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Assessing the Potential Efficacy of Glutaraldehyde for Biocide Treatment of Un-ballasted Transoceanic Vessels

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ABSTRACT. *Treating the ballast water of oceanic vessels with a biocide is one potential management strategy to reduce the number of nonindigenous species released into the Laurentian Great Lakes from NOBOB (no ballast on board) vessels. To evaluate biocide effectiveness, glutaraldehyde, a five-carbon dialdehyde widely used for its antimicrobial properties, was investigated. Biocide effectiveness was assessed for various organisms using 24 h acute toxicity bioassays in water-only and water-sediment environments. Acute studies indicate a 24 h* LC_{90} *value of 100 mg glutaraldehyde* L^{-1} *or less for most of the freshwater organisms tested. The main exception was the freshwater amphipod,* Hyalella azteca, *which was much more resistant to glutaraldehyde (24 h* LC_{90} *= 550 mg glutaraldehyde* L^{-1}*; 95% CI: 476–681). Biocide efficacy was also evaluated in water-sediment exposures. The presence of a test sediment (3% organic carbon) greatly increased lethal concentration estimates for the oligochaete* Lumbriculus variegatus, *but not for* H. azteca: *The 24 h* LC_{90} *for* L. variegatus *varied depending on the water-sediment ratio, and ranged from 61 mg glutaraldehyde* L^{-1} *(95% CI 52–78) for an 8:1 water-sediment ratio to 356 mg glutaraldehyde* L^{-1} *(95% CI 322–423) for a 2:1 water-sediment ratio. This indicates that the amount of sediments present in NOBOB vessels may have a significant impact on biocide efficacy. Experiments using material from actual NOBOB vessels generally corroborated data from the water-sediment experiments and suggest a potential treatment concentration of approximately 500 mg glutaraldehyde* L^{-1} *for short exposure periods (e.g., 24 h).*

INDEX WORDS: *Nonindigenous species, ballast water, biocide, glutaraldehyde, Laurentian Great Lakes.*

INTRODUCTION

Biological invasions have been an important component of the natural ecological and evolutionary processes of aquatic ecosystems. Human activities, however, have dramatically altered both the rate and scale of invasions, so that most are now associated with human activities such as aquaculture, recreation, and commerce (Courtenay and Taylor 1986, Mills *et al.* 1993). Invasions by nonindigenous aquatic organisms pose a particularly significant threat to the Laurentian Great Lakes. Over the past two centuries, more than 160 nonindigenous species have colonized this area (Mills *et al.* 1993, MacIsaac *et al.* 1999, Ricciardi and MacIsaac 2000).

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The establishment of these nonindigenous species has had profound ecological and economic impacts. Ecological impacts include habitat alteration (Busch and Larry 1996, Stewart *et al.* 1998), competition with, and predation on native organisms (Haag *et al.* 1993, Dermott 2001, French and Jude 2001, Lozano *et al.* 2001) and larger-scale alterations in biogeochemical cycles (Nicholls *et al.* 1999, Nicholls *et al.* 2001). The economic impacts have been substantial. Costs associated with controlling the zebra mussel, for example, have been estimated at over $100 million annually (Pimentel *et al.* 2000). Recent discoveries of several new nonindigenous species in the Great Lakes, such as the predatory cladoceran, *Cercopagis pengoi* (MacIsaac *et al.* 1999) and the amphipod, *Echinogammarus ischnus* (Dermott *et al.* 1998), demonstrate the urgent need to implement measures to eliminate or substantially reduce the release of nonindigenous species into this system.

