

METHODS FOR EVALUATING ZEBRA MUSSEL CONTROL PRODUCTS IN LABORATORY AND FIELD STUDIES

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ABSTRACT The zebra mussel, *Dreissena polymorpha*, is causing great economic hardship among industries located on Lake Erie because of its ability to colonize water intakes and thereby impede water flow. The zebra mussel has also impacted commercial fishing operations by filtering massive quantities of phytoplankton which would otherwise be available to support the growth of game fish. Currently there is great interest in devising methods to control the mussel in Lake Erie and in designing control programs to address a spectrum of diverse needs.

Critical to providing the information to support control programs is the generation of data that are of high quality and which were derived through the use of standardized methods and are thus comparable. In addition, methods must be available to screen candidate control agents in the laboratory as well as the field.

We report herein methods for measuring the acute toxicity of molluscicides to zebra mussel adults. In addition, a protocol for assessing efficacy of antibiofouling paints in the field is presented. Using these methods, a variety of molluscicidal and molluscistatic products were evaluated for their activity against zebra mussels. In general, the data indicate that there is a potentially large number of chemicals which will kill or repel the zebra mussel.

KEY WORDS: zebra mussel, *Dreissena*, molluscicide, molluscistat, standard protocols

INTRODUCTION

The zebra mussel, *Dreissena polymorpha*, is a Nonindigenous bivalve whose biofouling activity is causing profound economic hardship in the Great Lakes. Since the mussel was first introduced in North America, ostensibly in the ballast water from an ocean-going vessel (Herbert et al. 1989, Mackie et al. 1989), the mussel has spread to all of the Great Lakes (Roberts 1990) and has reached densities in Lake Erie in excess of 70,000 per square meter (McMahon and Tsou 1990). The mussel actively colonizes pipes and other hard substrates by secreting byssal threads with which it attaches to the surface. Frequently, the zebra mussels accumulate in masses sufficient to reduce water flow to critically low levels (McMahon et al. 1990). In addition, an individual mussel has the ability to filter a volume of one liter of water per day. In so doing, the mussels collectively remove huge amounts of phytoplankton from the water thus reducing zooplankton abundance and depriving juvenile fish of an important food source. The U.S. Fish and Wildlife Service has estimated that 3.7 billion dollars in commercial fishing revenues will be lost due to the zebra mussel (Mackie et al. 1989).

The severity of the zebra mussel problem has prompted considerable interest in devising methods to control it. At present, a variety of chemical control agents which include surfactants (McMahon and Tsou 1990), heavy metals (Dudnikov and Mikheev 1968), chlorine (Jenner 1984, Morton 1969), ozone (Jenner and Janssen-Mommen 1989) and ammonium nitrate (Shetsova et al. 1978) are being considered as zebra mussel control agents. While all methods are potentially important, it is readily apparent that no single method will satisfy the diverse array of needs for zebra

mussel control. Approaches to controlling the zebra mussel must mirror the diversity of settings in which the mussels can be found. In all cases, standardized methods must be available to provide a systematic approach to screening candidate chemicals for a variety of potential uses.

Of the various control methods available, two general approaches to chemical control appear viable. The first is the use of molluscicides to kill the mussels. A diverse arsenal of molluscicides has been developed in the Soviet Union and Europe in an effort to control zebra mussels (McMahon and Tsou 1990). In North America, compounds developed to control the biofouling Asiatic clam, *Corbicula fluminea* may also be effective against zebra mussels (McMahon and Tsou 1990). The second chemical control strategy is to develop molluscistatic products which do not kill the mussel but deter attachment. Chemicals which are irritating enough to deter attachment may be useful in preventing secondary infestation once the primary infestation has been removed. Importantly, both molluscicidal and molluscistatic chemicals could be incorporated into paints to protect solid supports from zebra mussel attachment.

We describe herein protocols for measuring the acute toxicity of molluscicides to adults which take into consideration the biology of the mussel. In addition, we describe methods for evaluating the efficacy of molluscicidal and molluscistatic antibiofouling paints. Using these methods, we have evaluated the effectiveness of a variety of chemicals against the zebra mussel in both laboratory and field settings.

