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Comparative responses of target and nontarget species to exposures of a copper-based algaecide

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ABSTRACT

In order for water resource managers to make informed, risk-based decisions for algaecide applications, data are needed regarding the relative sensitivities of targeted algal species and nontarget animal species. The objective of this research was to measure responses of eight target algal species (*Ankistrodesmus falcatus* Corda, *Cymbella tumida* Brebisson, *Desmidium* sp., *Eudorina elegans* Ehrenberg, *Haematococcus pluvialis* Flotow, *Microcystis aeruginosa* Kutzing, *Nostoc punctiforme* Kutzing, and *Pandorina charkowiensis* Korschikov) and five nontarget animal species (*Ceriodaphnia dubia* Richard, *Daphnia magna* Straus, *Hyallela azteca* Saussure, *Lepomis macrochirus* Rafinesque, *Pimephales promelas* Rafinesque) to exposures of a copper-based algaecide (a.i. copper 5%) in 96-h laboratory toxicity tests. The copper concentrations required to achieve control (i.e. 96-h EC_{90}) of the targeted algae were 110, 120, 180, 200, 620, 630, 720, and 730 µg/L for *C. tumida*, *A. falcatus*, *H. pluvialis*, *P. charkowiensis*, *E. elegans*, *N. punctiforme*, *M. aeruginosa*, and *Desmidium* sp., respectively. For the animal species, the copper concentrations that elicited a 96-h LC_{50} were 4.6, 48, 250, 390, and 67,000 µg/L for *D. magna*, *C. dubia*, *P. promelas*, *H. azteca*, and *L. macrochirus*, respectively. These results indicate a range of sensitivities to copper exposures within and between algal and animal species. Based on the sensitivities of specific target algae at a site, the risks to nontarget species could be significant (i.e. low margin of safety).

Key words: algae, algaecide, copper, fish, invertebrates, risk management, toxicity.

INTRODUCTION

Copper-based algaecides, in a variety of formulations, are widely used to control problematic algal blooms in water resources (Oliveira-Filho et al. 2004). Responses of algal species to different copper formulations can vary widely depending upon both constituents in water and characteristics of the algal species (Fitzgerald 1964, Murray-Gulde et al. 2002). As data regarding the sensitivities of algal species to exposures of copper algaecides become available, informed decisions can be made regarding the potential efficacy that can be expected from field applications.

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Potential adverse effects on nontarget species are also important factors influencing a decision to use algaecides to control the growth of nuisance algae in water. Laboratory experiments with sensitive nontarget species can identify potential risks of an algaecide application. By comparing the concentration of algaecide required to control the target algal species with the concentration eliciting adverse effects on nontarget species, water resource managers can make a treatment decision with an estimate of the margin of safety (MOS) associated with an application.

Although the aqueous toxicity of copper sulfate pentahydrate has been thoroughly studied (Kosalwat and Knight 1987, Nor 1987, Flemming and Trevors 1989, Masuda and Boyd 1993), toxicities of copper algaecide formulations differ significantly (Stauber and Florence 1987, Mastin and Rodgers 2000, Murray-Gulde et al. 2002). This research evaluated a water soluble liquid, copper-based algaecide/cyanobactericide containing 5.0% copper in a weakly chelated (copper-citrate and copper gluconate chelates) formulation of copper sulfate pentahydrate. It is a U.S. Environmental Protection Agency registered algaecide (EPA Reg. No. 7364-09-8959) that can be applied at a maximum concentration of 1.0 mg copper L^{-1} (5.31 gallons acre-ft^{-1}) to control a variety of algae and cyanobacteria. This copper based algaecideis a National Sanitation Foundation (NSF) certified algaecide (ANSI-NSF 60) and can be used in potable water reservoirs, irrigation conveyance systems, ponds, lakes, canals, ditches, and laterals. No post-application water use restrictions are present on the label (Algimycin®-PWF product label; Applied Biochemists 2006, 2007).