One of the more important means for transport of nonindigenous species into the Great Lakes is the ballast water of ocean-going vessels: Approximately 25% of the nonindigenous species that currently reside in the lakes are believed to have been introduced through this vector (Mills *et al.* 1993, MacIsaac *et al.* 1999, Ricciardi and MacIsaac 2000). The ballast water of vessels consists of either fresh or salt water that is pumped into tanks to help control trim, stability, structural loading, and maneuverability. Vessels can take on and discharge ballast water at different points during a voyage (NRC 1996), and organisms that are in the water column at the time of ballasting may be caught up in ballast water and transported long distances. Several studies have documented the diversity and quantity of organisms that can be present in ballast water (Carlton 1985, Hallegraeff and Bolch 1991, Gollasch *et al.* 2000, Olenin *et al.* 2000, Ruiz *et al.* 2000). Although survival of organisms during long transits tends to be low, the total volume of ballast water released is usually large, so that millions of organisms may be released into receiving waters (Olenin *et al.* 2000). In addition to the large amount of ballast water carried by vessels, there can also be a substantial amount of sediment that accumulates on the bottom of these tanks. Several studies have documented the presence of dinoflagellate cysts and diapausing eggs in ballast tank sediments (Hallegraeff *et al.* 1990, Hallegraeff and Bolch 1992, Bailey *et al.* 2003), which may provide another potential source of invasive nonindigenous species.

Because ballast water is a primary vector for introductions into the Great Lakes, decreasing the number of nonindigenous species carried by transoceanic vessels is one option for reducing the risk of future invasions. To accomplish this, several forms of ballast water management are currently being tested. One treatment option under consideration is the application of chemical biocides (or "biocide") to ballast water. The use of biocides as disinfectants for ballast water may be most promising for vessels classified as NOBOB (no ballast on board). Treatment of NOBOBs has greater potential because these vessels contain a relatively small amount of ballast material and undergo cross-transfer of lake water into ballast tanks, thereby diluting the effective concentration of biocide following treatment (Lubomudrov *et al.* 1997). Most of the transoceanic NOBOB vessels that enter the Great Lakes carry between 50 and 210 metric tons of unpumpable water and residual sediment (Farley 1996) and thus are a potential vector for the introduction of nonindigenous aquatic species. In addition, as many as 90% of the transoceanic vessels that enter the Great Lakes are classified as NOBOB and do not engage in high-seas ballast exchange (Colautti *et al.* 2003). Recent studies have documented that NOBOB vessels arriving in the Great Lakes can carry a large number of viable organisms in both residual water and sediments. These organisms include virus-like-particles and bacteria (Dobbs *et al.* 2003), rotifers, cyclopoids, harpacticoids, and cladocerans (van Overdijk *et al.* 2003), and diapausing invertebrate eggs (Bailey *et al.* 2003).

The biocide tested in this study was glutaraldehyde (1,5-pentanedial, CAS Registry No. 111-30-8), a five carbon dialdehyde. Glutaraldehyde is used in several commercial and industrial applications including for sterilizing heat sensitive dental and medical equipment, for treating water cooling towers and pulp mill effluent, and as a cross-linking agent in enzyme immobilization and X-ray film development. The National Research Council (1996) was the first to identify glutaraldehyde as a potentially promising non-oxidizing biocide for ballast water treatment. The non-oxidizing property of glutaraldehyde distinguishes it from other types of biocides (e.g., chlorine, bromine), which are oxidizing and may pose corrosion problems in a ballast tank environment. Glutaraldehyde's biocidal properties are attributed primarily to the reaction of its aldehyde moiety with the amino groups in proteins to form a Schiff base (Peters and Richards 1977).

The objective of this study was to develop the

toxicological data required for application to ballast systems. To satisfy this objective, several 24 hour acute mortality bioassays were conducted using a range of representative aquatic organisms under both water-only and water-sediment exposure conditions. In addition, two sets of experiments were conducted using sediment collected from transoceanic NOBOB vessels to assess potential efficacy under field conditions.

MATERIALS AND METHODS

Chemicals and Analytical Methods

Glutaraldehyde was obtained as a 50% solution (w/w) in water from Fisher Scientific (Fairfield, NJ, USA). For use in bioassays, a 0.1% stock solution was made in the same dilution water employed for the individual experiments. This stock was kept refrigerated at 4°C and was used for up to 4 weeks with no detectable decrease in glutaraldehyde concentration.