MATERIALS AND METHODS

Measurement of Acute Toxicity in Adult Dreissena

Adult zebra mussels were obtained by scuba divers from the bottom of Lake Erie. Adults were maintained in Lake Erie water

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with oxygenation during transport back to the laboratory. In the lab, adult *Dreissena* were maintained in 55 gallon aquaria filled with aged tap water from which chlorine had dissipated. Approximately 2,000-3,030 mussels were maintained in each aquarium under full aeration at 20°C. Cultures were maintained for three-four months by feeding the colony every other day a slurry of pelleted *Chlorella* suspended in 100 mL of water which was then frozen. Frozen *Chlorella* cubes were suspended over the aquaria; the water containing the alga dripped slowly into the water as the cube melted; *Chlorella* was removed from water by the filtering action of the mussels. The water in which the mussels were housed was checked daily for accumulation of ammonia and was replaced completely every 2 days to prevent ammonia intoxication. Culture water was removed from the aquaria and treated with .3-5 mL 1 N HCl to kill any veligers that may have been produced prior to disposal.

Static acute toxicity tests with *Dreissena* adults were initiated by putting 500 mL of aged tap water, pH 6.0 into 1 L beakers. Thereafter, the water in each beaker received 1 mL of a given concentration of test chemical dissolved in acetone (or appropriate solvent); controls received 1 mL of solvent: three replicates of each concentration were made. Range finding tests were conducted initially to identify 5 concentrations projected to give 5-95% mortality.

Twenty-four hours prior to use in toxicity tests, adult zebra mussels, approximately 1.5-2.0 cm in size were removed from the stock culture by cutting their byssal threads with a razor blade. Groups of 12-13 mussels were then placed on the bottom half of 9 cm diameter glass petri dishes; the latter were immersed in fresh tap water from which chlorine gas had been allowed to dissipate. Mussels were allowed to reattach themselves to the petri dishes over a 24 hour period. Those mussels which reattached to the petri dish were considered healthy and acceptable for use in toxicity tests. Mussels which did not attach to petri dishes within 24 hours were discarded. A minimum of 10 adult zebra mussels was used for each replicate. Into each of the previously prepared test beakers, a petri dish containing attached zebra mussels was submerged. Test beakers were then placed in a Forma Scientific (Marietta, OH) #37422 environmental chamber set at the appropriate temperature on a photoperiod of 14:10 hours. In most cases, a standard temperature of 24°C was used. Selection of the mussels as test subjects as well as the position of the test beakers in the environmental chamber were randomly selected and placed into the environmental chamber.

Mortality was assessed every 24 hours for the duration of the toxicity tests. The criterion for mortality was failure to respond to the touch of a probe. In most cases, dead mussels gaped open. However, mussels with closed shells were examined by inserting the probe between the two shells just above the incurrent siphon. Mussels which showed no adductor activity were considered dead. The test was invalidated if control mortality exceeded 20%. The duration of the toxicity tests ranged from 24-96 hours.

The water in all beakers containing zebra mussels was adjusted to pH 1.0 with 0.5 mL 1 N HCl following the cessation of the test. Beakers were held an additional 24 hours to ensure that survivors of the toxicity test were killed by reduced pH before disposal.

To assure that test conditions were suitable for the mussels during the toxicity tests, DO, pH, total alkalinity, total hardness and temperature were monitored in controls and the highest concentration of toxicant at the beginning of each test and every 24 hr thereafter.

For all toxicity tests, data were analyzed using Finney's (1971) probit analysis to estimate LC₅₀ values and 95% confidence limits. Mortalities of 0 or 100% were not used in probit analyses: in every case, at least 3 partial kill points were obtained. LC₅₀ values were considered to be significantly different where 95% confidence limits did not overlap.

Field Tests of Antibiofouling Paints and Substrates

To test the effectiveness of a variety of chemicals in preventing the attachment of the zebra mussels, 20 cm by 20 cm fiberglass panels were coated with a given antibiofouling product according to manufacturer's instructions. For each test, 9 treatment panels and 3 unpainted control panels were assembled. Thereafter, groups of 3 panels (either treatment or control) were attached at one end to a 1 m wooden dowel rod using 50 lb fishing line. At the opposite end, the panels were attached using 50 lb fishing line to a 1 m metal tube which was filled with dry Quikrete® cement. The assembly was then suspended in Lake Erie with the dowel rod at the water surface to mark the position of the submerged panels and the cement-filled metal tube as an anchor. The lengths of fishing line were adjusted so that the panels were suspended equidistance from the dowel rod and the metal tube anchor. In general, panels were anchored near Franz Theodore Stone Laboratory at Put-in-Bay on Lake Erie at a depth of 4-5 feet. At this depth, the panels were in contact with aquatic plants that had very high densities of adult zebra mussels.