This research focused on responses of potential target algal species as well as nontarget animal species to exposures of a copper-based algaecide. Specific objectives of this research were to: 1) measure responses of target algal species (i.e. *Ankistrodesmus falcatus*, *Cymbella tumida*, *Desmidium* sp., *Eudorina elegans*, *Haematococcus pluvialis*, *Microcystis aeruginosa*, *Nostoc punctiforme*, and *Pandorina charkowiensis*) to exposures of Algimycin®-PWF in 96-h laboratory toxicity tests; 2) review responses of nontarget animal species (i.e. *Ceriodaphnia dubia*, *Daphnia magna*, *Hyallela azteca*, *Lepomis macrochirus*, and *Pimephales promelas*) to exposures of Algimycin®-PWF in 96-h laboratory toxicity tests (Johnson et al. 2008); and 3) compare responses of target algal and nontarget animal species to Algimycin®-PWF exposures and calculate margins of safety associated with an applications.

Table 1. Description of experimental design of toxicity tests for five animal species exposed to Algimycin®-PWF (Johnson et al. 2008).

Animal species	Source of organisms	Age/Size of test organisms	Test method	Targeted initial copper concentrations as Algimycin®-PWF (µg Cu/ L)	Exposure chamber	Volume per replicate	Number of organisms tested per treatment concentration (n)
Daphnia magna	CU AARL[1]	≤24 hours	USEPA 1996b	Background, 1, 3, 5, 10, 30, 50, and 100	250 ml Beaker	200 ml	30
Ceriodaphnia dubia	CU AARL[1]	≤24 hours	Lewis et al. 1994	Background, 5, 10, 20, 30, 50, 70, 100, and 150	20 ml Vial	10 ml	10
Pimephales promelas	CU AARL[1]	≤24 hours	Lewis et al. 1994	Background, 10, 100, 200, 500, 750, 1000, 2000, and 3000	250 ml Beaker	200 ml	30
Hyalella azteca	CU AARL[1]	10 to 13 days (0.5 to 1.0 cm)	USEPA 1994	Background, 100, 200, 400, 600, 800, 1000, and 2000	250 ml Beaker	200 ml	30
Lepomis macrochirus	ARO[2]	Approx. 1.4 g (3 to 5 cm length)	USEPA 1996a	Background, 500, 1000, 5000, 10000, 15000, 20000, 40000, and 100000	38 L Tank	26 L	20

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[2]Aquatic Research Organisms (Hampton, NH 03842)

MATERIALS AND METHODS

Algal species used for these experiments were obtained from the University of Texas at Austin culture collection. All algae, with the exception of *M. aeruginosa*, were cultured in U.S. Environmental Protection Agency (USEPA) nutrient medium (Lewis et al. 1994) with decreased chelating agent, disodium ethylenediamine tetra-acetate (EDTA), to avoid copper sequestration. *Microcystis aeruginosa* was cultured in BG-11 nutrient media (Berberoglu et al. 2008). Glass beads (Sigma Chemical Co. St. Louis, MO 63178) were added to *C. tumida* growth vessels to provide a binding substrate and an essential micronutrient (Silica). Testing was initiated upon achieving sufficient densities (10^5 to 10^6 cells/ml; USEPA 1994, Franklin et al. 2000).

Animal care and testing followed standard protocols under supervision of an institutional animal care and use committee at Clemson University (an Association for Assessment and Accreditation of Laboratory Animal Care certified institution). *Lepomis macrochirus* were obtained from Aquatic Research Organisms (Hampton, NH) and held for 10 d before testing. *Pimephales promelas*, *H. azteca*, *D. magna*, and *C. dubia* were obtained from cultures at Clemson University that have been maintained over 30 years. A minimum of 20 organisms of each animal species were exposed to treatments in glass vessels of a sufficient size to eliminate potential density-mediated and water quality (i.e., dissolved oxygen, ammonia, etc.) impacts on exposures (Table 1; USEPA 1996a,b).

All organisms were cultured and tested at a temperature of 23 ± 2 C under a 16-h light/8-h dark photoperiod illuminated by cool white fluorescence lighting at an intensity of 3,100 ± 100 lux. Organisms were exposed to a range of concentrations of copper as Algimycin®-PWF in 96-h toxicity experiments (Tables 1 and 2; Lewis et al. 1994, CFR 2004, Johnson et al. 2008). Moderately hard laboratory water was used for testing of all organisms and water characteristics were measured prior to test initiation and at test conclusion according to standard methods (APHA 2005, Johnson et al. 2008).

