Island Sound sediment is relatively aerobic negating the need to use a nitrogen atmosphere when adding amendments. After mixing, the sediments were stored in the dark at 4°C for at least 12 days until used. Definitive amendment concentrations for the final toxicity testing dilution series were determined based on preliminary range finder toxicity studies. After amendments were completed, nominal copper concentrations ranged from 200 to 2000 mg Cu/kg (dry) sediment with measured concentrations, analyzed by inductively coupled plasma- atomic emission spectroscopy (ICP-AES), ranging from 160 to 2400 mg/kg (dry) sediment.

Sediment toxicity testing

Sediment toxicity testing of the parent copper materials was performed based on methods described in Ho et al. (2000). Briefly, toxicity tests with amended sediments were performed in 100 mL glass jars containing 20 grams of sediment (wet) and 60 mL of 30‰ RS. Ten 48 hr old cultured *A. bahia* and ten field-collected (0.5 – 0.7 mm) were added to each exposure chamber approximately 24 h after the RS and sediments were added. Mysids were cultured in our laboratory while the amphipods were collected from the nearby Narrow River estuary (Narragansett, Rhode Island, USA). Amphipods were held in the laboratory for less than five days before addition to the test. Tests were performed in triplicate without renewal (static) with gentle overlying water aeration. Mysids were fed newly hatched *Artemia salina* daily (0.14 g (wet)/mL) while the amphipods were not fed. Physicochemical measurements such as pH, salinity, temperature, and dissolved oxygen were monitored to ensure water quality was maintained. After seven days, tests were terminated, and organisms were sieved from the exposure jars, and assessed for mortality (missing organisms were considered mortalities).

Sediment extraction

Copper was extracted from the reference sediments amended with parent copper materials using methods described by Katz et al. (2017). Specifically, sediment samples were weighed in duplicate and placed in 15-mL trace metal clean polyethylene vials. Sediments were

digested using concentrated trace metal grade acids (J.T.Baker, Center Valley, PA, USA), nitric:hydrochloric:hydrofluoric acids at 3:2:1 mL/vial. Samples were allowed to stand for 20 min in the fume hood to digest organic matter and then sonicated in a water bath three times for one-hour intervals (25 °C) with samples vortexed after each interval. Samples were then transferred to a hot block (DigiPREP Jr; SCP Science, Champlain, NY, USA) and allowed to digest for one hour (100 °C) before cooling overnight in a contained negative pressure environment. American Society for Testing and Materials (ASTM) type I water (Milli-Q Element; Millipore, Billerica, MA) was added to maintain volumes at approximately 6 mL and for all subsequent dilutions. After cooling overnight, water was added as needed to bring volumes to 10 mL. Digests were vortexed again and sonicated for one hour at 25 °C. Digests were then centrifuged at 1500 g for ten minutes, decanted into trace metal clean polyethylene volumetric flasks, and diluted to 100 mL with water. Each digest was then filtered through a 0.4 µm Nuclepore filter (GE Healthcare Life Sciences, Marlborough, MA) in a trace metal clean polycarbonate filtration apparatus. Samples were then stored in trace metal grade 125 mL polyethylene sample bottles until analysis.

Inductively coupled plasma-atomic emission spectroscopy (ICP-AES)

To determine the total copper concentrations in sediment samples, each acidified subsample was analyzed for copper (Cu) at the 324.754 nm emission line against an external calibration using a Horiba Jobin Yvon Ultima 2 ICP-AES equipped with an AS500 autosampler (Kyoto, Japan). A single element Cu ICP standard (Ultra Scientific (North Kingstown, RI, USA), certified value of 1001 ± 2 mg/L) was used to make calibration standards at 0.12, 1.26, 5, 20, and 50 mg/L in 30 ‰ filtered seawater (FSW) and 2.5% nitric acid. Quality control was maintained by analyzing one of the calibration standards every 10 to 15 samples. These calibration checks did not deviate more than 20% from the reported value with a majority deviating less than 10%. The copper concentration in each sample was based on an average of three individual measurements by the instrument. The instrumental detection limit (dl) for copper was 2 µg/L with the limits of quantification four times the limit of detection.

Statistical analysis

Median lethal concentrations (LC50s) were calculated for both organisms (*A. bahia* and *A. abdita*) in the toxicity tests using the Toxicity Relationship Analysis Program (TRAP) version 1.30a (U.S. Environmental Protection Agency 2016b) based on the measured concentrations of copper in the sediments. Dose response curves were fitted using Sigma Plot 12.5 (Systat Software, San Jose, CA, USA) based on a four-parameter logistic curve fit.

