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**Toxicity of Candidate Molluscicides to Zebra Mussels
(*Dreissena polymorpha*) and Selected Nontarget Organisms**

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ABSTRACT. Many compounds have been marketed for control of zebra mussels (*Dreissena polymorpha*), but most compounds lack comparable toxicity data and have not been tested on nontarget organisms. We tested the toxicity of 18 chemicals to two sizes of zebra mussels, two nontarget fish (rainbow trout, *Oncorhynchus mykiss* and channel catfish, *Ictalurus punctatus*), and a unionid mussel (threehorn wartyback, *Obliquaria reflexa*) under standard conditions. Organisms were exposed to the chemicals for 48 h in "soft" reference water (pH 7.7, alkalinity 6×10^{-4} m/L (30 mg/L) as CaCO_3 , and total hardness 40 mg/L as CaCO_3) at 17°C. Zebra mussels and unionid mussels were held in untreated reference water for another 48 h after exposure to measure delayed mortality. The LC_{50} values and 95% confidence intervals were compared among test organisms. Potassium chloride, Bayluscide (a registered molluscicide), and Clamtrol CT-1 (a polyquaternary ammonium compound) were the most selective chemicals tested against zebra mussels. They were two to three times more toxic to zebra mussels than to the nontarget species. Most of the remaining chemicals lacked the desired toxicity or were more toxic to fish than to zebra mussels.

Index Words: Zebra mussel, molluscicides.

INTRODUCTION

Since introduction of the zebra mussel (*Dreissena polymorpha*) into Lake St. Clair in 1985, it has spread rapidly throughout the Great Lakes and into major rivers in the East, Midwest, and Southeast (*Dreissena polymorpha* Information Review 1992). The organisms have caused many economic, environmental, and ecological problems in North America. At present, chlorine is the chemical of choice for controlling zebra mussels in industrial and municipal water facilities in the United States and Europe (Greenshields and Ridley 1957, Jenner 1985, Claudi and Evans 1993). However, chlorine is a nonselective oxidizing agent and can form hazardous byproducts (e.g., trihalomethanes).

Concerns about the continued use of chlorine have prompted research into alternative chemicals

that are environmentally safer and more selective than chlorine. Economic considerations for a chlorine replacement include cost, availability, and potential for registration by the U.S. Environmental Protection Agency for aquatic use. A variety of compounds have been evaluated and marketed for use as molluscicides in zebra mussel control, including oxidizing agents, nonoxidizing biocides, heavy metals, and organic acids (Nalepa and Schloesser 1993). The available toxicity information for most of these chemicals is not comparable because the test procedures and conditions varied with the specific objectives of the laboratory or agency. Furthermore, the toxicity to nontarget organisms has been given minimal consideration during evaluations of chemicals for industrial use.

The objective of this study was to compare the toxi-

city of candidate chemicals to zebra mussels with toxicity to nontarget mussels and fish under standard test conditions. Data produced in our tests will be used to rank the chemicals for further evaluation based on efficacy and the effects on nontarget organisms.

MATERIALS AND METHODS

Eighteen chemicals (Table 1) were tested for their toxicity to two size classes of the zebra mussel, a unionid mussel (threehorn wartyback, *Obli-*

TABLE 1. Candidate molluscicides for zebra mussel control.