Responses of algal species measured included cell densities and chlorophyll *a* concentrations with treatments compared to untreated controls to determine differences. Cell densities were measured using an improved Neubauer hemocytometer (Hausser Scientific Co. Horsham, PA 19044) and chlorophyll *a* was measured fluorometrically using a SpectraMax®M2 spectrophotometer (Molecular Devices Corp. Sunnyvale, CA 94089; APHA 2005). The measured responses of *L. macrochirus*, *P. promelas*, *H. azteca*, *D. magna*, and *C. dubia* were differences in mortality in treatments versus controls (Johnson et al. 2008).

Stock solutions used for exposures in these experiments were prepared less than 4 prior to experiment initiation by diluting Algimycin®-PWF (Applied Biochemists, Inc., Germantown,WI) with NANOpure™ water. Exposure solutions were prepared from the stock solutions using moderately hard laboratory water. Exposure concentrations of copper as Algimycin®-PWF for all algal species tested were: background, 100, 200, 400, 600, 800, and 1,000 mg Cu L^{-1} in an exposure volume of 200 ml. Exposure concentrations of copper as Algimycin®-PWF for the animal species

Table 2. Description of experimental design of toxicity tests for eight algal species exposed to Algimycin®-PWF.

Algal species	Identification (UTEX culture)	Targeted initial copper concentrations as Algimycin®-PWF (µg Cu/ L)	Replicates per exposure	Initial cell densities ± st. dev. (cells/ ml)	Initial chlorophyll *a* concentrations ± st. dev. (µg/ L)
Ankistrodesmus falcatus	Stock 749	Background, 0.1, 0.2, 0.4, 0.6, 0.8, 1.0	4	$1.9 \times 10^5 \pm 3.0 \times 10^4$	54 ± 6
Cymbella tumida	LB FD96	Background, 0.1, 0.2, 0.4, 0.6, 0.8, 1.0	3	$1.2 \times 10^5 \pm 2.0 \times 10^4$	19 ± 3
Haematococcus pluvialis	2505	Background, 0.1, 0.2, 0.4, 0.6, 0.8, 1.0	4	$4.0 \times 10^5 \pm 1.5 \times 10^5$	47 ± 10
Pandorina charkowiensis	LB 840	Background, 0.1, 0.2, 0.4, 0.6, 0.8, 1.0	4	$1.4 \times 10^5 \pm 1.8 \times 10^4$	122 ± 20
Eudorina elegans	LB 1210	Background, 0.1, 0.2, 0.4, 0.6, 0.8, 1.0	4	$6.5 \times 10^5 \pm 4.4 \times 10^4$	68 ± 8
Nostoc Punctiforme	LB 1833	Background, 0.1, 0.2, 0.4, 0.6, 0.8, 1.0	4	$8.5 \times 10^5 \pm 1.8 \times 10^5$	51 ± 7
Microcystis aeruginosa	LB 2385	Background, 0.1, 0.2, 0.4, 0.6, 0.8, 1.0	4	$2.8 \times 10^5 \pm 4.0 \times 10^4$	99 ± 7
Desmidium sp.	LB 612	Background, 0.1, 0.2, 0.4, 0.6, 0.8, 1.0	4	$2.3 \times 10^5 \pm 4.5 \times 10^4$	121 ± 27

differed based on their sensitivities (in house screening level experimentation defined treatments for definitive testing).

Copper concentrations in exposure solutions were verified by measuring acid-soluble copper concentrations in samples prior to experiment initiation and at experiment conclusion (APHA 2005). Copper concentrations of exposure solutions for animal species were measured using a graphite furnace atomic absorption spectrometer (Perkin-Elmer 5100 PC, Waltham, MA; APHA 2005). Copper concentrations for algal experiments were measured using Inductively Coupled Plasma- Atomic Emission Spectrometry (ICP-AES) according to standard methods (APHA 2005).

Exposure–response relationships were developed for each organism. For the animal species, lowest observed effect concentrations (LOEC) and lethal concentration values for 50% of the exposed organisms (96-h LC_{50}) were calculated by probit or trimmed Spearman-Karber analysis. Effect concentration values for 50 and 90% decrease in chlorophyll *a* (96-h EC_{50}, EC_{90}) compared to untreated controls for the algal species were calculated using regression analysis (Sigma Plot 11.0 2008). Responses of species were compared using ANOVA, with differences identified with a Tukey's post hoc test.