The panels remained in the lake for a 3 month period and were withdrawn from the lake every 10-14 days to determine whether any adult zebra mussels had attached. After the number of adults attached to control and treated panels had been scored, the panels were resuspended in Lake Erie. The data were expressed as the number of adult zebra mussels attached to control and treated panels. Statistical differences between treatments and controls were analyzed using analysis of variance (ANOVA) (SAS 1982); means were separated using Duncan's (1951) Multiple Range Test.

Chemicals

The chemical control agents, both molluscicidal and molluscistatic, which have been evaluated are listed in Table 1. Also provided is information on chemical purity, formulation, source and type of test performed.

RESULTS

Acute Toxicity Tests

The zebra mussel is susceptible to the toxic action of chemicals belonging to diverse chemical classes. The toxicity of Buckman 6002, a polyquaternary ammonium derivative, was measured both as a function of concentration and time. A dose-time-response was clearly evident; the LC₅₀ for Buckman 6002 declined from 1670 µg/L at 24 hr to 830 µg/L at 96 hr (Table 2). The molluscicide, 2-(thiocyanomethylthio) benzothiazole (Buckman 6009) proved to be even more toxic to adult zebra mussels with acute toxicity values ranging from 794 µg/L at 24 hr to 653 µg/L at 96 hr.

The effect of temperature on toxicity was measured using Calgon H-130 in acute toxicity tests with zebra mussel adults (Table 3). An increase in temperature from 24°C to 28°C generally lead to an increase in the toxicity of H-130 although the differences were not always statistically different. For instance, the marked de-

TABLE 1.
Compounds used in laboratory and field tests against *Dreissena polymorph.*

Source	Product	Active Ingredient	Test Type
Calgon	H-130	didecyl dimethyl ammonium chloride	acute toxicity
Fermenta	chlorothalonil	tetrachloroisophthalonitrile	acute toxicity, field
Philadelphia Resins	TBT	tributyltin methacrylate	field
Philadelphia Resins	cuprous oxide	cuprous oxide	field
Buckman	6002	Poly[oxyethylene-(dimethyl-iminio)ethylene-(dimethyl-iminio)ethylene dichloride	acute toxicity
Buckman	6009	2-(thiocyanomethylthio)] benzothiazole	acute toxicity

crease in the 96 hr LC₅₀ from 697 µg/L at 24°C to 275 µg/L at 28°C was not statistically different due to the large 95% confidence limits reported for the 24°C, 96 hr LC₅₀. However, the statistically significant increases in toxicity between temperatures at 48 and 72 hr clearly conveyed the effect of increasing temperature on toxicity. As with Buckman 6002, the toxicity of Calgon H-130 was seen to be both concentration and time-dependent (Table 3).

Field Tests with Antibiofouling Paints

Chlorothalonil was applied as an antibiofouling paint in three different formulations denoted 1, 2 and 3, respectively. All contained up to 19.5% chlorothalonil as the active ingredient. Within one hour of being set out into Lake Erie, 3 adult zebra mussels were detected on the surface of a single panel treated with formulation 2. However, the mussels did not secrete a byssal thread attachment and were gone by the time the panels were next inspected. No other adult zebra mussels were detected on the panels treated with any formulation of chlorothalonil throughout the 3 month test period (Table 4). Among control panels, the number of adult mussels attached varied from a low of 0 for formulation 3 on August 27 to a high of 75 adults on formulation 3 on October 22. The number of mussels attached to control panels varied considerably over time. In some cases, mussels were seen to attach and then later detach leaving remnants of the byssal threads behind as evidence of the mussel having been present. The data entries in Tables 4 and 5 thus reflect only adult mussels present on a particular date and not cumulative attachment over time.

The two heavy metals (TBT and cuprous oxide) were seen to be effective in deterring attachment of adult zebra mussels during the 3 month field season (Table 5). No adult zebra mussels were detected on any of the treated panels at any time during the field

season. However, adult mussels regularly attached to the control panels. As with the chlorothalonil control panels, adult zebra mussels attached to control panels and later detached, leaving behind byssal threads. It should be noted, that due to a shortage of materials only 3 treated panels of each type were used in these assays.

DISCUSSION

Critique of Methods

Various components of the zebra mussels' biology are relevant to the assessment of acute toxicity and must therefore be accounted for in designing protocols for estimating LC₅₀s. Of obvious importance are a variety of water quality parameters which include water type, oxygen content, pH and temperature,

The use of a reconstituted standard reference water, i.e. distilled, deionized water to which known concentrations of salts are added back would have the benefit of eliminating variability in important water quality parameters. However, the standard reference water prescribed by USEPA (1975) cannot be used since two of the major components, KH₂PO₄ and KCl, are lethal to the zebra mussels at the recommended concentrations (Fisher et al. 1991). The mussels survive readily in aged tap water and distilled water. The former was chosen for use in these tests until a suitable reconstituted water can be found.

