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Responses of *Lepomis macrochirus*, *Pimephales promelas*, *Hyalella azteca*, *Ceriodaphnia dubia*, and *Daphnia magna* to Exposures of Algimycin® PWF and Copper Sulfate Pentahydrate

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ABSTRACT

To make risk-based decisions, water resource managers need information that indicates potential responses of non-target species to algaecide applications. This research was conducted to determine the responses of select non-target species to exposures of two commonly used copper-based algaecides (Algimycin® PWF and copper sulfate pentahydrate). Bluegill (*Lepomis macrochirus*), fathead minnow (*Pimephales promelas*), scud (*Hyalella azteca*), and two water fleas (*Ceriodaphnia dubia* and *Daphnia magna*) were exposed to a series of concentrations of chelated copper as Algimycin® PWF and copper sulfate pentahydrate in 96-hour toxicity experiments. For exposures of both copper algaecides, *D. magna* was the most sensitive species, followed by *C. dubia*, *P. promelas*, *H. azteca*, and *L. macrochirus*. *Daphnia magna*, *C. dubia*, *P. promelas*, and *H. azteca* responded similarly to both Algimycin® PWF and copper sulfate pentahydrate. However, copper sulfate pentahydrate was 29 times more toxic than Algimycin® PWF to *L. macrochirus*. These results indicate a great difference in sensitivities to algaecide exposures within and among animal species. For these aqueous exposures to copper algaecides, the microcrustaceans *C. dubia* and *D. magna*, were clearly more sensitive than the fish species *L. macrochirus* and *P. promelas*. These laboratory data provide conservative estimates of field exposures and must be translated to field situations due to copper speciation. To minimize risks in practical situations, water resource managers should consider timing of algaecide applications in terms of partial treatments, extent of target species infestation, duration of exposures, availability of refugia, spawning seasons, as well as fecundity of the non-target species in the aquatic system.

Key words: algaecide, amphipod, bluegill, fathead minnow, risk management, toxicity, water flea.

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INTRODUCTION

Problematic algal species may grow to densities that adversely impact water quality and impair desirable species in aquatic systems (Boyd 1990, Chorus 2000, Briand et al. 2003). Recreation and other uses of water resources may also be affected by algal blooms, leading to declines in property values (Henderson et al. 2003). Some algal species can produce toxins that directly affect survival, growth, and reproduction of aquatic vertebrates and invertebrates (Shilo 1967, Nguyen et al. 2000, Briand et al. 2003) as well as mammals such as humans (Briand et al. 2003), while others can produce compounds such as methylisoborneol or geosmin, causing taste and odor problems in water supplies and "off" flavor in fish (Brown and Boyd 1982, Tucker 2000). As water resources are used more intensively and extensively, we become acutely aware of the presence and impacts of problematic algae. When problematic algae interfere with critical uses of water resources, such as for domestic water supply, water resource managers often seek to intervene and control their growth. The rapid onset, intensity, and extent of an algal bloom may render mechanical, physical, or biological remediation techniques impractical. In such instances, chemical remediation through algaecides may be efficient and effective for mitigating risks posed by these algae. To better understand potential risks that algaecide applications may pose for non-target species, we need more information regarding responses of sentinel non-target species to algaecide exposures.

Several of the available algaecides for controlling growth of problematic species are formulated from copper salts. Previous research has illustrated that copper formulations are not the same (Murray-Gulde et al. 2002). In addition to environmental factors, the form of the active ingredient plays an important role in the efficacy of the treatment as well as any impacts on non-target species (Morris and Russell 1973). Target algal species differ significantly in their responses to algaecide exposures. Responses of relatively sensitive, sentinel aquatic species to acute algaecide exposures under controlled laboratory conditions have indicated that invertebrates are generally more sensitive than vertebrates (Murray-Gulde et al. 2002). These laboratory data are useful for predicting potential risks to non-target species in the field posed by algaecide applications.

Aquatic species often used in laboratory toxicity tests include: bluegill (*Lepomis macrochirus* Rafinesque), fathead minnow (*Pimephales promelas* Rafinesque), scud (*Hyalella azteca* Saussure), and two water fleas (*Ceriodaphnia dubia* Richard and *Daphnia magna* Straus). These sentinel species are used because of their widespread occurrence in freshwater ecosystems, ease of culture, sensitivity to chemicals such as algaecides, and prior use in toxicity testing (USEPA 1984, 1994, 1996a, 1996b). If the concentration of algaecide required for control of the target algae is less than the concentration required to elicit adverse effects from non-target species, a margin of safety exists. This margin of safety may differ between vertebrate and invertebrate aquatic species as well as between chemical forms of algaecide.