An important consideration for field use of algaecides is the margin of safety (MOS) for nontarget species (Murray-Gulde et al. 2002). Effective algaecide concentrations for control of algae were compared to toxicity data for nontarget species of fish and invertebrates to calculate margins of safety. For this study, a MOS was calculated as follows:

$$\text{MOS} = \frac{\text{Concentration eliciting adverse effects for nontarget organisms (96–h } LC_{50})}{\text{Effective concentration for control of algae (96–h } LC_{50})} \quad [1]$$

Thus a MOS < 1 indicates potential risks for nontarget species, while a MOS ≥ 1 indicates less potential for adverse effects on nontarget species (Table 3).

RESULTS

Measured acid-soluble copper concentrations were within 93 to 109% of target copper concentrations, therefore LOEC, LC_{50}, and EC_{90} values were calculated from the target copper concentrations. The lowest exposure concentration that was significantly different from the control was reported as the LOEC for the nontarget animal species. Nontarget animal species were not fed, or fed minimally, during testing to maximize the bioavailability of copper by decreasing available ligands (Sprague 1985, Kim et al. 1999). Exposure water (moderately hard laboratory water) characteristics remained relatively constant throughout the duration of the experiments (pH 7 ± 1.5, DO 8 ± 2 mg O_2 L^{-1}, temperature 23 ± 2 C, conductivity 130 to 350 uS cm^{-1}, alkalinity 40 to 80 mg as $CaCO_3$ L^{-1}, hardness 40 to 90 mg as $CaCO_3$/L).

All species of algae tested had a 90% decrease in chlorophyll *a* content compared to untreated controls following exposures to Algimycin®-PWF at concentrations of $\leq$ 730 µg Cu L^{-1} in 96-h toxicity tests. Chlorophyll *a* concentrations and cell densities for all algal species significantly decreased at the EC_{90} values following the 96-h exposures (Figures 1 and 2). 96-h EC_{90} values ranged from 110 µg Cu L^{-1} for *C. tumida* to 730 µg Cu L^{-1} for *Desmidium* sp. (Table 4). *Cymbella tumida* and *A. falcatus* were more susceptible to copper than *H. pluvialis* and *P. charkowiensis* and those four species were more susceptible than *E. elegans*, *N. punctiforme*, *M. aeruginosa* and *Desmidium* sp. ($\alpha = 0.05$).

In 96-h static, nonrenewal exposures of Algimycin®-PWF, *L. macrochirus* was the least sensitive animal species with an LC_{50} of 67,000 µg Cu L^{-1} followed by *H. azteca* with an LC_{50} of 390 µg L^{-1}, and *P. promelas* with an LC_{50} of 250 µg L^{-1}. *C. dubia* and *D. magna* were the most sensitive species to Algimycin®-PWF exposures with LC_{50} values of 48 µg Cu L^{-1} and 4.6 µg Cu L^{-1}, respectively (Table 5). The LOEC values were: 29,400 µg Cu L^{-1} for *L. macrochirus*, 100 µg Cu L^{-1} for *H. azteca*, 10 µg Cu L^{-1} for *P. promelas*, 15 µg Cu L^{-1} for *C. dubia*, and 1 µg Cu L^{-1} for *D. magna* (Table 5).

DISCUSSION

To decrease ambiguity in experiments, water constituents remained relatively constant allowing comparative responses to copper exposures based on algal characteristics. In this study, the diatom and three green algal species were more susceptible than the blue-green algae to Algimycin®-PWF exposures. Gibson (1972) reported that a blue-green alga (*Anabaena flos-aquae*) was more sensitive than a green alga (*Scenedesmus quadricauda)* to copper sulfate exposures, though sensitivities may differ for different copper formulations or species of algae. In this study, the planktonic algal species were more susceptible to Algimycin®-PWF exposures in comparison to the three colonial algal species and filamentous algal species tested. All species of algae tested were susceptible to Algimycin®-PWF below the maximum label rate (1 mg Cu L^{-1}) with a 96-h exposure

