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Comparison of the Toxicity of Two Chelated Copper Algaecides and Copper Sulfate to Non-Target Fish

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Abstract New pesticide products are reviewed by the United States Environmental Protection Agency for possible effects to non-target aquatic organisms. The required toxicity data are for the active ingredient only, and fail to include toxicity of the mixture of other ingredients found in these pesticides. These ingredients may increase the toxicity of the active ingredient to non-target organisms. Our study compares the toxicity of two formulations of chelated copper algaecides with each other, and to a copper sulfate algaecide. We were particularly interested in the effects of a surfactant that is present in one of the formulations. We found that copper becomes less toxic to fish (e.g. fathead minnow 48-h LC50 = 0.90 mg/L) when it is chelated, providing an additional margin of safety to non-target fish compared to copper sulfate. However, inclusion of a surfactant to the formulation resulted in increased toxicity (e.g. fathead minnow 48-h LC50 = 0.30 mg/L).

Keywords Brook trout · Fathead minnow · Surfactant · Toxicity · LC50 · EC50

All new pesticide products are reviewed by the USEPA for possible effects to non-target aquatic organisms (40 CFR Part 158 2002). Data submitted include toxicity studies for at least two species of freshwater fish, as well as at least one aquatic invertebrate. The required toxicity data are for the active ingredient only, and rarely include toxicity data on mixtures of the active ingredient with other chemicals, which may increase the toxicity of the active ingredient to target pests (i.e., synergists). Synergists have been shown to increase the toxicity of a pesticide to non-target aquatic life (e.g. piperonyl butoxide used to synergize pyrethroid insecticides for mosquito control (Paul et al. 2005)). In addition, "end use" products also contain various "inert ingredients" designed to keep the active ingredients stable, dissolved or in solution, to assist in penetrating target algal species, or some other desirable property. These "inert ingredients" may also alter the toxicity of a pesticide to non-target organisms.

Copper sulfate has been used as an algaecide for decades, and most algaecides are copper-based materials (Mastin and Rodgers 2000). Copper sulfate, when dissolved in water, dissociates into copper ions and sulfate ions, and results in high levels of bioavailable copper. Copper is quite toxic to many fish and aquatic invertebrate species, especially in ionic form (Mastin and Rodgers 2000). Newer copper-based algaecides have been designed to increase their effectiveness against various algae species, as well as reduce copper's non-target effects. Captain™ (SePRO Corporation, Carmel, IN, USA) is a copper-based algaecide, which uses copper carbonate to produce a copper ethanolamine complex. This complex is supposed to reduce the toxicity of the copper mixture to fish. In addition, these chelated copper algaecides may target algae more effectively by passing through algal cell walls and membranes more readily. SEPRO has a newer formulation of this algaecide called Captain™ XTR. Captain™ XTR contains the same copper ethanolamine complex and adds a proprietary surfactant designed to penetrate the protective sheath of problem algal species such as *Lyngbya*, *Pithophora*, and *Microcystis*. The

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effect of this surfactant on the toxicity of Captain™ to fish has not been explored. In our study we compare the toxicity of copper sulfate, Captain™, and Captain™ XTR on two species of freshwater fish.

Materials and Methods

Captain™ XTR contains a chelated form of copper (9.1 % Copper) and a surfactant. Captain™, contains a chelated form of copper (9.1 % Copper) without the addition of a surfactant. The two Captain algaecides are "ready-to-use" formulations which do not require further dilution, but may be diluted to ensure even application, prior to application. We also conducted tests using a Copper Sulfate Pentahydrate Fine Crystal (Old Bridge Chemical Inc Old Bridge, NJ, USA) which is an algaecide containing 25.2 % copper. This pesticide is dissolved in some manner prior to application to a pond or lake.

Toxicity test procedures follow those of the USEPA (2002). Trout used in our toxicity tests were New York State Rome Lab strain domestic brook trout (*Salvelinus fontinalis)*. Tests were conducted on two different size classes of brook trout; mean length of 53 mm (mean weight = 1.5 g), and mean length of 89 mm (mean weight = 7.0 g). Fathead minnows (*Pimephales promelas*) used in this study were shipped from Aquatic Biosystems Inc. (Fort Collins, CO), acclimated for at least 1 week, and were 45 days old (mean length = 20 mm, mean wt = 0.09 g). Neither species was fed throughout the duration of the toxicity tests.

