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 algaecide is 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\textsuperscript{-PWF product label}; Applied Biochemicals 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\textsuperscript{-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\textsuperscript{-PWF} in 96-h laboratory toxicity tests (Johnson et al. 2008); and 3) compare responses of target algal and nontarget animal species to Algimycin\textsuperscript{-PWF} exposures and calculate margins of safety associated with an applications.
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 1. Description of Experimental Design of Toxicity Tests for Five Animal Species Exposed to Algimycin-PWF (Johnson et al. 2008).

<table>
<thead>
<tr>
<th>Animal species</th>
<th>Source of organisms</th>
<th>Age/Size of test organisms</th>
<th>Test method</th>
<th>Targeted initial copper concentrations as Algimycin-PWF (lg Cu/L)</th>
<th>Exposure chamber</th>
<th>Volume per replicate</th>
<th>Number of organisms tested per treatment concentration (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Daphnia magna</em></td>
<td>CU AARL 1/CU AARL 2</td>
<td>24 hours</td>
<td>USEPA 1994</td>
<td>Background, 1, 3, 5, 10, 30, 50, 70, 200, 400, 600, 1,000</td>
<td>250 ml Beaker</td>
<td>200 ml</td>
<td>30</td>
</tr>
<tr>
<td><em>Ceriodaphnia dubia</em></td>
<td>CU AARL 1/CU AARL 2</td>
<td>24 hours</td>
<td>Lewis et al. 1994</td>
<td>Background, 1, 3, 5, 10, 30, 50, 70, 200, 400, 600, 1,000</td>
<td>20 ml Vial</td>
<td>10 ml</td>
<td>10</td>
</tr>
<tr>
<td><em>Pimephales promelas</em></td>
<td>CU AARL 1/CU AARL 2</td>
<td>24 hours</td>
<td>Lewis et al. 1994</td>
<td>Background, 1, 3, 5, 10, 30, 50, 70, 200, 400, 600, 1,000</td>
<td>250 ml Beaker</td>
<td>200 ml</td>
<td>30</td>
</tr>
<tr>
<td><em>Hyalella azteca</em></td>
<td>CU AARL 1</td>
<td>10 to 13 days</td>
<td>Lewis et al. 1994</td>
<td>Background, 1, 3, 5, 10, 30, 50, 70, 200, 400, 600, 1,000</td>
<td>250 ml Beaker</td>
<td>200 ml</td>
<td>30</td>
</tr>
<tr>
<td><em>Lepomis macrochirus</em></td>
<td>ARO2</td>
<td>Approx. 1.4 g</td>
<td>USEPA 1994</td>
<td>Background, 500, 1000, 5000, 10,000, 20,000, 40,000, 100,000</td>
<td>38 L Tank</td>
<td>26 L</td>
<td>20</td>
</tr>
</tbody>
</table>

1. Clemson University Aquatic Animal Research Laboratory
2. Aquatic Research Organisms (Hampton, NH 03842)
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). Copper concentrations of exposure solutions prior to experiment initiation and at experiment duration of the experiments (pH 7 ± 1.5, DO 8 ± 2 mg O₂ L⁻¹, temperature 23 ± 2 °C, conductivity 130 to 350 μS cm⁻¹, alkalinity 40 to 80 mg as CaCO₃ L⁻¹, hardness 40 to 90 mg as CaCO₃/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 ≤ 730 μg Cu L⁻¹ in 96-h toxicity tests. Chlorophyll a concentrations and cell densities for all algal species significantly decreased at the EC₉₀ values following the 96-h exposures (Figures 1 and 2). 96-h EC₉₀ 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. charkwienensis and those four species were more susceptible than E. elegans, N. punctiforme, M. aeruginosa and Desmidium sp. (α = 0.05).

In 96-h static, nonrenewal exposures of Algimycin™-PWF, L. macrochirus was the least sensitive animal species with an LC₅₀ of 67,000 μg Cu L⁻¹ followed by H. azteca with an LC₅₀ of 390 μg L⁻¹, and P. promelas with an LC₅₀ of 250 μg L⁻¹. C. dubia and D. magna were the most sensitive species to Algimycin™-PWF exposures with LC₅₀ values of 18 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.
durations. 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 algicides 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 algicide 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 algicides 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 Trewors 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 algicides 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 algicide 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 algicide 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 algicide. Translation of laboratory algicide 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 algicide 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 3. Margins of Safety Associated with Algimycin®-PWF Exposures for Five Animal Species Compared with Eight Algal Species. Margins of Safety Were Defined as the Ratio of the Concentration of Algicide That Adversely Affects a Highly Sensitive Nontarget Animal Species (96-h LC50 Value) to the Concentration Required to Control the Growth of the Algal Species (EC90). A MOS of ≥ 1 Indicates Less Potential for Nontarget Species Risks.