Common or Trade Name (Source)	Chemical Name (Compound Class)	% Active Ingredient
Antimycin (Aquabiotics)	isovaleric acid, 8-ester wityh 3-formamido-N-(7-hexyl-3-yl) salicylamide	98
Bayluscide (Mobay Corp.)	2,5'-dichloro-4'-nitro-salicylanilide	70
Baythroid (Mobay Corp.)	cyano(4-fluoro-e-phenoxy phenyl) methyl 3-(2,2-dichloroetheny)-2,2'-dimethyl-cyclopropanecar-carboxylate (pyrethrum)	93
Bulab 6002 (Buckman)	poly[oxyethylene-(dimethyl-iminio) ethylene-(dimethyl-iminio) ethylene dichloride] (polyquaternary ammonium)	60
Bulab 6009 (Buckman)	2-(thiocyanomethylthio) benzothiazole	30
Calgon H-130 (Calgon Corp.)	didecyl dimethyl ammonium chloride (polyquaternary ammonium)	50
Calgon DMDACC (Calgon Corp.)	poly(dimethyl diallyl ammonium chloride) (polyquaternary ammonium)	100
Clamtrol CT-1 (Betz Chemical)	n-alkyl dimethyl benzyl ammonium chloride and dodecylguanidine hydrochloride (polyquaternary ammonium)	13
Copper sulfate (J. T. Baker)	cupric sulfate, 5-hydrate (CuSO ₄)	100
Endod (H. Lee, University of Toledo)	mixture of polycyclic aglycone (sapogenin) isolated from <i>Phytolacca dodecandra</i>	NR
Juglone (Aldrich Chem. Co.)	5-hydroxy-1,4-naphthoquinone	98
KML V2 (KML Inc.)	chitosan	2
KML V54 (KML Inc.)	chitosan	5.4
Noxfish (Penick Corp.)	1,2,12,12a-tetrahydro-2-isopropenyl 8,9-dimethoxy-[1]benzopyrano-[3,4]furo [2,3-b] [1] benzo pyran-6 (6aH) one (rotenone)	5
Potassium chloride (Fisher Scientific)	potassium chloride (KCl)	99
Potassium permanganate (Pfaltz & Bauer)	potassium permanganate (KMnO ₄)	100
Salicylanilide I (Aldrich Chem. Co.)	2-hydroxy-N-phenylbenzamide (salicylanilide)	100
TFM (Hoescht)	3-trifluoromethyl-4-nitrophenol	36

NR - Not reported by source.

quaria reflexa), and two fish species (rainbow trout, *Oncorhynchus mykiss*, and channel catfish, *Ictalurus punctatus*). Chemicals were selected based on previous applications for water treatment or for control of other aquatic organisms. The list included registered fishery chemicals, commercial biocides, and compounds recently reported as probable molluscicides. Tests with zebra mussels were conducted at Ohio State University in Columbus, Ohio; tests with fishes and unionid mussels were conducted at the National Fisheries Research Center in La Crosse, Wisconsin.

Test Organisms

Zebra Mussels

Adult zebra mussels were collected by divers from Lake Erie near South Bass Island, Put-in-Bay, Ohio. Mussels were held at 10°C in 2000-L aquaria containing dechlorinated municipal water (pH 7.7, alkalinity 1.6×10^{-3} m/L (80 mg/L as CaCO₃), hardness 120 mg/L as CaCO₃) that was constantly aerated and filtered through carbon. Zebra mussels were fed a daily diet of Tetra-Min® (Tetra Sales, U.S.A., Bladesburg, VA) fish food. Before exposures, two size classes of adult zebra mussels (5–8 mm and 20–25 mm shell length) from the stock culture were placed in glass petri dishes and allowed to attach. The petri dishes were preconditioned in aquaria containing test water for 2–3 d to promote the growth of a biofilm that presumably facilitates attachment of the mussels' byssal threads (Claudi and Evans 1993). Mussels that did not attach to the dishes within 24 h were considered unsuitable for testing and were discarded. Zebra mussels were exposed in 3.8-L glass jars containing 2.5 L test water without aeration.

Native Mussels and Fish

Adult unionid mussels (20–45 mm length) were collected, by snorkeling and diving, from Navigation Pool 7 of the Mississippi River. Mussels were maintained at 12°C in flowing well water [pH 7.9, alkalinity 2.14×10^{-3} m/L (107 mg/L as CaCO₃), hardness 134 mg/L as CaCO₃] on a sand substrate and fed a daily diet of Microfeast® (Zeigler Brothers, Inc., Gardners, PA) and live green algae (*Ankistrodesmus* and *Scenedesmus*). Mussels were

held for at least 1 week before testing to monitor handling mortality.