New York State Rome Fish Hatchery (Rome, NY) spring water was used as the dilution water (pH = 8.10, hardness = 132 mg/L $CaCO_3$) for all toxicity tests. Hardness and pH were measured at the beginning of each test series and did not vary from that of spring water. Dissolved oxygen (DO) was monitored at each "fish count" interval. Test containers were lightly aerated after the first 24-h until the termination of the tests. DO never dropped below 60 % saturation. For experiments using brook trout, 20-L glass containers were used, with 16 L of test solution. Brook trout tests were conducted at 13 ± 1°C. For experiments using fathead minnows, 2-L glass containers were used, with 1.5 L of test solution. Fathead minnow tests were conducted at 21 ± 1°C.

The two Captain™ formulations were mixed directly into the test containers; however, the Copper Sulfate Pentahydrate Fine Crystals (approximately 4.0 g of the product) were dissolved into 100 mL of distilled water prior to being applied to the test containers. The actual weight of crystals dissolved into the water was recorded (weighed on an analytical balance with a precision of 1 mg) in order to calculate the strength of these stock solutions. All algaecides and stock solutions were measured and added using a certified digital pipette (precision = 0.1 μL). Test concentrations used for all fathead minnow experiments were 0.1, 0.2, 0.4, 0.8, 1.6, 3.2, and 6.4 mg/L (as elemental copper), with an additional test group at 0.05 mg/L for fathead experiments conducted using Captain™ XTR. For all brook trout experiments, concentrations used were 0.13, 0.2, 0.3, 0.45, 0.68, and 1.0 mg/L (as elemental copper), with an additional test group at 0.087 mg/L for all brook trout experiments conducted using copper sulfate, and for smaller brook trout experiments conducted using Captain™ XTR. Control containers were filled with spring water.

All tests were conducted with five brook trout or five fathead minnows in each test chamber and at least six replicates per concentration, with the number of replicates varying between experiments and concentrations. Mortality and behavior were observed every 24 h after the start of the experiment, with additional counts at 6 h (fathead minnow), and 8 h (brook trout). At the end of each time period, mortality and intoxication were recorded, and dead fish were removed, measured, and weighed (brook trout only). Each test was conducted for 96 h. Fish were defined as intoxicated if they were unable to remain upright or exhibited jerky uncontrolled movements. Effective concentrations (EC) were based on the number of dead or intoxicated fish.

LC50s and EC50s were calculated using nominal concentrations and the probit (Finney 1978) or trimmed Spearman-Karber (Hamilton et al. 1977) methods. The calculated LC50s and EC50s were used to compare the toxicities of the formulations and copper sulfate, all based on nominal concentrations of copper in the products.

Results and Discussion

Captain™ XTR was consistently more toxic to larger brook trout and fathead minnows. All LC50 and EC50 values are presented in Tables 1 and 2. There was no mortality in any of the control containers. Captain™ LC50 values for fathead minnows, were 2–3 times greater than Captain™ XTR LC50 values (and at least an order of magnitude greater in the 6-h exposure tests), with copper sulfate LC50 values similar to that of Captain™ XTR. Captain™ toxicity to larger brook trout was only slightly lower than that of Captain™ XTR, with copper sulfate toxicity similar to Captain™ XTR based upon EC50 values. Captain™ and Captain™ XTR toxicity to smaller brook trout was nearly the same, except in the short 8-h exposures. In general, brook trout were more sensitive to both formulations of Captain™ (Fig. 1) with, in nearly all cases, EC50 values significantly ($p < 0.05$) lower than those of the fathead minnow.