To further develop and expand information on potential responses of non-target species to exposures of two widely used copper-containing algaecides (Algimycin® PWF and copper sulfate pentahydrate), the objectives of this research were to: (1) compare responses of non-target sentinel vertebrate and invertebrate species to aqueous exposures of Algimycin® PWF; (2) compare responses of non-target sentinel vertebrate and invertebrate species to aqueous exposures of copper sulfate pentahydrate; and (3) contrast responses of *L. macrochirus*, *P. promelas*, *H. azteca, C. dubia*, and *D. magna* to exposures of Algimycin® PWF and copper sulfate pentahydrate.

MATERIALS AND METHODS

Lepomis macrochirus were obtained from Aquatic Research Organisms (Hampton, NH) and held for 10 days before testing. *Pimephales promelas*, *H. azteca*, *C. dubia*, and *D. magna* were cultured at Clemson University. These cultures have been maintained over the past 30 years, and the species have originated from many commercial cultures and cultures in universities throughout the United States. All organisms were cultured and tested at a temperature of 23 ± 2 C under a 16 hour light/8 hour dark photoperiod. A minimum of 20 organisms of each species was exposed to each treatment in glass vessels of the appropriate size for the organism (Table 1). The measured response of *L. macrochirus*, *P. promelas*, *H. azteca*, *C. dubia*, and *D. magna* was a difference in mortality in treatments versus controls. Reproduction data were also collected for *C. dubia* in 7-day toxicity experiments to determine the potential for reproductive effects of Algimycin® PWF and copper sulfate pentahydrate. To discern potential effects of the mass of the test organisms on responses of organisms to exposures, mass of *L. macrochirus*, *P. promelas*, and *H. azteca* was measured using an A&D GR-202 dual range (0.00001 g) balance (A&D Engineering, Inc., San Jose, CA), and mass of *C. dubia*, and *D. magna* was estimated from Anderson and Benke (1994) and Sterner and Robinson (1994), respectively. Water characteristics (i.e., pH, alkalinity, hardness, conductivity, dissolved oxygen [DO], and temperature) were measured prior to test initiation and at test conclusion using standard methods (APHA 1998).

Organisms were exposed to a series of concentrations of copper as Algimycin® PWF and copper sulfate pentahydrate in 96-hour toxicity experiments (Table 1; Lewis et al. 1994, CFR 2004). Stock solutions used for these experiments were prepared less than 4 hours prior to experiment initiation by dissolving Algimycin® PWF (Applied Biochemists, Inc., Germantown, WI) and copper sulfate pentahydrate (Acros® Organics, Fisher Scientific, Pittsburgh, PA) in NANOpure water (Table 2). Exposure solutions were prepared from the stock solutions using moderately hard laboratory water (pH 7 ± 1.5, DO 8 ± 2 mg O_2/L, temperature 23 ± 2 C, conductivity

TABLE 1. TEST CONDITIONS AND METHODS FOR *LEPOMIS MACROCHIRUS* (BLUEGILL), *PIMEPHALES PROMELAS* (FATHEAD MINNOW), *HYALELLA AZTECA* (SCUD), *CERIODAPHNIA DUBIA* (WATER FLEA) AND *DAPHNIA MAGNA* (WATER FLEA) TOXICITY EXPERIMENTS.

	L. macrochirus	*P. promelas*	*H. azteca*	*C. dubia*	*D. magna*
Method	USEPA 1996a	Lewis et al. 1994	USEPA 1994	Lewis et al. 1994	USEPA 1996b
Source of organisms	ARO[1]	Clemson University Aquatic Animal Research Laboratory			
Age/size of test organisms	Aprox. 1.4 g/3 to 5 cm length	≤24 hours	10-13 days 0.5-1.0 cm	≤24 hours	≤24 hours
Organism holding time	~10-12 days	≤4 hours	≤4 hours	≤4 hours	≤4 hours
Daily feeding	None	None	None	200 uL YCT: Algae[2]	None
Algimycin® PWF (ug Cu/L) exposure concentrations	Background to 100,000	Background to 3,000	Background to 2,000	Background to 150	Background to 100
Copper sulfate (ug Cu/L) exposure concentrations	Background to 16,000	Background to 3,000	Background to 2,000	Background to 150	Background to 100
Test type	Static	Static	Static	Static Renewal	Static
Test duration	96 hours	96 hours	96 hours	96 hours & 7day	96 hours
Exposure chamber	38 L Tank	250 mL Beaker	250 mL Beaker	20 mL Vial	250 mL Beaker
Volume per replicate	26 L	200 mL	200 mL	10 mL	200 mL
Organisms per replicate	10	10	10	1	10
Replicates per exposure	2	3	3	10	3
Response	Mortality	Mortality	Mortality	Mortality & reproduction	Mortality

[1]Aquatic Research Organisms (Hampton, NH 03842).
[2]YCT (yeast: cerophyll: trout chow): Algae (*Raphidocelis subcapitata* (synonymous with *Selenastrum capricornutum*).