Fish (0.8–1.2 g) were obtained from the National Fisheries Research Center–La Crosse and maintained in 5,000-L fiberglass tanks supplied with flowing well water and fed *ad libitum* a daily diet of Silver Cup® (Sterling Nelson and Sons, Murray, UT). Fish were taken off feed 4 d before tests. Unionid mussels and fish were exposed in 20-L glass jars containing 15 L of test water without aeration.

Toxicity Testing

Static tests were conducted following procedures of the Committee on Methods for Acute Toxicity Testing with Aquatic Organisms (1975). Chemical concentrations were based on the active ingredient present in the formulation. Test water was reconstituted "soft" water of pH 7.7, alkalinity 6×10^{-4} m/L (30 mg/L as CaCO₃), and total hardness 40 mg/L as CaCO₃. Tests were conducted at 17°C in a constant temperature water bath or in an environmental chamber. Animals were acclimated to the test temperature by increasing water temperature 3°C in a 24-h period. Dissolved oxygen, pH, and temperature were measured daily in control, low, middle, and the highest exposure concentrations. Total alkalinity and hardness of the exposure water were measured at the beginning of each test. Tests were rejected if oxygen levels fell below 60% saturation or the control mortality exceeded 10%. Treatments of 10 animals per vessel were duplicated for unionid mussels and fish, and triplicated for zebra mussels. Exposures were 48 h for all tests. Mortality was recorded at 48 h and was defined for bivalves as failure to respond to a blunt probe and for fish as lack of gill movement. Zebra mussels and threhorn mussels clamped the valves in response to some chemicals producing highly variable mortality counts at 48 h. Therefore, mussels were held in untreated water for 48 h after exposure to measure delayed mortality.

Statistical Analyses

The LC₅₀ (lethal concentrations producing 50% mortality) values and 95% confidence intervals were calculated using primarily the probit methods of Spearman-Kärber (Hamilton *et al.* 1977). The LC₅₀ values for seven of the fish tests (Table 2)

¹Mention of brand names does not constitute endorsement by the U.S. Government.

TABLE 2. 48-h exposure toxicity of candidate molluscicides to zebra mussels and nontarget fish and mussels. Concentrations are based on the percent active ingredient of the formulation.

Compound	48-h LC ₅₀ and 95% confidence interval (mg/L)				
	Zebra Mussel		Rainbow Trout	Channel Catfish	Threehorn Wartyback
	20-25 mm	5-8 mm	0.8-1.2 g	0.8-1.2 g	30-50 mm
Antimycin	>1 ^a	>1 ^a	0.00003 ^b 0.00002-0.00004	0.00460 ^b 0.00390-0.00530	>1.0 ^a
Bayluscide	0.017 0.015-0.018	0.015 0.014-0.017	0.047 0.043-0.051	0.043 0.045-0.047	0.051 0.043-0.061
Baythroid	>100 ^a	>100 ^a	0.00057 0.00051-0.00065	0.00200 0.0019-0.00250	>10 ^a
Bulab 6002	>60 ^a	>60 ^a	0.044 ^b 0.041-0.048	3.350 ^b 2.820-3.960	>60 ^a
Bulab 6009	>15 ^a	>15 ^a	0.021 ^b 0.018-0.026	0.049 ^b 0.037-0.065	>15 ^a
Calgon H-130	0.85 0.64-1.14	1.12 0.85-1.48	0.75 0.71-0.79	0.71 0.69-0.73	6.12 4.85-7.73
Calgon DMDACC	>50 ^a	>50 ^a	0.64 0.53-0.77	1.90 1.58-2.28	>50 ^a
Clamtrol CT-1	0.738 0.577-0.944	0.290 0.229-0.369	2.120 ^b 1.610-2.800	0.830 ^b 0.750-0.920	20.877 16.681-26.103
CuSO ₄	5.38 3.65-7.93	>40 ^a	0.042 0.034-0.052	0.733 0.622-0.865	>20 ^a
Endod	>10 ^a	9.51 8.50-10.65	1.31 1.12-1.53	1.60 1.23-2.08	>30 ^a
Juglone	5.800 3.830-8.800	>10 ^a	0.024 0.025-0.027	0.015 0.013-0.017	>20 ^a
KML V2	>100 ^a	>100 ^a	0.38 0.33-0.43	0.37 0.35-0.40	>100 ^a
KML V54	>100 ^a	>100 ^a	0.50 ^b 0.41-0.62	0.92 ^b 0.26-3.19	>100 ^a
Noxfish (rotenone)	0.219 0.131-0.365	0.165 0.147-0.185	0.0020 0.0018-0.0023	0.0073 0.0060-0.0080	>1.0 ^a
KCl	150 129-175	147 132-163	1,610 ^b 1,223-2,119	720 ^b 588-882	>2,000 ^a
KMnO ₄	>40 ^a	>40 ^a	1.86 1.61-2.14	1.59 1.29-1.95	>100 ^a
Salicylanilide I	0.0370 0.0300-0.0460	0.0200 0.0170-0.0230	0.0055 0.0048-0.0064	0.0042 0.0037-0.0048	>0.1 ^a
TFM	2.37 1.85-3.05	2.08 1.23-3.55	1.85 1.58-2.16	1.40 1.25-1.56	1.87 1.71-2.06