RESULTS AND DISCUSSION

Sediment toxicity

Physicochemical measurements of water column temperature, salinity and pH performed during the study were within established ranges. Control survival for the mysids and amphipods in the LIS reference sediment was 90% for both species. Toxicity testing of the sediments amended with conventional copper and nanocopper resulted in mysid LC50s ranging in value from 708 to > 2400 mg Cu/kg (dry sediment) for CuSO₄ and MCA, respectively, and 258 to 1070 mg Cu/kg (dry sediment) for CuCO₃ and CuPro for the

amphipod, respectively (Table 1) (dose-response curves are presented in Supplemental Data Figure S1). The amphipods demonstrated greater sensitivity to the exposures with lower LC50s for every material tested, suggesting that the causes of toxicity remained associated with the sediment phase rather than readily dissolving into the water column. The commercial products (e.g., CuPro and Kocide) demonstrated the least toxicity, with the reagent forms of copper (CuCO_3 and CuSO_4) showing the greatest toxicity. Specifically, except for the MCA, the CuCO_3 , ACQ, and CuSO_4 reflected relatively similar toxicity values by species: for these three materials, LC50s ranged from 708 to 819 mg Cu/kg (dry sediment) and 258 to 326 mg Cu/kg (dry sediment) for the mysid and amphipods, respectively. Curiously, the MCA values (i.e., >2400 mg Cu/kg (dry sediment) for mysids and 526 mg Cu/kg (dry sediment) for the amphipods) were relatively larger, especially for the mysids, indicating this form of nanocopper was less toxic.

When Ho et al. (2000) discussed the experimental design for the testing system used in this investigation which simultaneously exposes two marine organisms one benthic and the other epibenthic, they noted differential exposures could occur as the organisms experience different environmental phases during the test. Specifically, the mysids are exposed primarily via the water column while the amphipod's exposure is through a combination of interstitial and water column waters. As a result, if a chemical is particle reactive, like copper (Campbell 1995), the exposure in the sediments may be greater (i.e., there are greater copper concentrations in the sediment interstitial waters) than in the water column.

As discussed in the Introduction, the form of copper also dictates the magnitude of toxicity to aquatic organisms with ionic copper being the most toxic form (Campbell 1995). For ionic copper to become available from the products compared in this study dissolution of the conventional copper salts and release from the nano-copper particles had to first occur. Reagent copper sulfate is amongst the most readily dissolving of the conventional salts used in this study under marine salinity and pH conditions (Kester 1986) and generated some of the greatest toxicity in the sediment test (e.g., LC50s were 708 and 326 mg/kg dry sediment for the mysid and amphipod, respectively). Conversely, copper hydroxide, the form of copper used in the two commercial pesticides (CuPro 2005 T/N/O and Kocide 3000), is relatively resistant to dissolution under marine conditions (Kester 1986) and produced some of the lowest sediment toxicity (e.g., LC50s were 1240 – 1290 mg/kg dry sediment and 677 – 1070 mg/Kg dry sediment for the mysid and amphipod, respectively). Similarly, the nanoparticle form of the copper carbonate used in the MCA, which resulted in very low relative toxicity to the mysids, may have slowed the dissolution process for ionic copper compared to the conventional salts. Consequently, very speculatively as we did not measure ionic copper directly but relied on measurements of total copper in the sediment to reflect exposure, the ability of the ionic copper to form may have explained the differences in toxicity observed between the products compared

Consideration of other constituents

As shown in Table 1, some of the materials tested (e.g., MCA, ACQ, and Kocide) contained other potentially active constituents including 2-propenoic acid, monoethanolamine, quaternary ammonium, and tebuconazole. Review of the toxicity of these chemicals to

aquatic organisms (Table 2) indicates toxicity in the high $\mu\text{g/L}$ to mid-mg/L concentration range. Given that tebuconazole (in MCA), monoethanolamine (ACQ), and 2-propenoic acid (in Kocide) are present as relatively small fractions of the parent materials (e.g., 0.62% to 10%) and cause toxicity at relatively high concentrations (e.g., 470 to 97,000 $\mu\text{g/L}$), we suspect they contributed very little to the observed toxicity. In contrast, copper was the major constituent of all the materials tested and is, as discussed earlier, very toxic to many aquatic organisms including *A. bahia* and *A. abdita* with water-only LC50s of approximately 150 and 90 $\mu\text{g/L}$, respectively (U.S. Environmental Protection Agency 2016a). Note that because water concentrations of copper were not measured in this investigation, these water-only toxicity values cannot be directly compared to concentrations in the exposure chambers (which are based on measured sediment concentrations). Of the other potentially active constituents, quaternary ammonium is of more concern as a possible toxicant as it is known to cause toxicity to aquatic organisms in the low to mid- $\mu\text{g/L}$ concentration range (Table 2). However, despite being present in ACQ, it represented < 1% of the constituents. Consequently, we suspect its contribution to adverse effects in the sediment toxicity test with ACQ was overwhelmed by the toxicity of copper present in or released from the materials.