Table 1 LC50s (95 % confidence limits), expressed in mg Cu/L, from standard toxicity tests of Captain™, Captain™ XTR, and copper sulfate for brook trout and fathead minnow

Lethal concentrations (mg Cu/L) (95 % confidence interval)							
Species	Formulation	6 h	8 h	24 h	48 h	72 h	96 h
Fathead minnow	Captain™	>6.40	N/A	1.04 (0.90–1.20)	0.90 (0.77–1.04)	0.81 (0.69–0.94)	0.69 (0.59–0.81)
	Captain™ XTR	0.67 (0.61–0.74)	N/A	0.48 (0.43–0.53)	0.41 (0.36–0.46)	0.31 (0.27–0.37)	0.24 (0.20–0.28)
	Copper Sulfate	0.90 (0.78–1.02)	N/A	0.60 (0.55–0.65)	0.44 (0.39–0.51)	0.34 (0.29–0.39)	0.28 (0.25–0.33)
Smaller brook trout	Captain™	N/A	0.84 (0.77–0.92)	0.32 (0.29–0.36)	0.21 (0.18–0.24)	0.19 (0.16–0.21)	0.18 (0.16–0.21)
	Captain™ XTR	N/A	>1.00	0.45 (0.39–0.51)	0.22 (0.20–0.24)	0.21 (0.19–0.24)	0.20 (0.19–0.22)
	Copper Sulfate	N/A	>1.00	0.41 (0.36–0.45)	0.26 (0.24–0.28)	0.25 (0.23–0.27)	0.25 (0.23–0.27)
Larger brook trout	Captain™	N/A	>1.00	0.71 (0.65–0.77)	0.39 (0.35–0.43)	0.36 (0.33–0.39)	0.36 (0.33–0.39)
	Captain™ XTR	N/A	>1.00	0.50 (0.45–0.56)	0.32 (0.29–0.35)	0.31 (0.28–0.34)	0.31 (0.28–0.34)
	Copper Sulfate	N/A	1.15 (0.99–2.22)	0.53 (0.49–0.57)	0.48 (0.44–0.52)	0.48 (0.44–0.52)	0.48 (0.44–0.52)

Table 2 EC50 s (95 % confidence limits), expressed in mg Cu/L, from standard toxicity tests of Captain™, Captain™ XTR, and copper sulfate for brook trout and fathead minnow

Effective concentrations (mg Cu/L) (95 % confidence interval)							
Species	Formulation	6 h	8 h	24 h	48 h	72 h	96 h
Fathead minnow	Captain™	2.63 (2.30–3.02)	N/A	1.01 (0.87–1.17)	0.90 (0.77–1.04)	0.81 (0.69–0.94)	0.68 (0.58–0.80)
	Captain™ XTR	0.56 (0.51–0.61)	N/A	0.39 (0.34–0.44)	0.30 (0.26–0.34)	0.20 (0.17–0.23)	0.15 (0.12–0.17)
	Copper Sulfate	0.71 (0.64–0.79)	N/A	0.52 (0.47–0.58)	0.35 (0.30–0.40)	0.25 (0.21–0.28)	0.20 (0.18–0.23)
Smaller brook trout	Captain™	N/A	0.84 (0.77–0.88)	0.24 (0.22–0.26)	0.23 (0.20–0.26)	0.21 (0.17–0.25)	0.18 (0.16–0.21)
	Captain™ XTR	N/A	0.61 (0.55–0.68)	0.21 (0.19–0.24)	0.18 (0.17–0.20)	0.18 (0.16–0.20)	0.17 (0.15–0.19)
	Copper Sulfate	N/A	0.58 (0.52–0.64)	0.27 (0.24–0.30)	0.22 (0.20–0.25)	0.21 (0.18–0.23)	0.20 (0.18–0.24)
Larger brook trout	Captain™	N/A	0.85 (0.79–0.92)	0.46 (0.42–0.51)	0.33 (0.30–0.35)	0.32 (0.30–0.35)	0.31 (0.28–0.34)
	Captain™ XTR	N/A	0.57 (0.51–0.64)	0.37 (0.34–0.40)	0.29 (0.27–0.32)	0.29 (0.28–0.34)	0.29 (0.26–0.32)
	Copper Sulfate	N/A	0.37 (0.33–0.42)	0.28 (0.24–0.32)	0.27 (0.23–0.31)	0.26 (0.22–0.30)	0.24 (0.21–0.28)