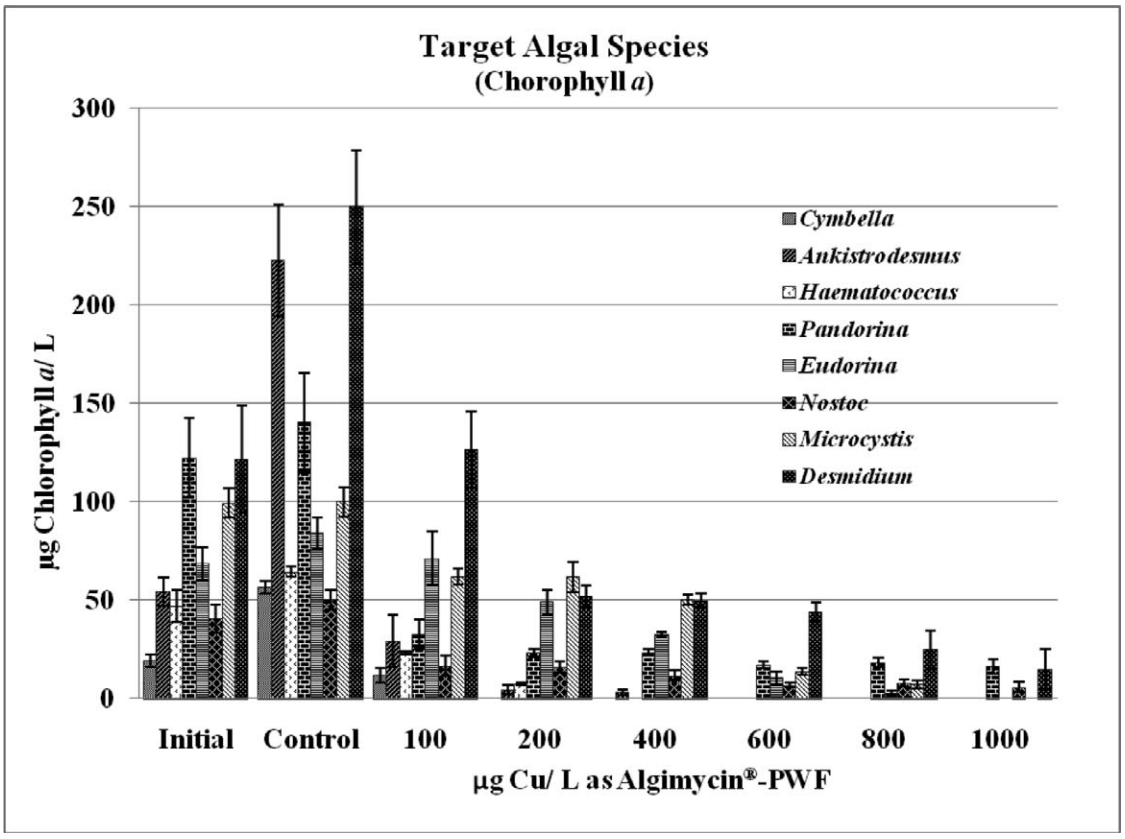

Figure 1. Responses, in terms of chlorophyll *a*, of algal species exposed to Algimycin® PWF in 96-h laboratory toxicity tests.