TABLE 2. CHEMICAL CHARACTERISTICS OF ALGIMYCIN® PWF AND COPPER SULFATE PENTAHYDRATE.

	Algimycin® PWF[1]	Copper (II) sulfate pentahydrate[2]
Manufacturer	Applied Biochemists	Acros® Organics
Identification	7364-09-8959 (EPA Reg. No)	7758-99-8 (CAS No)
Concentrate pH	1.8	NA
Active Ingredient	5% Cu 95% Inert Ingredients	25% copper by weight
	Copper citrate	$CuSO_4 \times 5H_2O$
	Copper gluconate chelates	249.68 formula weight
Appearance	Blue viscous liquid	Blue crystalline
Water Solubility	Complete	31.6 g/100 mL @ 0 C, 203.3g/100 mL @ 100 C
Odor	Slight amine (citrus)	NA
Specific Gravity	1.2 @ 27 C	NA

[1]Applied Biochemists, Inc. (2002, 2006).
[2]USEPA (1986).

130-350 µS/cm², alkalinity 40-80 mg $CaCO^3$/L, hardness 40-80 mg $CaCO^3$/L). Exposure concentrations of copper as Algimycin® PWF for *L. macrochirus* were: background, 500, 1000, 5000, 10000, 15000, 20000, 40000, and 100000 ug Cu/L. Exposure concentrations of copper as copper sulfate pentahydrate for *L. macrochirus* were: background, 500, 1000, 2000, 4000, 6000, 8000, 10000, and 16000 ug Cu/L. Exposure concentrations of copper as Algimycin® PWF and copper sulfate pentahydrate for *P. promelas* and the invertebrate species were the same for both algaecides: background for *P. promelas*—10, 100, 200, 500, 750, 1000, 2000, and 3000 ug Cu/L; background for *H. azteca*—100, 200, 400, 600, 800, 1000, and 2000 ug Cu/L; background for *C. dubia*—5, 10, 20, 30, 50, 70, 100, and 150 ug Cu/L; and background for *D. magna*—1, 3, 5, 10, 30. 50, and 100 ug Cu/L.

Copper concentrations in exposure solutions were verified by measuring acid-soluble copper concentrations in samples of exposure solutions prior to experiment initiation and at experiment conclusion (APHA 1998). Prior to measurement, all samples for acid-soluble copper concentrations were acidified to *p*H ≤ 2 using technical-grade grade hydrochloric acid. Copper concentrations of exposure solutions were measured using a graphite furnace atomic absorption spectrometer (Perkin-Elmer 5100 PC, Waltham, MA; APHA 1998).

Exposure-response curves were developed for each organism and algaecide. Lethal concentration values for 50% of the organisms (LC_{50}) were calculated by probit or trimmed Spearman-Karber analysis. Lowest observable effect concentrations (LOECs) were determined from the exposure-response curves using regression and ANOVA.

RESULTS AND DISCUSSION

Water characteristics such as pH, hardness, ionic strength, and dissolved organic carbon can influence the toxicity of different forms of copper (Erickson et al. 1996). Experimental conditions were controlled to minimize the potential for influence of water characteristics on these exposures (Table 3). In these laboratory experiments, the test organisms were exposed to copper in 'clean water,' meaning they were not fed or were fed minimally (Table 1), which maximizes the bioavailability of copper. Under actual algaecide treatments in the field, bioavailability of copper is depleted by sorption to algae and other ligands (Sprague 1985, Taylor et al. 1998, Kim et al. 1999). Copper concentrations were measured in untreated controls with a background copper concentration of 0.99 ug Cu/L. Measured acid-soluble copper concentrations were within 97% to 102% of target copper concentra-

TABLE 3. MEASURED WATER CHARACTERISTICS FOR ALGIMYCIN® PWF AND COPPER SULFATE 96-HOUR TOXICITY TESTS FOR *LEPOMIS MACROCHIRUS*, *PIMEPHALES PROMELAS*, *HYALELLA AZTECA*, *CERIODAPHNIA DUBIA* AND *DAPHNIA MAGNA*.