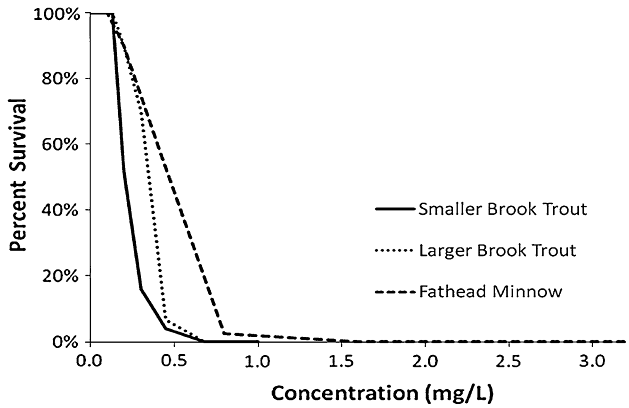

Fig. 1 Percentage of test fish surviving after 48 h of exposure to various concentrations of Captain™ XTR. An example of the general order of sensitivity observed through most of the tests conducted with the three "types" of fish

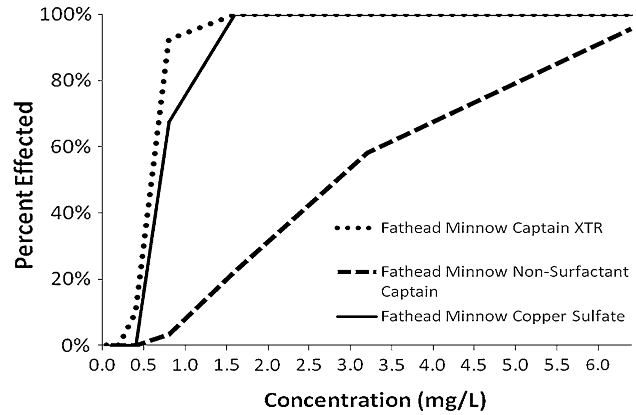

Fig. 2 Percentage of fathead minnows adversely affected (death or abnormal swimming) across test concentrations after 6 h of exposure

Richards and Beitinger (1995) found LC50s for copper sulfate to larger (20–55 mm) fathead minnows of 0.31 mg/L at a temperature of 22°C. Our results compare quite favorably to this. Benson and Birge (1988) determined a 96-h LC50 of 0.21 mg/L (95 % CL = 0.16–0.28) to fathead minnows (length = 40 mm) using copper sulfate conducted in water with hardness of 100 mg/L $CaCO_3$. This LC50 is not significantly different than the LC50 we found in our tests. In a study conducted by Mayer and Ellersieck (1986), copper sulfate toxicities were calculated for various freshwater species. Rainbow trout weighing an average 1.6 g were shown to have LC50s of 0.150 mg/L and 0.135 mg/L for 24- and 96-h respectively. These results are consistent with our brook trout toxicity tests. Fathead minnows weighing 1.2 g had 24- and 96-h LC50s of 1.68 and 0.838 mg/L respectively. Their tests on fathead minnow were conducted in very hard water (272 mg/L $CaCO_3$) and with substantially larger fish. It is possible that copper toxicity was reduced by the high water hardness in these studies. When conducting copper toxicity studies on fathead minnows, our LC50 s for 24- and 96-h were 0.60 and 0.28 respectively. These LC50s are substantially lower than the LC50s determined by Mayer and Ellersieck, and may be due to differences in water hardness. McKim and Benoit (1971) studied the long-term effects of copper exposure on survival, growth, and reproduction of brook trout. They found brook trout (age = 14 months) to have a 96-h LC50 of 0.11 mg/L when tested in softer water (pH = 7.5 and hardness = 45 mg/L $CaCO_3$). The 96-h LC50 obtained by McKim and Benoit is considerably lower than our 96-h LC50 (0.48 mg/L) calculated after conducting copper sulfate tests on our larger brook trout, and may be due to the lower pH and hardness of their test waters.

In our experiments with fathead minnows, Captain™ was significantly less toxic than copper sulfate ($p < 0.05$), demonstrating that chelated copper algaecides can be less toxic to non-target fish. This stands in contrast to our experiments with young brook trout where Captain™ toxicity was nearly the same to that of copper sulfate. For larger brook trout we found Captain™ toxicity to be lower than that of copper sulfate up until 24 h. However, from that point on, Captain™ toxicity was significantly ($p < 0.05$) higher than that of copper sulfate indicating that chelated copper algaecides may not be consistently safer than ionic copper algaecides (copper sulfate).