Glutaraldehyde concentrations are reported as mg glutaraldehyde L^{-1} and were determined using a spectrophotometric assay with 3-methyl-2-benzothiazonlinone hydrazone hydrochloride as the color-developing agent (Sawicki *et al.* 1962, Pakulski and Benner 1992). Actual glutaraldehyde concentrations were estimated from a standard curve consisting of three concentrations (0.5 mg L^{-1}, 1 mg L^{-1}, and 8 mg L^{-1}) run during each analysis. The range of this method was 0.5 mg L^{-1}-8.0 mg L^{-1} of glutaraldehyde, and samples taken from test vessels often had to be diluted so as to fall within this sensitivity range.

Test Organisms

Five different species were used for acute mortality tests: the freshwater amphipod *Hyalella azteca*, the freshwater oligochaete *Lumbriculus variegatus*, the freshwater cladocerans, *Daphnia magna* and *Ceriodaphnia dubia*, and the marine bacterium *Vibrio fischeri*.

The *H. azteca* were obtained from an in-house culture, originating from the United States Geological Survey (USGS), Columbia Environmental Research Center (Columbia, Missouri, USA). Organisms were maintained on a coiled plastic substrate (coarse, washable filter media, Aquatic Ecosystems, Apopka, FL, USA) in a 37-L glass aquarium held at 24°C under low intensity light with a light:dark photoperiod of 16:8 h. Culture water was taken from the Huron River, Michigan, USA (total hardness: 165 mg L^{-1} as $CaCO_3$; total alkalinity: 3.996 meq L^{-1}; pH: 8.2–8.6). This water was filtered using a Cole Palmer submicrofilter (Vernon Hills, IL, USA) and flushed through the tank at a flow rate of approximately 4 L per day. The organisms were fed ¼ g of ground Tetramin® fish food (TetraWerke, Melle, Germany) twice a week. The animals used for toxicity tests were those retained by a 710 μm mesh and thus classified as adults (U.S. EPA 1999).

The stock of *L. variegatus* used in experiments was acquired from the US Environmental Protection Agency (U.S. EPA), Midcontinent Ecology Division (Duluth, Minnesota, USA). The culture was maintained in a 37-L glass aquarium on a substrate of shredded, unbleached paper towel. Organisms were held at room temperature (21 ± 2°C) under low intensity (20 lux), gold fluorescent light (λ > 500 nm). Well water (total hardness: 500 mg L^{-1} as $CaCO_3$; total alkalinity: 5.00 meq L^{-1}; pH: 7.2–7.8) was used for the culture and was acquired from the USGS, Great Lakes Science Center (Ann Arbor, MI). The culture was continuously aerated, and unfiltered well water was flushed through the tank at a rate of approximately 2 L per day. Worms were fed 3 g of Trout Chow (Purina Brand®, St. Louis, MO, USA) three times per week. The organisms used for toxicity tests were from the same general size class, averaging approximately 4 cm in length.

Cultures of *D. magna* were maintained in 4-L borosilicate beakers containing artificial hard reconstituted water (U.S. EPA 1993) and kept in an incubator at 24°C on a 16:8 h photoperiod. The water used for the media was Ann Arbor (Michigan, USA) city water that was passed through a carbon filtration system, then filtered through a Ropure® ST reverse osmosis system (Barnstead, Dubuque, IA, USA) and a Nanopure® UV ultrapure water system (Barnstead, Dubuque, IA, USA). Water was aerated for at least 2 days prior to use. The final hardness and alkalinity of the water were 140 mg L^{-1} as $CaCO_3$ and 1.998 meq L^{-1}, respectively. Media was changed approximately every 48 hours. Cultures of *C. dubia* were maintained in artificial, moderately hard, reconstituted water (U.S. EPA 1993). The water source was identical to that described for *D. magna*; however, the total amount of salts added was lower, resulting in water with a final hardness and alkalinity of 80 mg L^{-1} as $CaCO_3$ and 1.1988 meq L^{-1}, respectively. Media was changed approximately every 72 hours, and organisms were maintained at 24°C, with a 16:8 h

photoperiod. Both *D. magna* and *C. dubia* cultures were fed a combination of *Pseudokirchneriella subcapitata* (formerly *Selenastrum capricornutum*; Hindák 1990) and a yeast, Cerophyll®, trout chow mixture (U.S. EPA 1993). Food was added after each water change.