Species	pH	DO mg O_2/L	Conductivity uS/cm	Alkalinity mg $CaCO_3$/L	Hardness mg $CaCO_3$/L
			Algimycin® PWF		
L. macrochirus	8.2 ± 0.2	8 ± 1	170-330	50-110	60-120
P. promelas	7.7 ± 0.3	8 ± 1	310-360	58-72	80-120
H. azteca	7.8 ± 0.1	8 ± 1	307-327	56-66	84-92
C. dubia	8.0 ± 0.2	8 ± 1	130-350	60-70	80-110
D. magna	7.7 ± 0.5	8 ± 1	300-360	55-70	80-110
			Copper Sulfate Pentahydrate		
L. macrochirus	8.2 ± 0.2	8 ± 1	170-330	50-110	60-120
P. promelas	7.7 ± 0.3	8 ± 1	310-360	58-72	80-120
H. azteca	7.8 ± 0.1	8 ± 1	301-320	56-64	84-88
C. dubia	8.0 ± 0.2	8 ± 1	130-350	60-70	80-110
D. magna	7.7 ± 0.5	8 ± 1	300-360	55-70	80-110

tions, thus LC_{50} and LOEC values were calculated from the target copper concentrations. In instances where LOEC values could not be calculated or determined from linear regression analysis (*P. promelas*, *H. azteca*, and *D. magna*), the lowest exposure concentration significantly different from the control was reported as the LOEC.

Organism Responses to Algimycin® PWF

In 96 hour static, nonrenewal exposures of Algimycin® PWF, *L. macrochirus* was the least sensitive species with an LC_{50} of 67,000 ± 7,000 ug/L, followed by *H. azteca* with an LC_{50} of 390 ± 90 ug/L, and *P. promelas* with an LC_{50} of and 250 ± 70. *Ceriodaphnia dubia* and *D. magna* were the most sensitive species to Algimycin® PWF with LC_{50} values of 48 ± 5 ug/L and 4.6 ± 0.7 ug/L, respectively (Table 4; Figures 1-5). The LOEC values were: 29,360 ug/L for *L. macrochirus*, 10 ug/L for *P. promelas*, 100 ug/L for *H. azteca*, 15 ug/L for *C. dubia*, and 1 ug/L for *D. magna* (Table 4; Figures 1-5).

To further discriminate the responses of these species to aqueous exposures of Algimycin® PWF, slopes of the change in response with change in concentration (potency slopes) were calculated for the linear portion of the exposure-response curve. *L. macrochirus* was relatively insensitive with a slope of 0.001% mortality/ug/L; *P. promelas* and *H. azteca* had the same slope of 0.1% mortality/ug/L. For the two more sensitive species, *C. dubia* and *D. magna*, the potency slopes revealed differences of 1.23% mortality/ug/L and 6.64% mortality/ug/L, respectively.

Organism Responses to Copper Sulfate Pentahydrate

In 96-hour, static, non-renewal exposures of copper sulfate pentahydrate, *L. macrochirus* was the least sensitive species with an LC_{50} of 2,640 ± 90 ug/L, followed by *H. azteca* with an LC_{50} of 400 ± 70 ug/L, and *P. promelas* with an LC_{50} of and 230 ± 50 ug/L. *Ceriodaphnia dubia* and *D. magna* were the most sensitive species to copper sulfate pentahydrate, with LC_{50} values of 42 ± 6 ug/L and 5.0 ± 0.6 ug/L, respectively. The LOEC values were: 363 ug/L for *L. macrochirus*, 10 ug/L for *P. promelas*, 100 ug/L for *H. azteca*, 14 ug/L for *C. dubia*, and 1 ug/L for *D. magna* (Table 4).

To further discriminate the responses of these species to aqueous exposures of copper sulfate pentahydrate, slopes of the change in response with change in concentration (potency slopes) were calculated for the linear portion of the exposure-response curves. *Lepomis macrochirus* was relatively insensitive with a slope of 0.04% mortality/ug/L; *P. promelas* and *H. azteca* had similar slopes 0.12 and 0.09% mortality/ug/L, respectively. For the two most sensitive species, *C. dubia* and *D. magna*, the potency slopes revealed differences of 1.31% mortality/ug/L and 8.61% mortality/ug/L, respectively.

Reproductive Response of *C. dubia*

In 7-day, static non-renewal tests using *C. dubia*, no reproductive impairment was recorded in exposures of either Algimycin® PWF or copper sulfate pentahydrate in comparison to control organisms (average of 14.2 neonates/surviving adult). In *C. dubia* 7-day experiments measuring reproductive effects, Murray-Gulde et al. (2002) found the LOEC was 50 ug/L for copper sulfate, and 200 ug/L for both Clearigate® and Cutrine®-Plus (Table 5).