Compared with Captain™, Captain™ XTR exhibits a greater intoxicating effect, especially during short exposure times (Fig. 2). Throughout the bulk of our experiments, it was clear that the original Captain™ formulation was less toxic to our test fish. This demonstrated that the formulation with the surfactant was substantially more toxic to non-target aquatic organisms than the original Captain™ formulation, which did not contain a surfactant. The addition of the surfactant in Captain™ XTR increases the risk to non-target aquatic life.

Mastin and Rodgers (2000) examined the toxicity of two chelated copper algaecides, and copper sulfate, to several aquatic species including fathead minnows. They reported 48-h LC50s of 0.255 and 0.480 mg/L for the two chelated copper algaecides to fathead minnows. They also report a 48-h LC50 of 0.019 mg/L for copper sulfate, which is an order of magnitude lower than the 48-h LC50 we found. Their tests were conducted with younger fish and were conducted in softer water than our tests. Interestingly, the LC50s they found for the chelated copper algaecides were 15–20 times greater than copper sulfate to fathead minnows, while our values were about two times greater. However, the large difference that they observed with fish did not occur with the three invertebrate species that they tested.

Copper sulfate is applied to ponds and lakes at rates up to 1 mg/L. Captain™ and Captain™ XTR are applied at rates from 0.2 to 1.0 mg/L depending on the density and species of algae to be controlled. So, application rates are well within the rates which we have demonstrated to be lethal to trout and fathead minnows. These application rates are, in fact, at the levels that are toxic to these fish based upon the EC50s we found in short (6 or 8 h) exposures (the exception being Captain™ to fathead minnows). This suggests that there is little "margin of safety" between application rates and toxic levels, yet fish kills are not a common occurrence.

The biotic ligand model (BLM) (DiToro et al. 2001, Santore et al. 2001) provides some insight into why field applications of copper algaecides are not as toxic to fish as might be expected based strictly upon laboratory toxicity tests. In water, the copper ion binds to various ligands that are present. These ligands can be inorganic or organic. In addition, other cations present in the water will compete with copper for these binding sites. Toxicity in an aquatic animal is correlated with the accumulation of copper on active sites (biotic ligands), and these sites are in "competition" for copper ions with other ligands. The BLM proposes that toxicity occurs when a critical threshold of physiologically active sites on an aquatic organism bind with copper.

One potential shortcoming of the BLM is that it assumes equilibrium conditions. These most likely do not exist at the beginning of an algaecide treatment, with the rapid release of copper to the treated lake. Copper sulfate is dissolved into its ionic form at the being of a treatment, whereas the copper in a Captain™ treatment is released in a chelated form. This might make our short exposure toxicity tests (6 or 8 h) particularly useful in evaluating potential effects on fish in a lake. It was in the short exposure tests where we noted the greatest difference in the toxicity to fathead minnows of copper sulfate and Captain™.

We evaluated the BLM for its applicability to water quality criteria in New York State (NYS). We found that pH, alkalinity, and dissolved organic carbon (DOC) had the greatest influence on predicted copper toxicity using data from lakes in NYS (Simonin et al. 2008). Traditionally, pH, alkalinity, and hardness have been considered when making decisions about treatment levels with copper sulfate in lakes in NYS, but DOC has not. High levels of DOC, often found in lakes treated with algaecides, may reduce the toxicity of copper to fish, providing an extra level of protection to non-target aquatic life. The uptake of copper by algae and other plants present (other biotic ligands) in treated water bodies may also contribute to reduced toxicity in lakes. We are currently unaware of any state that uses the BLM to determine appropriate treatment levels with copper-based algaecides. Since most of the water quality parameters used in the BLM are not required for obtaining a algaecide treatment permit, it is very important that the amount of these products applied be no higher than what is needed to control the particular problem algae.

Since all of our tests were conducted in the same water, comparisons between formulations (our primary purpose) and between the two species remain valid. All Captain™ experiments using fathead minnows produced LC50 values notably higher than the experiments conducted with copper sulfate alone. However, the opposite was found when a surfactant is added into the formulation. All Captain™ XTR experiments produced LC50 values substantially lower than the experiments conducted with copper sulfate alone. It appears, overall, that copper becomes less toxic to non-target fish when it is chelated, providing an additional margin of safety to non-target fish. However, once the surfactant is added to the formulation, the toxicity of the copper is increased to fish.

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