^aLess than 50% mortality in the highest test concentration.

^bLC₅₀s were computed using the methods of Litchfield and Wilcoxon (1949).

were calculated using the methods of Litchfield and Wilcoxon (1949) because the Spearman-Kärber method failed to calculate 95% confidence intervals for these data. The 48-h exposure LC₅₀ values were calculated for all species (Table 2) and 48-h post-exposure LC₅₀ values were calculated for unionid and zebra mussels (Table 3). The LC₅₀ values were considered significantly different when the 95% confidence intervals did not overlap.

A selectivity ratio was calculated for each compound by dividing the LC₅₀ value of the most sensitive nontarget organism by the 48 h post-exposure LC₅₀ value of the least sensitive size of zebra mussels (Table 4). The 48 h LC₅₀ value generally underestimated final mussel mortality because of the valve clamping response. Therefore, the 48 h post-exposure LC₅₀ value was considered the more accurate estimate of mortality for mussels.

TABLE 3. 48-h post-exposure toxicity of candidate molluscicides to zebra mussels and threhorn mussels. Concentrations are based on the percent active ingredient of the formulation.

Compound	48-h post-exposure LC ₅₀ and 95% confidence interval (mg/L)		Threhorn Wartyback 30-50 mm
	Zebra Mussel		
	20-25 mm	5-8 mm	
Antimycin	2.34 1.55-3.52	4.93 2.00-12.17	>1.0 ^a
Bayluscide	0.0197 0.0181-0.0215	0.0153 0.0138-0.0169	0.0445 0.0407-0.0488
Baythroid	>100 ^a	>100 ^a	>10 ^a
Bulab 6002	>60 ^a	>60 ^a	>60 ^a
Bulab 6009	>15 ^a	>15 ^a	>15 ^a
Calgon H-130	0.38 0.33-0.44	0.59 0.50-0.70	3.72 2.73-5.06
Calgon DMDACC	>50 ^a	>50 ^a	>50 ^a
Clamtrol CT-1	0.339 0.293-0.391	0.144 0.092-0.220	7.700 6.620-8.940
CuSO ₄	2.54 1.16-4.01	2.07 1.00-4.32	11.76 7.86-17.59
Endod	7.58 7.14-8.06	7.06 6.45-7.72	17.96 14.31-22.23
Juglone	4.30 3.27-5.66	7.77 5.92-10.20	8.40 7.40-9.52
KML V2	>100 ^a	>100 ^a	>100 ^a
KML V54	>100 ^a	>100 ^a	>100 ^a
Noxfish (rotenone)	0.228 0.157-0.329	0.149 0.129-0.172	0.518 0.421-0.636
KCl	150 129-175	147 132-163	>2,000 ^a
KMnO ₄	>40 ^a	>40 ^a	>100 ^a
Sal I	0.0270 0.0220-0.0330	0.0170 0.0150-0.0190	0.0712 0.0599-0.0846
TFM	2.37 1.85-3.05	2.08 1.23-3.55	1.87 1.71-2.06

^aLess than 50% mortality in the highest test concentration.