Contrast of Species Responses to Exposures of Algaecides

Typically, vertebrate species are less sensitive to exposures of copper than invertebrate species (USEPA 1984, Mastin and Rodgers 2000, Murray-Gulde et al. 2002). In this study both vertebrate species (*L. macrochirus* and *P. promelas*) were much less sensitive than the microcrustaceans (*D. magna* and *C. dubia*); however, *H. azteca* responded similarly to exposures of Algimycin® PWF and copper sulfate pentahydrate as *P. promelas* (Table 4).

To discern any influence of the mass of the test animal on the observed response to copper algaecide exposures, the exposure mass ratio was calculated as the mass of copper in an exposure vessel per mass of organism. The exposure mass ratio was calculated for *L. macrochirus*, *P. promelas*, *H. azteca*, *C. dubia*, and *D. magna* at both the maximum and minimum target exposure concentrations (Table 6, Figure 6. This exposure mass ratio indicates the potential for partitioning of copper from the aqueous phase to non-target organisms assuming all of the copper partitions to the animals and allows comparison of the responses of the organisms independent of mass. *Daphnia magna,* the most sensitive species, had the lowest mass ratios (0.03 to 1.61 ug Cu/mg organism), indicating that it was the most sensitive species to copper exposures independent of mass. *Lepomis macrochirus*, the least sensitive species, had the second smallest mass ratios (0.93 to 7.43 ug Cu/mg organism), suggesting that its larger size may have influenced its tolerance of copper exposures. However, the laboratory exposures of Algimycin® PWF and copper sul-

TABLE 4. ALGIMYCIN® PWF AND COPPER SULFATE LC_{50} VALUES AND LOEC ESTIMATES FOR 96 HOUR TOXICITY TESTS (UG CU/L) ON. *LEPOMIS MACROCHIRUS*, *PIMEPHALES PROMELAS*, *HYALELLA AZTECA*, *CERIODAPHNIA DUBIA* AND *DAPHNIA MAGNA*.

	Algimycin® PWF		Copper Sulfate Pentahydrate	
Species	LC_{50}	LOEC	LC_{50}	LOEC
L. macrochirus	67,000 ± 7,000	29,360	2,640 ± 90	363
P. promelas	250 ± 70	10	230 ± 50	10
H. azteca	390 ± 90	100	400 ± 70	100
C. dubia	48 ± 5	15	42 ± 6	14
D. magna	4.6 ± 0.7	1	5.0 ± 0.6	1

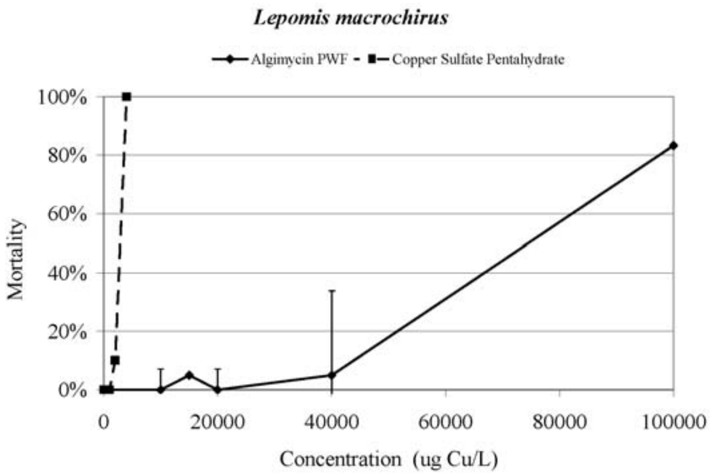

Figure 1. Effects of Algimycin® PWF and copper sulfate pentahydrate in 96-hour exposures for *Lepomis macrochirus* (mortality).