Experiments employing the daphnids used both 24 h neonates and "adult" organisms. The neonates were obtained from adult organisms that were separated no more than 24 hours prior to the initiation of the bioassay. Adult organisms consisted of organisms that were of reproductive age. For our cultures, *C. dubia* became reproductive after 3 to 4 days, and *D. magna* became reproductive after approximately 14 days. These adult organisms were separated by two different sieve mesh sizes: 600 μm for *D. magna* and 300 μm for *C. dubia*.

For the Microtox® assay, freeze-dried samples of the bioluminescent bacterium *V. fischeri* were obtained from Azur Environmental (Carlsbad, CA, USA). Vials of the bacteria were maintained at approximately –20°C prior to use. Tests were conducted in Ann Arbor city water that was passed through the reverse osmosis and ultrapure water systems (i.e., the same treatment employed for the daphnids). The water was then aerated for 2 days to remove any excess chlorine or chloramine residuals, and total chlorine concentrations were measured prior to testing.

Test Sediment

The sediment used for bioassays to evaluate the effect of sediment on glutaraldehyde toxicity was collected from Gallup Park (Ann Arbor, MI). Sediments were taken from a nearshore area in the northeast section of Geddes Pond, just east of Huron Parkway, in a slow moving, protected embayment. The organic carbon content of the sediment was determined after removing carbonates with 1 N HCl, re-drying the sample, and measuring carbon on a CE Instruments 1110 CHN analyzer (ThermoQuest, Italia, Milan, Italy).

Test Conditions

All of the experiments were conducted in the same water used for the cultures with two exceptions: Experiments using the amphipod *Hyalella azteca* were conducted in Huron River water that was also filtered through a 0.22 μm Millipore filter (Bedford, MA, USA). During the spring and summer months, when particulate matter was abundant, the water was additionally prefiltered through a 0.45 μm Millipore filter prior to final filtration through the 0.22 μm filter. For *L. variegatus*, the well water was filtered through both a 0.45 μm and a 0.22 μm Millipore filter prior to use.

Test Methods

Water-only Bioassays

Twenty-four-hour static water-only bioassays were conducted according to ASTM (1998) and U.S. EPA (1993) protocols. Organisms were added to vessels at a maximum biomass-loading rate of 0.5 g L^{-1}. In general, six concentrations were tested per experiment with five replicates per concentration. Each replicate contained five organisms. Experiments were performed at least twice, depending on data quality. The controls consisted of the same dilution water as the treatments. Samples to measure glutaraldehyde concentrations were taken at the beginning of the experiment (t = 0 h) and at the end of the experiment (t = 24 h).

Once organisms were isolated for the experiments, they were randomly distributed into test vessels. For experiments with *H. azteca*, a small square of cotton gauze (approximately 2 cm × 2 cm) was added to each test vessel to serve as substrate. Oligochaetes were isolated from the tank and transferred to a dissecting tray containing filtered well water. Worms were then gently separated from culturing material prior to distribution into test containers.

All water-only experiments were conducted in the dark in an incubator held at ± 1°C of the temperature of the cultures (i.e., 19°C for *L. variegatus*, and 24°C for *H. azteca*, *D. magna*, and *C. dubia*). At the end of the experiment, the temperature, pH, dissolved oxygen (DO), and biocide concentration were measured in all of the test chambers. Organism condition was assessed in terms of alive/dead or mobile/immobile. Test conditions in which DO dropped below 3 mg L^{-1} or temperature varied by more than 2°C over the 24 h period were automatically discarded from analysis.