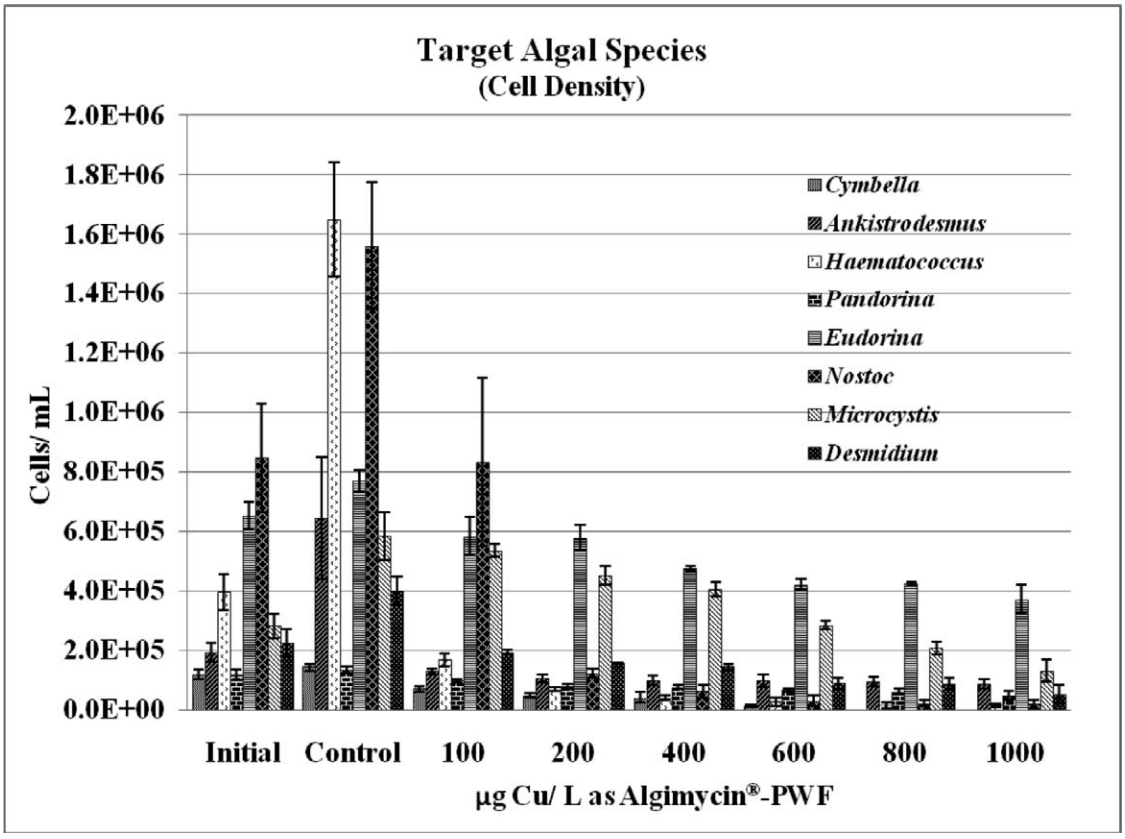

Figure 2. Responses, in terms of cell densities, of algal species exposed to Algimycin® PWF in 96-h laboratory toxicity tests.

Table 3. Margins of safety associated with Algimycin®-PWF exposures for five animal species compared with eight algal species. Margin of safety was defined as the ratio of the concentration of algaecide that adversely affects a highly sensitive nontarget animal species (96-h LC_{50} value) to the concentration required to control the growth of the algal species (EC_{90}). A MOS of ≥ 1 indicates less potential for nontarget species risks.

	Cymbella tumida	*Ankistrodesmus falcatus*	*Haematococcus pluvialis*	*Pandorina charkowiensis*	*Eudorina elegans*	*Nostoc punctiforme*	*Microcystis aeruginosa*	*Desmidium* sp.
Daphnia magna	0.04	0.04	0.03	0.02	0.007	0.007	0.006	0.006
Ceriodaphnia dubia	0.44	0.40	0.27	0.24	0.077	0.076	0.067	0.066
Pimephales promelas	2.3	2.1	1.4	1.3	0.40	0.39	0.35	0.34
Hyalella azteca	3.5	3.3	2.2	2.0	0.63	0.62	0.54	0.53
Lepomis macrochirus	609	558	372	335	108	106	93	92

duration. Other algal species and higher algal densities may possess different or altered susceptibility to Algimycin®-PWF exposures. By understanding the susceptibility of algal species to different concentrations of algaecides in laboratory exposures, there is an enhanced prediction of site-specific responses following field applications.

The animal species tested differed by orders of magnitude in their sensitivities to Algimycin®-PWF. The planktonic crustaceans (*C. dubia* and *D. magna*) were more sensitive than the fish species (*L. macrochirus* and *P. promelas*). These results are in agreement with previous studies that found *C. dubia* and *D. magna* were more sensitive to chelated copper exposures than *P. promelas* (Mastin and Rodgers 2000, Murray-Gulde et al. 2002).These laboratory data provide conservative estimates of potential responses to field exposures and require translation to specific field situations because of copper speciation and affinity. In laboratory exposures with animal species, there are typically no competing organic ligands to bind the copper applied, which would be present in applications at natural sites. These competing ligands would include the target algal species as well as other particulates and dissolved organic carbon (Playle et al. 1993, Erickson et al. 1996, Santore et al. 2001).