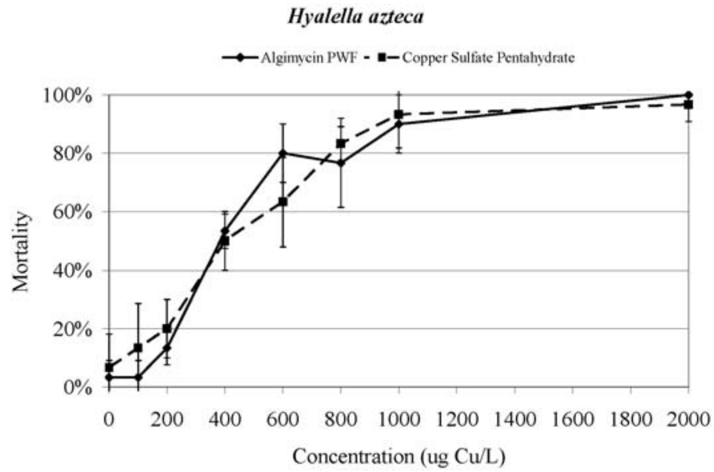

Figure 3. Effects of Algimycin® PWF and copper sulfate pentahydrate in 96-hour exposures for *Hyalella azteca* (mortality).

fate pentahydrate required to elicit responses from *L. macrochirus* (LOEC of 29,360 and 363 mg Cu/L, respectively) were well in excess of realistic field application rates (1 mg Cu/L). *Pimephales promelas* had exposure mass ratios (0.40 to 40.08 ug Cu/mg organism), which was less than *H. azteca* (6.25 to 125.0 ug Cu/mg organism), further supporting the notion that *P. promelas* is more sensitive than *H. azteca*. *Ceriodaphnia dubia* was the second most sensitive species to both algaecides; however, it had the greatest ratio (119.0 to 1,190.5 ug Cu/mg organism). Because *C. dubia* is sensitive to copper exposures, the large mass ratio of *C. dubia* indicates that although mass may influence the sensitivity of a species to a copper exposure, it is not the only indicator of sensitivity.

Differences Between Copper Sulfate Pentahydrate and Chelated Copper Algaecides

The results of this study are in agreement with other studies that examined non-target species toxicity for copper sulfate and two chelated copper algaecides (Clearigate® and Cutrine®-Plus; Table 5). Murray-Gulde et al. (2002) found *C. dubia* were more sensitive to copper exposures than *P. promelas*. Mastin and Rodgers (2000) found *D. magna* were more sensitive to copper exposures than *P. promelas*. Both studies also found that chelated copper algaecides generally were less toxic to fish and invertebrate species than copper sulfate (Table 5).

For aquatic algaecides containing copper, an important goal is to maximize control of target species while minimizing risks to non-target species (Murray-Gulde et al. 2002). In comparison to the copper ion, chelation of copper, as in the case of Algimycin® PWF, can increase the stability of copper in a water column by decreasing the potential for precipitation as well as increase binding to algal cell membranes (Fitzgerald and Faust 1963, Flemming and Trevors 1989, Murray-Gulde et al. 2002). Stauber and Florence (1987) concluded that organo-copper complexes were much more toxic to algae than ionic copper. Chelated algaecides that have an affinity for the target algal species will potentially produce a greater dose of copper to the active sites on or in algal cells

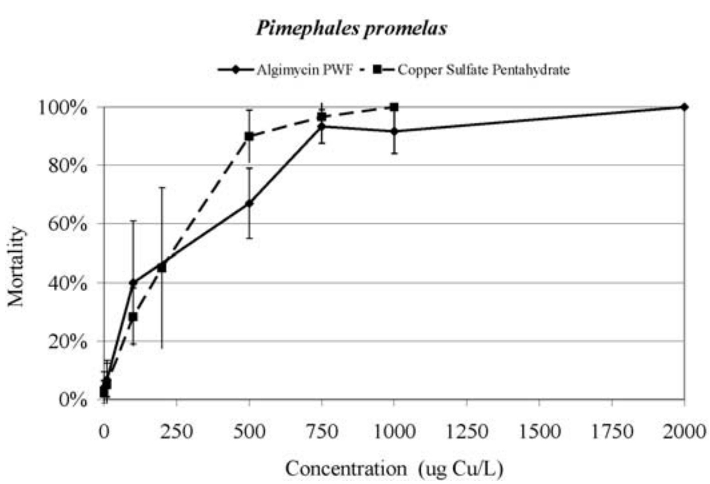

Figure 2. Effects of Algimycin® PWF and copper sulfate pentahydrate in 96-hour exposures for *Pimephales promelas* (mortality).