Experiments using *V. fischeri* were conducted with a Microtox™ Model 500 toxicity analyzer. The basic test protocol (Microbics 1992) was followed. Briefly, the appropriate aliquot of osmotically adjusted sample dilutions was added to bacterial suspensions, and light output measurements were made at 5 and 15 min intervals. Light readings were corrected according to changes in the

dilution control (blank) to allow for natural time-dependent drifts in light output and small dilution effects. Toxicity was then assessed as the percent inhibition of light emission from treated aliquots compared to the controls.

Water-sediment Bioassays

Water-sediment bioassays were performed in a similar manner to water-only exposures. Biomass was kept the same as in the water-only exposures, and experiments were maintained for 24 h in unlit incubators held at the temperature of the cultures (± 1C). In contrast to water-only bioassays, water-sediment experiments were conducted using 250 mL Erlenmeyer flasks and employed only two freshwater organisms (*L. variegatus* and *H. azteca*). Experiments were started by weighing out an approximate volume of sediment required to achieve the desired water-sediment (V:V) ratio. Three different ratios were tested: 8:1, 4:1, and 2:1 water-sediment. Samples for glutaraldehyde analysis were taken at the beginning of the experiment (from the stock solutions), at 1 to 4 hours after glutaraldehyde addition (from the individual test vessels), and at the end of the experiment (from the individual test vessels).

For experiments using *L. variegatus*, the oligochaetes were added to the sediment first and allowed to burrow for approximately 20 minutes prior to toxicant addition. The appropriate concentration of biocide was then added to the test chambers, accounting for dilution due to the volume of sediments. Thus, the concentrations were set for the total volume of sediment plus water of the exposure systems. For *H. azteca*, the organisms were added to the test chambers after the addition of the toxicant. Once the experiment was initiated, test flasks were placed on an orbital shaker set at 66 revolutions per minute (rpm) to simulate the mixing that might occur in a ballast tank. This amount of mixing was not enough to resuspend test sediments.

At the end of the experiment, the contents of the flasks were sieved through a 90 µm sieve to retain organisms. For *L. variegatus*, organisms were removed from the sieve and placed in a pan with either deionized water or unfiltered Huron River water. Organism status was then assessed and recorded. Recovery rates of *L. variegatus* from sediments (based on controls) were approximately 98%. For *H. azteca*, organisms were also removed from the sieve and placed in deionized water. Organism status was assessed in terms of mobility (either mobile or immobile). In some cases, organisms were directly removed from the flasks and placed in a separate container to facilitate counting. Recovery of *H. azteca* from sediments averaged 97% for controls.

Ballast Tank Simulation Experiments

Two experiments were conducted to assess glutaraldehyde efficacy using sediments collected from transoceanic NOBOB vessels. The ballast tank material was collected manually from two different vessels that were docked at Cleveland Harbor (first experiment) and Hamilton Harbour (second experiment). For the first experiment, the sediment with associated water was collected from a double bottom tank on the starboard side. The sample for organic carbon analysis was lost. For the second experiment, the sample was collected from the forepeak tank on the starboard side. The organic carbon content was analyzed only for the second sample and was 2.2 ± 0.01%.

Ballast tank simulation experiments were started by placing 1 L of ballast sediment into each of four 20 L clear Nalgene® polycarbonate carboys. The approximate water-sediment ratio was 4:1 (V:V). Once the sediment was in place, 2 L of prefiltered Huron River water were added. To assess toxicity, 30 individuals each of *L. variegatus* and *H. azteca* were added to the carboys. The oligochaetes were allowed to burrow for approximately 60 minutes prior to glutaraldehyde addition. Two liters of a glutaraldehyde solution were then added to the carboys to yield an approximate concentration of 500 mg L^{-1}. After biocide addition, the carboys were placed on an orbital shaker set at 45 rpm, which provided sediment agitation and some resuspension. Carboys were maintained in the dark, at a temperature of 21°C. After 24 h, the water was siphoned from the carboys, and the remaining material (mostly sediments with a small amount of overlying water) was diluted with 20 L of filtered Huron River water to simulate dilution associated with re-ballasting. The experiment was then maintained for an additional 10 days to permit grow-out of any viable organisms. During this period, carboys were kept at 21°C on a 16:8 h light:dark cycle. The solutions were gently aerated during this period to prevent anaerobic conditions from developing. At the end of the grow-out period, the contents of the carboys were sieved (using a 90 µm sieve), and organism status was determined (alive/dead or mobile/immobile).