The primary purpose for applying a copper-based algaecide in a water resource is to control the targeted algal species, although potential risks to nontarget species should be considered prior to application (Murray-Gulde et al. 2002). Chelated copper algaecides can increase the stability of copper in the water column by decreasing the potential for precipitation as well as increase binding of the copper to algal cells (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 at the active sites on or in algal cells and consequently increase control at lower treatment concentrations. When an algaecide is applied, the target algae serve as ligands rapidly uptaking and binding the applied copper which may decrease the bioavailable fraction for some nontarget organisms in the field (Crist et al. 1990, Levy et al. 2007). Since initiation of an algaecide application often occurs in response to a large amount of algae biomass, copper sorbed to algae increases with this density and the amount available in exposures to nontarget organisms is likely decreased. Experiments in this research sought to identify the maximum potential risks for nontarget organisms by exposing them in waters with no detectable organic matter present and at a highly sensitive life stage. This provides a conservative MOS value with nontarget species risks likely "worst case" and not representative of risks observed in typical field applications of this algaecide. Translation of laboratory algaecide efficacy results to the field has been supported, upon achieving a similar exposure (Bishop and Rodgers 2011).

The susceptibility of algal and animal species to algaecide exposures can differ significantly. The copper concentrations in typical laboratory animal toxicity tests remain relatively constant and the water does not contain measurable amounts of algae or particulate matter and may subsequently overestimate response compared with a typical field situation (Sprague 1985, Kim et al. 1999). In applications of Algimycin®-PWF for many algal species the margin of safety is minimal for *P. promelas*, *H. azteca*, *C. dubia*, and *D. magna*. Therefore, use rates need to be selected based upon the minimum amount required to control the observed density of the targeted algal species (Mastin et al. 2002, Murray-Gulde et al. 2002). Risks can be further decreased or mitigated through efficient application techniques and use of efficacious exposure concentrations.

Table 4. Algimycin®-PWF 96-h EC_{50} and EC_{90} values for algal toxicity tests (µg Cu/ L) along with regression analysis equations and fit probability.

	Toxicity values (µg Cu/ L)				
Algal species	96-h EC_{50}	96-h EC_{90}	95% confidence interval (EC_{90})	Regression equation (y=)	R squared
Cymbella tumida	100	110	80–120	$56.7^{\wedge}$–16.5x	0.9981
Ankistrodesmus falcatus	50	120	100–140	$222.6^{\wedge}$–20.2x	0.9819
Haematococcus pluvialis	90	180	160–220	$64.5^{\wedge}$–10.5x	0.9978
Pandorina charkowiensis	60	200	160–320	$140.2^{\wedge}$–12.1x	0.8360
Eudorina elegans	300	620	570–690	$88.3^{\wedge}$–3.0x	0.9500
Nostoc punctiforme	40	630	460–1,200	$46.3^{\wedge}$–5.7x	0.7904
Microcystis aeruginosa	290	720	640–830	$96.3^{\wedge}$–2.6x	0.9289
Desmidium sp.	50	730	590–1,000	$202.2^{\wedge}$–4.3x	0.8487

Table 5. Algimycin®-PWF 96-h LOEC and LC_{50} values for animal toxicity tests (µg Cu/ L; Johnson et al. 2008).

	Toxicity values (µg Cu/ L)		
Animal species	LOEC	96-h LC_{50}	95% confidence interval
Daphnia magna	1	4.6	3.9–5.3
Ceriodaphnia dubia	15	48	43–53
Pimephales promelas	10	250	180–320
Hyalella azteca	100	390	300–480
Lepomis macrochirus	29,400	67,000	60,000–74,000

Understanding the potential risks from applying this copper-based product to nontarget organisms is a critical aspect of the algae management decision matrix. Future experimentation may involve exposures of both nontarget and target species simultaneously to identify copper affinity and effects, as well as to measure the amount of copper sorbed by algae.

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