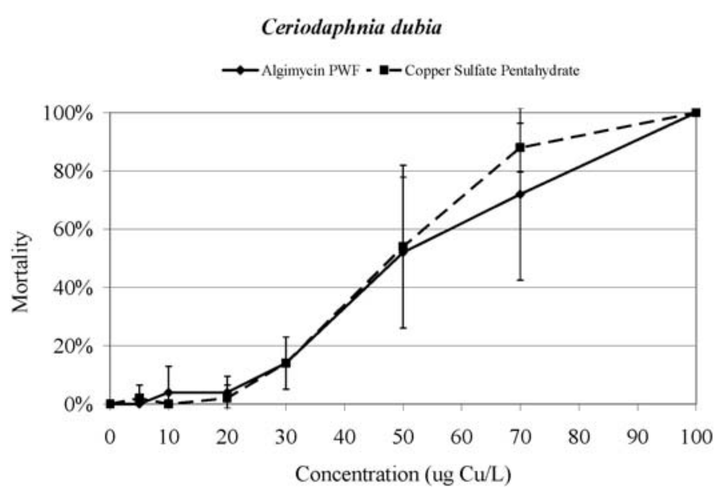

Figure 4. Effects of Algimycin® PWF and copper sulfate pentahydrate in 96-hour exposures for *Daphnia magna* (mortality).

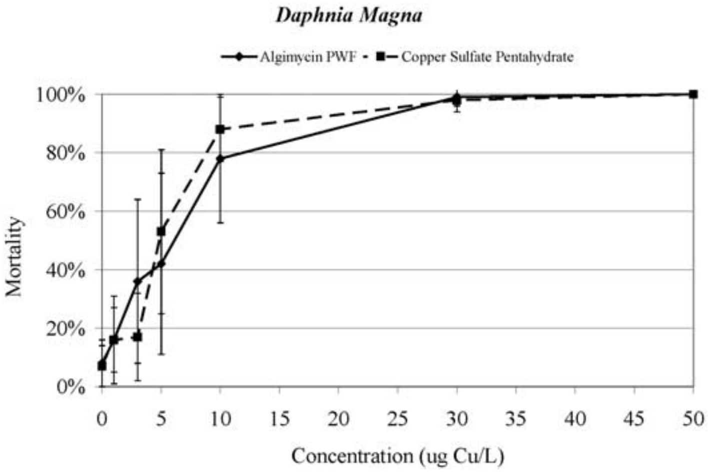

Figure 5. Effects of Algimycin® PWF and copper sulfate pentahydrate in 96-hour exposures for *Ceriodaphnia dubia* (mortality).

and thus greater control of algae at lower overall environmental copper concentrations. Also, copper sorbed to algal cells will be less bioavailable to non-target species (Clearwater et al. 2002). The lower the bioavailable copper concentrations in a water column after an algaecide exposure, the greater the margin of safety will be for non-target species.

RECOMMENDATIONS AND SUMMARY

For *L. macrochirus,* copper sulfate pentahydrate is about 29 times more toxic than Algimycin® PWF (Table 4; Figure 1). Straus and Tucker (1993) found that copper sulfate was more toxic to channel catfish than a chelated form of copper. The margins of safety with these algaecides are minimal for *P. promelas*, *H. azteca*, *C. dubia*, and *D. magna*, indicating the need for selecting algaecide use rates based upon the minimum amount required to control existing targeted algal species and algal cell density (Murray-Gulde et al. 2002; Figures 2-5). Water resource managers should consider the type of water body, water chemistry (e.g., pH, alkalinity, hardness, dissolved oxygen), use of the water body, and weather conditions before algaecide applications. Managers may also need to consider partial treatments, duration of exposures, availability of refugia, spawning seasons, fecundity of the non-target species as well as presence of endangered species in the aquatic system.

These results indicate a great difference in sensitivities to algaecide exposures within and among animal species. For these aqueous exposures to copper algaecides, the microcrustaceans *C. dubia* and *D. magna* were clearly more sensitive than the fish species *L. macrochirus*, *P. promelas*. These laboratory data provide conservative estimates of field exposures and require translation to field situations due to copper spe-

TABLE 5. LC_{50} VALUES FOR COPPER SULFATE AND CHELATED COPPER COMPOUNDS (UG CU/L).