TABLE 4. Selectivity ratios of candidate molluscicides, calculated as lowest nontarget LC_{50} value divided by highest zebra mussel 48 h post-exposure LC_{50} value.

Compound	Selectivity ratio
Potassium chloride	4.8
Clamtrol CT-1	2.45
Bayluscide	2.18
Calgon H-130	1.20
TFM	0.59
Endod	0.17
Salicylanilide I	0.16
Copper sulfate	0.017
Noxfish	0.009
Juglone	0.002
Antimycin	<0.0001

RESULTS

Zebra Mussels

The 18 chemicals were divided into three groups based on their molluscicidal activity. The chemicals with the greatest toxicity to zebra mussels ($LC_{50} \leq 1$ mg/L; Tables 2 and 3) ranked as follows: Bayluscide > Salicylanilide I (Sal I) > Noxfish > Clamtrol CT-1® > Calgon H-130®. Chemicals that were intermediate in toxicity (LC_{50} 1–150 mg/L; Tables 2 and 3) were TFM > copper sulfate > antimycin > Juglone > Endod > potassium chloride. Potassium chloride was about 20 times less toxic to zebra mussels than other chemicals in this group. The third group contained chemicals that caused insufficient mortality of the test organisms to calculate an LC_{50} value (i.e., Buckman Bulab 6002®, Buckman Bulab 6009®, Calgon DMDACC®, KML V2®, KML V54®, and potassium permanganate; Tables 2 and 3) or that were insoluble in water at concentrations above 100 mg/L (Baythroid).

We did not find a distinct size effect in the toxicity of chemicals to zebra mussels. The LC_{50} values for six chemicals were significantly different among mussel size classes, but four were more toxic to small mussels, and two were more toxic to large mussels.

Delayed mortality of mussels occurred in seven of the compounds (Calgon H-130, Clamtrol CT-1, copper sulfate, Endod, Juglone, Noxfish, and Sal I) and resulted in lower LC_{50} values at 48 h post-exposure (Table 3).

Native Mussels and Fish

Threehorn mussels were generally less sensitive to the candidate chemicals than zebra mussels. The

most toxic chemicals to the threehorn mussel were Bayluscide, Sal I, and Noxfish (LC_{50} values < 1 mg/L; Tables 2 and 3). Chemicals with LC_{50} values ranging from 1 to 10 mg/L included Calgon H-130, Clamtrol CT-1, Juglone, and TFM. Endod and copper sulfate produced LC_{50} values between 10 and 20 mg/L. An LC_{50} value could not be estimated for antimycin, Bulab 6002, Bulab 6009, Calgon DM-DACC, KML V2, KML V54, potassium chloride, and potassium permanganate due to insufficient mortality of the test organisms. Baythroid was insoluble in water at the concentrations tested. Delayed mortality of threehorn mussels occurred in eight of the compounds (Bayluscide and those listed above for zebra mussels) and resulted in lower LC_{50} values at 48 h post-exposure (Table 3).

The toxicity of the candidate chemicals was generally greater to fish than to threehorn or zebra mussels. Rainbow trout and channel catfish were significantly more sensitive than zebra mussels to all of the chemicals tested except Bayluscide, potassium chloride, Calgon H-130, Clamtrol CT-1, and TFM (Table 2). The LC_{50} values of Calgon H-130 and TFM were similar for all test species. Bayluscide, Clamtrol CT-1, and potassium chloride were the only compounds that were more toxic to zebra mussels than to rainbow trout and channel catfish.

DISCUSSION

Bayluscide was the most toxic chemical to zebra mussels; it was 2.2 to 2.8 times more selective for zebra mussels than for the next most sensitive organism tested (Table 4). Clamtrol CT-1 was less toxic, but it demonstrated greater sensitivity for zebra mussels than Bayluscide (Table 4). Clamtrol CT-1 was 2.4 to 5.8 times more toxic to zebra mussels than to the next most sensitive organism tested but was 22.7 to 53.5 times more toxic to zebra mussels than to the most resistant species tested.