The survival of *Dreissena* in natural water is limited when oxygen concentrations fall below 7 ppm. Adult *Dreissena* are known to be sensitive to reductions in O₂ content (Mikheev 1968). Thus, O₂ content must be monitored during toxicity tests in order to assure validity of the results. Each treatment beaker was aerated during toxicity tests, to maintain oxygen levels compatible with

TABLE 2.
Toxicity of molluscicides to zebra mussel adults.

Compound	Test		95% C.L.
	Duration	LG ₅₀ (µg/L)	
Chlorothalonil	24	730	680-777
Buckman 6002	24	1670	1360-2220
Buckman 6002	72	980	790-1030
Buckman 6002	96	830	760-910
Buckman 6009	24	794	683-867
Buckman 6009	48	679	494-756
Buckman 6009	72	672	467-744
Buckman 6009	96	653	398-733

TABLE 3.
Toxicity of calgon H-130 to adult zebra mussels.

Temperature °C	Exposure Time (hr)	LC ₅₀ * µg/L	95% C.L.
28	24	928 ^{de}	819-1082
28	48	596 ^c	521-667
28	72	413 ^b	354-469
28	96	275 ^a	217-323
24	24	1103 ^e	1008-1268
24	48	826 ^d	780-873
24	72	765 ^d	714-812
24	96	697 ^{abcde}	27-1025

* LC₅₀ values followed by the same letter are not significantly different as determined by overlapping 95% C.L.

TABLE 4.
Field evaluation of chlorothalonil as an antibiofouling paint.

Panel Type Formulation	Number of Adult Mussels Attached*					
	8/9	8/27	9/10	9/17	10/2	10/22
Chlorothalonil 1	0	0	0	0	0	0
Control	0	41	10	19	13	0
Chlorothalonil 2	1	0	0	0	0	0
Control	0	9	14	21	0	5
Chlorothalonil 3	—	0	0	0	0	0
Control	—	—	40	33	16	75

* Panels were set out in Lake Erie on 8/9/90 with the exception of formulation 3 which were set out on 8/27. Panels were read after 1 hr of submersion of 8/27 and then on the dates indicated.

zebra mussel survival. This procedure kept dissolved oxygen levels in the range of 7.4-10.8 ppm which was sufficient to prevent oxygen depletion from becoming a cause of death. However, it should be noted that dead mussels had to be removed from the beakers at least every 24 hours in order to avoid a reduction in oxygen concentration in response to decaying zebra mussels. Likewise, the pH of the water in which the tests were performed was important. Zebra mussels will tolerate a range of pHs which may be as broad as 5.0-8.5 (Fisher et al. 1991). However, control mortality began to increase when the pH level of the aged tap water was experimentally set below 5.0 while pH values in the range of 5.5-8.0 did not affect survival of control adults. The pH of aged tap water consistently fell in the range of 5.8 to 6.0 and this pH level was used throughout because it required no amendment with buffer and because the mussels readily tolerated this pH level.

Water temperature is a variable which is an extremely important arbiter of molluscicidal activity in *Dreissena*. An increase in temperature leads to an increase in metabolism and a concomitant increase in oxygen demand (Mikheev 1968). Zebra mussels are thus more sensitive to oxygen deprivation at higher temperatures than they are at lower ones. In the present study, oxygen levels are maintained at levels greater than 7.0 ppm even at higher temperatures. Thus, the potential interaction between oxygen and temperature was probably not responsible for changes in molluscicidal activity. However, there was a significant increase in the toxicity of Calgon H-130 with a temperature increase of 4°C (Table 3). This finding is consistent with other studies on adult *Dreissena* in which the toxicity of a variety of molluscicidal compounds increased with an increase in water temperature (Jenner 1984, Lyakhov 1968, Mikheev 1968). Because significant changes in tox-

TABLE 5.
Field Evaluations of TBT and cuprous oxide as antibiofouling paints,

Paint Type	Number of Adult Zebra Mussels Attached				
	8/9	9/10	9/17	10/2	10/22
TBT (12%)	0	0	0	0	0
Cuprous Oxide (42.4%)	0	0	0	0	0
Control	40	14	21	21	77

icity can occur over a narrow temperature range, temperature must be carefully controlled in toxicity determinations,