Organism	Algaecide	Test duration	Concentration ug Cu/L	Citation
L. macrochirus	Copper sulfate	96-h LC_{50}	2,640	Current study
		96-h TL_{50}[1]	1,100	Benoit, 1975
	AlgimycinPWF	96-h LC_{50}	67,000	Current study
P. promelas	Copper sulfate	96-h LC_{50}	230	Current study
		96-h LC_{50}	675	Murray-Gulde et al. 2002
	AlgimycinPWF	96-h LC_{50}	250	Current study
	Cutrine-Plus	96-h LC_{50}	1,115	Murray-Gulde et al. 2002
		48-h LC_{50}	255	Mastin and Rodgers 2000
	Clearigate	96-h LC_{50}	481	Murray-Gulde et al. 2002
		48-h LC_{50}	480	Mastin and Rodgers 2000
H. azteca	Copper sulfate	96-h LC_{50}	400	Current study
		48-h LC_{50}	433	Mastin and Rodgers 2000
	AlgimycinPWF	96-h LC_{50}	390	Current study
	Cutrine-Plus	48-h LC_{50}	248	Mastin and Rodgers 2000
	Clearigate	48-h LC_{50}	158	Mastin and Rodgers 2000
C. dubia	Copper sulfate	96-h LC_{50}	42	Current study
		96-h LC_{50}	60	Murray-Gulde et al. 2002
	AlgimycinPWF	96-h LC_{50}	48	Current study
	Cutrine-Plus	96-h LC_{50}	92	Murray-Gulde et al. 2002
	Clearigate	96-h LC_{50}	56	Murray-Gulde et al. 2002
D. magna	Copper sulfate	96-h LC_{50}	5	Current study
		48-h LC_{50}	19	Mastin and Rodgers 2000
	AlgimycinPWF	96-h LC_{50}	5	Current study
	Cutrine-Plus	48-h LC_{50}	11	Mastin and Rodgers 2000
	Clearigate	48-h LC_{50}	29	Mastin and Rodgers 2000

[1]TL_{50}—Tolerable Limit for 50 percent of the organisms.

TABLE 6. EXPOSURE MASS RATIOS (UG CU/MG ORGANISM) FOR *LEPOMIS MACROCHIRUS*, *PIMEPHALES PROMELAS*, *HYALELLA AZTECA*, *CERIODAPHNIA DUBIA*, AND *DAPHNIA MAGNA* USING MAXIMUM AND MINIMUM COPPER CONCENTRATIONS FOR EACH SPECIES.

Species	Average weight per organism (mg)	Volume (mL per organism)	Min. conc. ug Cu/L	Min. ug Cu/mg organism	Max. conc. ug Cu/L	Max. ug Cu/mg organism
L. macrochirus[1]	1400	2600	500	0.93	100,000	7.4
P. promelas[2]	0.499	20	10	0.40	2,000	40.0
H. azteca[3]	0.32	20	100	6.25	2,000	125.0
C. dubia[4]	8.40×10^{-4}	10	10	119.05	100	1190.0
D. magna[5]	0.62	20	1	0.03	50	1.6

[1]Wet weight measured in experiment.
[2]Dry weight measured in experiment.
[3]Dry weight measured in experiment.
[4]Dry weight, adult (Anderson and Benke 1994).
[5]Dry weight, neonate (Sterner and Robinson 1994).

ciation. In practical situations, water resource managers consider timing of algaecide applications in terms of partial treatments, extent of target infestation, duration of exposures, availability of refugia, spawning seasons, as well as fecundity of the non-target species in the aquatic system. Furthermore, the target algae serve as ligands, rapidly uptaking and binding the applied copper, rendering it unavailable to non-target organisms in the field. This is in contrast to the relatively constant exposures in 'clean water' laboratory tests (Sprague 1985, Taylor et al. 1998, Kim et al. 1999). While the margins of safety calculated from these laboratory bioassays for these algaecides and sensitive non-target species seem small or nonexistent, risks can be reduced or mitigated through the efficacious use of the algaecides and the skill of the applicator.

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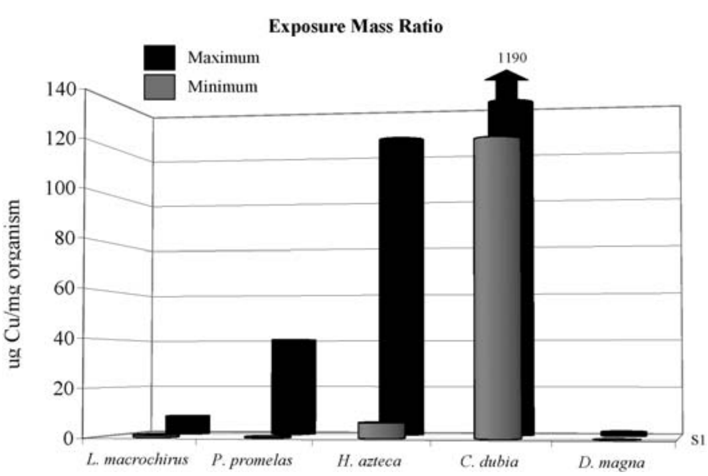

Figure 6. Exposure mass ratios (ug Cu/mg organism) for *Lepomis macrochirus*, *Pimephales promelas*, *Hyalella azteca*, *Ceriodaphnia dubia*, and *Daphnia magna* using maximum and minimum copper concentrations for each species.

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