Data Analysis

Results from acute bioassays were analyzed by estimating the 24 h LC_{50} (lethal concentration for 50% of tested organisms) and LC_{90} (lethal concentration for 90% of tested organisms) values and associated 95% confidence intervals with logit analysis using SYSTAT Version 10 (SPSS, Inc., Chicago, IL). Mortality plots for bioassays were made using Sigma Plot 4.0 (SPSS, Inc., Chicago, IL). For the Microtox™ experiment, the IC_{83} (inhibitory concentration for 83% of the tested population) was estimated because it is considered to be the highest impact point that can be reliably determined. The IC_{83} was obtained by calculating a log-linear plot of sample concentration versus percent light decrease (or more precisely, by log-log plotting of gamma versus concentration, where gamma is the corrected ratio of the amount of light lost relative to the controls). All Microtox™ calculations were done using MicrotoxOmni™ (Strategic Diagnostics, Inc., Newark, Delaware).

RESULTS

Test Conditions

For acute water-only exposures, measured glutaraldehyde concentrations were essentially constant over the 24 h exposure with an average change of ± 3% (SD 10%) between the beginning of the exposure period (t = 0 h) and the end (t = 24 h). This difference reflects the measurement error of the analytical method in addition to errors associated with sample dilution. For the water-only exposures, the initial concentration of the samples was used to calculate the LC values.

The organic carbon content of the sediment used to evaluate the impact of sediment on glutaraldehyde toxicity averaged 2.6 ± 0.4%. Other physical characteristics of the selected sediment were not assessed as it was assumed that the major determinant of efficacy would be organic carbon content since glutaraldehyde is known to react with organic amines. Possible background contaminant levels were not determined as persistant sediment-associated contaminants generally require longer exposures to achieve toxic responses, e.g., sediment bioassays for acute response are of 10 d duration (U.S. EPA 1999), and there were no known specific sources of contamination in the watershed. The high survival rate of control organisms and the short exposure duration of the experiments suggest that background sediment contamination likely did not have a large impact on toxicity results.

For the water-sediment exposures, the glutaraldehyde concentration exhibited a decline from the start to the end of the experiment of 27% (SD 15%). In contrast to the water-only exposures, this decline in concentration is attributed primarily to the dilution of glutaraldehyde into the sediments and to reactions of glutaraldehyde with the sediments and associated organic material. The LC values for these experiments were based on water samples collected between 1 to 4 hours after glutaraldehyde addition.

Temperature ranges for the different experiments were within 1°C of culture temperatures and did not fluctuate more than 1°C over the course of the 24 h exposure. Dissolved oxygen levels were greater than 5 mg L^{-1} for every experiment and did not fluctuate substantially over the course of 24 h. Similarly, pH values were relatively constant for each water type and did not fluctuate appreciably over the course of the experiments.

Water-only Bioassays

The estimated LC_{50} and LC_{90} values for the combined results of independent experiments are based on measured concentrations of glutaraldehyde (Table 1). Most of the lethal concentrations generated from the independent experiments using the same species demonstrated good agreement, with significant overlap of the 95% confidence intervals for both the LC_{50} and the LC_{90} values . The single exception was one of the water-only experiments employing *H. azteca*. For the two independent experiments, there was significant overlap of the LC_{90} estimates, but not of the LC_{50} estimates (the first experiment had 95% CI of 200–283 mg L^{-1}, while the second experiment had 95% CI of 288–377 mg L^{-1}).