<table>
<thead>
<tr>
<th>Algal species</th>
<th>C. tumida</th>
<th>Anostomus falcatus</th>
<th>H. azteca</th>
<th>P. promelas</th>
<th>D. magna</th>
<th>C. dubia</th>
<th>Nostoc punctiforme</th>
<th>Microcystis aeruginosa</th>
<th>Desmidium sp.</th>
</tr>
</thead>
<tbody>
<tr>
<td>96-h LC50 (%)</td>
<td>0.04</td>
<td>0.04</td>
<td>0.03</td>
<td>0.02</td>
<td>0.007</td>
<td>0.007</td>
<td>0.007</td>
<td>0.006</td>
<td>0.006</td>
</tr>
<tr>
<td>96-h LC90 (%)</td>
<td>0.04</td>
<td>0.04</td>
<td>0.03</td>
<td>0.02</td>
<td>0.007</td>
<td>0.007</td>
<td>0.007</td>
<td>0.006</td>
<td>0.006</td>
</tr>
</tbody>
</table>

### Table 4. Algimycin®-PWF 96-h EC50 and EC90 Values for Algal Toxicity Tests (μg Cu/L) Along with Regression Analysis Equations and Fit Probability.

<table>
<thead>
<tr>
<th>Algal species</th>
<th>96-h EC50 (μg Cu/L)</th>
<th>96-h EC90 (μg Cu/L)</th>
<th>95% confidence interval (EC90)</th>
<th>Regression equation (y=)</th>
<th>R squared</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cymbella tumida</td>
<td>100</td>
<td>110</td>
<td>80–120</td>
<td>56.7–16.5x</td>
<td>0.9981</td>
</tr>
<tr>
<td>Anostomus falcatus</td>
<td>50</td>
<td>120</td>
<td>100–140</td>
<td>222.6–20.2x</td>
<td>0.9819</td>
</tr>
<tr>
<td>H. azteca</td>
<td>90</td>
<td>180</td>
<td>160–220</td>
<td>64.5–10.5x</td>
<td>0.9978</td>
</tr>
<tr>
<td>P. promelas</td>
<td>60</td>
<td>200</td>
<td>160–320</td>
<td>140.2–12.1x</td>
<td>0.8360</td>
</tr>
<tr>
<td>Nostoc punctiforme</td>
<td>300</td>
<td>620</td>
<td>570–690</td>
<td>88.3–3.5x</td>
<td>0.9500</td>
</tr>
<tr>
<td>M. aeruginosa</td>
<td>40</td>
<td>630</td>
<td>460–1,200</td>
<td>46.3–5.7x</td>
<td>0.7094</td>
</tr>
<tr>
<td>Desmidium sp.</td>
<td>290</td>
<td>720</td>
<td>640–830</td>
<td>96.3–2.6x</td>
<td>0.9289</td>
</tr>
</tbody>
</table>

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TABLE 5. Algimycin-PWF 96-h LOEC and LC50 VALUES FOR ANIMAL TOXICITY TESTS (µg Cu/L; Johnson et al. 2008).

<table>
<thead>
<tr>
<th>Animal species</th>
<th>LOEC  (µg Cu/L)</th>
<th>96-h LC50 (µg Cu/L)</th>
<th>95% confidence interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>Daphnia magna</td>
<td>1</td>
<td>4.6</td>
<td>3.9–5.3</td>
</tr>
<tr>
<td>Ceriodaphnia dubia</td>
<td>15</td>
<td>48</td>
<td>45–53</td>
</tr>
<tr>
<td>Pimephales promelas</td>
<td>10</td>
<td>250</td>
<td>180–320</td>
</tr>
<tr>
<td>Hyalella azteca</td>
<td>100</td>
<td>390</td>
<td>300–480</td>
</tr>
<tr>
<td>Lepomis macrochirus</td>
<td>29,400</td>
<td>67,000</td>
<td>60,000–74,000</td>
</tr>
</tbody>
</table>

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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LITERATURE CITED