Clamtrol CT-1 offers several advantages relative to Bayluscide; it shows greater selectivity for zebra mussels, particularly over threehorn mussels, and is effective at a concentration (1.0 mg/L) that is suitable for use in a control program. However, Bayluscide is effective at concentrations well below 1.0 mg/L, and it has already been registered once by the U.S. Environmental Protection Agency. The cost of reregistration for Bayluscide would probably be much less than the initial registration of Clamtrol CT-1.

The other chemicals with LC_{50} values around 1 mg/L lacked the desired selectivity. Calgon H-130

and TFM were similarly toxic to zebra mussels and fish, whereas Noxfish and Sal I were three to four times more toxic to fish than to zebra mussels (Table 4). Potassium chloride yielded the desired selectivity but was toxic to zebra mussels only at very high concentrations. Potassium chloride treatments at these concentrations (>100 mg/L) would cost three to four times more than chlorine treatment, and therefore, be economically unfeasible.

The seven chemicals for which an LC₅₀ value was not obtained could not be ranked; further evaluation of these chemicals may be warranted under different exposure conditions. For example, Martin *et al.* (1993) and McMahon *et al.* (1993) reported Bulab 6002 and 6009 were toxic to zebra mussels at 1–4 mg/L in exposures greater than 100 h. Use of these chemicals may be feasible in a closed system, such as an industrial or municipal water intake.

Persistent chemicals or compounds, such as chlorine, that form hazardous byproducts are poor choices for environmental applications. Substantial information exists on the degradation and byproducts of the two lampricides, Bayluscide and TFM (NRCC 1985); TFM is significantly degraded by photolysis in the aquatic environment (Carey and Fox 1981). Bayluscide is strongly adsorbed to sediment and rapidly degraded (NRCC 1985, Ho and Gloss 1987). Salicylanilide I is structurally similar to Bayluscide, and we expect its chemical degradation to be similar. The plant molluscicide, endod, reportedly loses toxicity in 1 to 2 days and rapidly biodegrades (Lee *et al.* 1993). The nonoxidizing biocides, Clamtrol CT-1 and Calgon H-130, can be adsorbed onto substrates, such as bentonite clay, and degraded microbially to nontoxic products (Barton 1993; L. A. Lyons, Betz Laboratories, Inc., unpublished data). However, the fate of bentonite-molluscicide complexes has not been adequately investigated, and difficulties in mixing the clay with water could prevent efficient detoxification (Bargar 1991).

Selective formulation and judicious application of a chemical can reduce the risk to nontarget organisms and decrease the amount and expense of treatment. For example, Bayluscide is available as a 5% granule that is designed to release the chemical at the sediment-water interface. There have also been experimental formulations of TFM to target lamprey larvae in benthic areas of lakes (T.D. Bills, U. S. Fish and Wildlife Service, personal communication, April 1993). Selective treatment of adult zebra mussels could be similarly enhanced by localized bottom releases of a toxicant because the mus-

sels are sedentary, epifaunal, and gregarious. Chemicals demonstrating the desired selectivity, such as potassium chloride, may require more cost-effective delivery methods to make them competitive with chlorine.

We tested only adult mussels, but chemical control is essential for all life stages of the zebra mussel. Targeting veligers may be the most efficient strategy for preventing colonization. Although veligers are assumed to be more sensitive to chemical stress, information in the literature suggests that differences in sensitivity among life stages are chemical specific (S. W. Fisher, unpublished data; McMahon *et al.* 1993; Klerks *et al.* 1993; van Benschoten *et al.* 1993). Research involving early stages will be imperative in future evaluations of candidate chemicals.

The use of chemicals should not be the foremost solution for zebra mussel control, but a selective toxicant that targets the mussel by mode of action and method of application can significantly reduce the hazard to nontarget species. The toxicants currently in use or being field tested have yet to show specific effectiveness against zebra mussels. The selectivity of these compounds needs to be evaluated on a wider range of nontarget species across various life stages. Control strategies and environmental concerns will vary for an industrial intake pipe, a fish hatchery, or a navigation lock. A single chemical will probably not be applicable for all situations; hence, we must continue to investigate other control options and develop an integrated approach to zebra mussel control.

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