Data from the water-only bioassays indicate that organism sensitivity to glutaraldehyde varied greatly. The least sensitive (most resistant) organism tested was *H. azteca* (Fig. 1). The LC_{90} for *H. azteca* was 550 mg L^{-1} (95% CI: 476–681). The most sensitive organism was *C. dubia*, with a 24 h LC_{90} value of 11 mg L^{-1} (95% CI: 11–13) for neonates and 12 mg L^{-1} (95% CI: 11–14) for adults. These data indicate that *C. dubia* is approximately 32 times more sensitive to glutaraldehyde than *H. azteca* based on the 24 h LC_{50} estimate. The rest of the organisms had LC_{90} values that were equal to, or substantially less than, 100 mg L^{-1}. The single

TABLE 1. Combined concentration-response data from water-only and water-sediment experiments. Data from individual bioassays for the same species have been grouped in order to generate the lethal concentrations and associated 95% confidence intervals.

Organism	# of Exps	Temp (°C)	H_2O:Sed Ratio	LC50 (mg L^{-1})	95% CI (mg L^{-1})	LC90 (mg L^{-1})	95% CI (mg L^{-1})
H. azteca	2	24	1:0	289	257–318	550	476–681
H. azteca	2	24	8:1	286	237–327	543	476–653
H. azteca	2	24	4:1	323	286–354	563	499–685
H. azteca	2	24	2:1	325	194–412	738	628–932
L variegatus	2	19	1:0	11.1	10.3–11.5	12.9	12.3–13.9
L variegatus	2	19	8:1	31	27–35	61	52–78
L variegatus	2	19	4:1	79	70–86	134	117–170
L variegatus	2	19	2:1	230	198–252	356	322–423
C. dubia neonates	2	24	1:0	9	8–9	11	11–13
C. dubia adults	2	24	1:0	10	9–11	12	11–14
D. magna neonates	2	24	1:0	14	13–15	18	16–21
D. magna adults	2	24	1:0	56	51–61	102	89–125
V. fischeri	2	10	1:0			IC83 = 11	8–14

exception was *D. magna* adults, which had an LC_{90} of 102 mg L^{-1} (95% CI: 89–125), while all of the other organisms tested had LC_{90} values less than 20 mg L^{-1}.

V. fisheri also proved sensitive to glutaraldehyde. The 5 min IC_{83} value averaged 78 mg L^{-1}, and the 15 min IC_{83} averaged 11 mg L^{-1} (Table 1). The decrease in IC values over time indicated increased inhibition over longer exposure periods.

Results from the 24 h bioassays also indicate the potential for variations in sensitivity of different life stages. Both *C. dubia* neonates and adults demonstrated similar sensitivity to glutaraldehyde. Neither the 24 h LC_{50} nor the 24 h LC_{90} value were significantly different (based on overlap of 95% CI intervals of LC values: Table 1, Fig. 2a). In contrast, neonates of *D. magna* were substantially more sensitive than adults to glutaraldehyde (Fig. 2b). Neonates had a 24 h LC_{50} value of 14 mg L^{-1} (95% CI: 13–15). Adults were 4 times less sensitive than neonates, with a 24 h LC_{50} value of 56 mg L^{-1} (95% CI: 51–61).

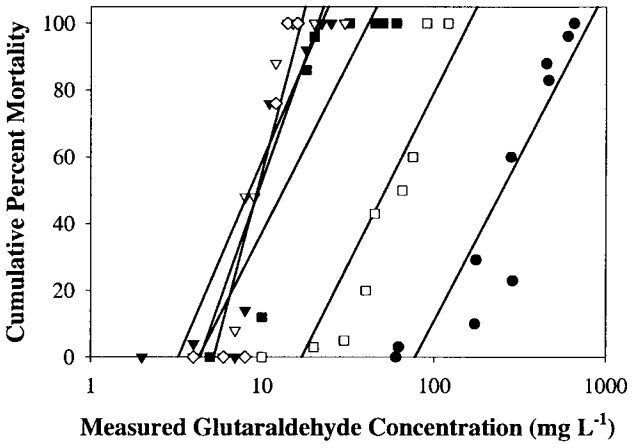

***FIG. 1. Comparison of 24 h acute toxicity data from water-only exposures. Data have been log-transformed in order to highlight differences in organism sensitivity to glutaraldehyde.* ● H. azteca, □ D. magna *adults,* ■ D. magna *neonates,* ∇ C. dubia *adults,* ▼ C. dubia *neonates,* ◇ L. variegatus.**

