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. 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 biofouling 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...
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 realign 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 provided is information on chemical purity, formulation, source and type of test performed.

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 incumbent 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 equidistant 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 molluscsicidal, 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-
increase 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 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.

Table:<ref>

### TABLE 1.

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

<table>
<thead>
<tr>
<th>Source</th>
<th>Product</th>
<th>Active Ingredient</th>
<th>Test Type</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calgon</td>
<td>H-130</td>
<td>didecyldimethylammonium chloride</td>
<td>acute toxicity</td>
</tr>
<tr>
<td>Fermenta</td>
<td>chlorothalonil</td>
<td>tetrachloroisophthalonitrile</td>
<td>acute toxicity, field</td>
</tr>
<tr>
<td>Philadelphia Resins</td>
<td>TBT</td>
<td>tributyltin methacrylate</td>
<td>field</td>
</tr>
<tr>
<td>Philadelphia Resins</td>
<td>cuprous oxide</td>
<td>cuprous oxide</td>
<td>field</td>
</tr>
<tr>
<td>Buckman</td>
<td>6002</td>
<td>Poly[oxyethylene-(dimethyl-iminio)ethylene-(dimethyl-iminio)ethylene dichloride]</td>
<td>acute toxicity</td>
</tr>
<tr>
<td>Buckman</td>
<td>6009</td>
<td>2-(thiocyanomethylthio) benzothiazole</td>
<td>acute toxicity</td>
</tr>
</tbody>
</table>

### 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 3.

**Toxicity of calgon H-130 to adult zebra mussels.**

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>Exposure Time (hr)</th>
<th>LC₅₀ (µg/L)</th>
<th>95% C.L.</th>
</tr>
</thead>
<tbody>
<tr>
<td>28</td>
<td>24</td>
<td>928</td>
<td>819-1082</td>
</tr>
<tr>
<td>28</td>
<td>48</td>
<td>596*</td>
<td>521-667</td>
</tr>
<tr>
<td>28</td>
<td>72</td>
<td>413*</td>
<td>354-469</td>
</tr>
<tr>
<td>28</td>
<td>96</td>
<td>275*</td>
<td>217-323</td>
</tr>
<tr>
<td>24</td>
<td>24</td>
<td>1103</td>
<td>1008-1268</td>
</tr>
<tr>
<td>24</td>
<td>48</td>
<td>826*</td>
<td>780-873</td>
</tr>
<tr>
<td>24</td>
<td>72</td>
<td>765*</td>
<td>714-812</td>
</tr>
<tr>
<td>24</td>
<td>96</td>
<td>6.97*</td>
<td>27-1025</td>
</tr>
</tbody>
</table>

* LC₅₀ values followed by the same letter are not significantly different as determined by overlapping 95% C.L.
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 LC_{50} 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 LT_{50} 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, Lukin 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 antifoulants (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 (Davies and White 1985), its halflife 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.

ACKNOWLEDGMENT

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LITERATURE CITED