CONCLUSIONS

Toxicity of the sediments amended with the copper and nanocopper products ranged from 708 to > 2400 mg Cu/kg (dry sediment) for CuSO_4 and MCA for the mysid, respectively, and 258 to 1070 mg Cu/kg (dry sediment) for CuCO_3 and CuPro for the amphipod, respectively. The simple copper salts (e.g., CuCO_3 and CuSO_4) were found to have caused the most toxicity. The presence of other potentially toxic constituents (e.g., tebuconazole, quaternary ammonium) did not appear to contribute substantially to observed toxicity with copper overwhelming any of the adverse effects of the other toxicants. This type of toxicity data is limited in the scientific literature for marine organisms and should be useful effects information for the conduct of ecological risk assessments on conventional and nanomaterial forms of copper including commercial products.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Table 1.

Toxicity of copper products evaluated with the marine mysid (*Americamysis bahia*) and amphipod (*Ampelisca abdita*) in sediment toxicity tests with amended reference sediment.

Type of Product	Source	Usage	Copper (%)	Form of Copper	Other Constituents (%)	LC50 based on measured copper (mg/kg (dry) sediment) (95% confidence intervals)	
						Species	
						<i>A. bahia</i>	<i>A. abdita</i>
Micronized Copper Azole (MCA)	P Evans, University of British Columbia, Vancouver, Canada	Lumber treatment chemical	34.5	CuCO ₃ (suspension)	Tebuconazole 0.62% Other inert ingredients >90%	> 2400	526 (347 – 704)
CuCO ₃	NanoComposix, San Diego, CA, USA	Reagent nanocopper	8.8 (mg/ml)	CuCO ₃ (liquid)	NA	792 (NC)	258 (NC)
Alkaline Copper Quaternary (ACQ)	P Evans, University of British Columbia, Vancouver, Canada	Lumber treatment chemical	41.1	CuO (liquid)	Monoethanolamine < 6% Quaternary ammonium < 1% Other inert ingredients >90%	819 (721 – 917)	301 (222 – 379)
CuSO ₄	Sigma-Aldrich, St Louis, MO, USA	Reagent copper (non-nano)	40	CuSO ₄ (powder/salt)	NA	708 (NC)	326 (254 – 398)
CuPro 2005 T/N/O (U.S. EPA registration # 6769037)	SePRO Corporation, Carmel, IN, USA	Fungicide/Bactericide	42	CuOH ₂ (powder)	Quartz <2% Other inert ingredients 46.2%	1240 (1150 – 1320)	1070 (979 – 1170)
Kocide 3000 (U.S. EPA registration # 352–662)	DuPont, Wilmington, DE, USA	Fungicide/Bactericide	36	CuOH ₂ (powder)	Clay 1–5% 2-Propenoic acid 5–10% Other inert ingredients 38.9–47.9%	1290 (1230 – 1360)	677 (551 – 802)

NA Not applicable.

NC Not calculated.

Table 2.

Toxicity of other active constituents present in copper products based on literature values.

Constituent	Product	Organism	Endpoint	Value (mg/L)	Reference
Monoethanolamine	Alkaline Copper Quaternary (ACQ)	Alga (<i>Phaeodactylum tricornutum</i>)	EC50	24.7	Libralato et al. (2010)
		Mollusc (<i>Crassostrea gigas</i>)	EC50	27.6	
		Mollusc (<i>Mytilus galloprovincialis</i>)	EC50	18.2	
		Crustacean (<i>Artemia franciscana</i>)	EC50	43.0	
2-Propenoic acid	Kocide 3000	Crustacean (<i>Americamysis bahia</i>)	LC50	97	Staples et al. (2000)
Quaternary ammonium	Alkaline Copper Quaternary (ACQ)	Crustacean (<i>Daphnia magna</i>)	EC50	0.023 – 0.37 ^a	Zhang et al. (2015)
Tebuconazole	Micronized Copper Azole (MCA)	Crustacean (<i>Americamysis bahia</i>)	LC50	0.47	U.S. EPA (1988)
		Mollusc (<i>Crassostrea virginica</i>)	LC50	2.7	
		Fish (<i>Cyprinodon variegatus</i>)	LC50	5.9	

^a Range of observed toxicity values for several forms of quaternary ammonium.