Two elements of adult zebra mussel behavior can have an important effect on the outcome of toxicity tests. The first of these is their filtering activity which can be continuous or intermittent. In addition, there is some evidence that zebra mussels will close their shells in response to the presence of chemicals or in response to unfavorable environmental conditions. This limits their exposure to the chemical and leads to variability in toxicity results (McMahon et al. 1990). The proposed test method minimizes this source of variability by maintaining a temperature consistent with optimal filtering activity (Mikheev 1968) and providing an acclimation period for the mussels to adapt to the test environment. The mussels were observed to be actively filtering 90-95 percent of the time when filtering activity was monitored every 30 min for 8 hr. The other important behavioral factor is the requirement for attachment of the adult mussels to a hard surface with byssal threads. It is disruptive to the mussel and potentially detrimental to cut the byssal threads in order to evaluate the condition of the mussels (McMahon et al. 1990). However, it was desirable to take multiple readings of the mussels' condition. The necessity of severing the byssal threads to take readings was eliminated by allowing the mussels to attach to a glass petri dish. The latter could be submerged and then retrieved affording an evaluation of the mussels' condition which did not require detachment.

The method employed for field testing of antibiofouling paints appeared to be successful (Tables 4, 5). Although the only panel type tested was fiberglass, other substrates such as concrete, wood or aluminum could have been substituted. The use of an adequate number of replicate panels was of paramount importance to this assay. There was sufficient variability in attachment of adults to control panels, both between replicates and at different time periods, that replication was essential to accuracy. In addition, the importance of taking multiple readings over an extended period of time was clearly demonstrated by the variability in attachment to control panels.

Since environmental conditions are extremely important to zebra mussel survival, the field assay could be improved by evaluating attachment of adults at different depths for which temperature, oxygen content and light quality will vary. Future evaluation of antibiofouling paints should also include a microscopic evaluation of veliger settling and attachment to control and treated panels.

Evaluation of Molluscicide Efficacy

A variety of structurally diverse chemicals were acutely toxic to adult zebra mussels (Tables 2, 3). All four molluscicides tested against adult *Dreissena* produced LC₅₀ values in the range of 275-1670 µg/L. Toxicity was responsive to concentration, time of exposure and temperature. As each of these variables increased, toxicity was likewise accentuated.

Adult zebra mussels proved to be much more sensitive to Buckman 6002 in the current study than is reported elsewhere. McMahon et al. (1990) measured LT₅₀ values for adult *Dreissena* exposed to Buckman 6002 at levels between 0.5-8 mg/L. At an exposure concentration of 2 mg/L, 50% mortality was achieved after approximately 200 hours of exposure. However, our findings indicate that 50% mortality will occur within 24 hours at a concentration of 1.6 mg/L (Table 2). The discrepancies in these findings may be attributed primarily to the fact that the tests conducted

by McMahon et al. (1990) were performed at 20°C as opposed to 24°C used in the present study. The ability of elevated temperature to increase toxicity is evident with Calgon H-130 (Table 3) and has also been reported for chlorine (Jenner 1984, Greenshields and Ridley 1957), heavy metals (Dudnikov and Mikheev 1968, Lukanin 1968) and ammonium nitrate (Shevtsova et al. 1978). The utility of combining a slightly elevated temperature with molluscicide treatment in contained settings such as pipes bears investigation.

Just as a variety of chemicals have proven efficacious in killing adult zebra mussels, so too are many chemicals effective in deterring attachment to solid surfaces. Heavy metals such as copper and tributyltin oxide (TBT) have been used widely as marine antifouling agents (Jenner and Janssen-Mommen 1989). These same chemicals appear to have application against the zebra mussel (Table 5). However, because heavy metals have in general and TBT in particular have been identified as significant pollutants with undesirable nontarget effects (Friberg et al. 1979), the finding that chlorothalonil prevents attachment of adult zebra mussels is important (Table 4). Although chlorothalonil is toxic to fish (Da-

vies and White 1985), its half-life in nonsterile aqueous systems containing sediments is less than 3 days (Walker et al. 1988). Chlorothalonil appears to be useful in several paint formulations and may prove to be effective in preventing zebra mussel attachment.

In conclusion, it is clear from these data that a variety of chemicals are effective in killing zebra mussels and preventing attachment. The procedures described herein should facilitate screening of a large number of candidate chemicals for determining efficacy in controlling *Dreissena* both in laboratory tests and in the field. This, in turn, will promote the accumulation of a body of data from which chemicals, having different merits, can be evaluated for use in zebra mussel control in a variety of different settings.

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