Water-sediment Bioassays

The results from the water-sediment bioassays differed considerably between the two organisms tested. For *H. azteca*, the 24 h LC_{90} values for the three different water-sediment ratios did not significantly differ (based on overlap of the 95% CI). Further, the LC values for the water-sediment bioassays were comparable to those derived for the water-only exposures (Table 1, Fig. 3).

For *L. variegatus*, the 24 h LC_{90} values were substantially higher in the water-sediment exposures for all the ratios tested (Table 1, Fig. 4). In this case, the water-sediment ratio had a large impact on biocide efficacy: a decreasing ratio of water-to-sediment (less water, more sediments) resulted in a substantial increase in the 24 h LC values. The 24 h LC_{50} estimate for the 4:1 ratio was 2.6 times higher than that for the 8:1 ratio, and the estimate for the

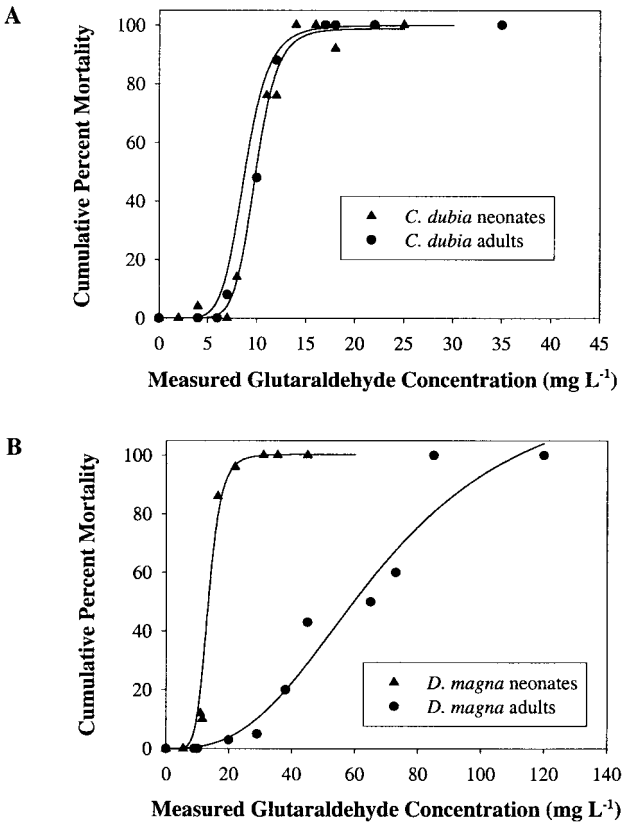

FIG. 2. Comparison of concentration-response data for the two different life stages of the cladocerans: A) Data for* C. dubia, *B) Data for* D. magna. *The lines for the concentration-response data represent the best fit of the data using a logistic (3-parameter) model.

2:1 ratio was 7.4 times higher than that for the 8:1 ratio.

Ballast Tank Simulations

After the 10 day grow-out period, survival of control organisms was > 100% for *L. variegatus* (due to organism reproduction) and > 78% for *H. azteca*. The survival rate of *L. variegatus* in the 500 mg L^{-1} treatments averaged 5.6% (SD 5%), and survival rates for *H. azteca* were zero in all of the exposures. No other macro-organisms that may have come from diapausing eggs were observed in the treatments after the 10-d grow-out period.

Glutaraldehyde concentrations decreased dramatically after dilution with Huron River water. Samples of water taken for analysis indicated that glutaraldehyde concentrations dropped to approximately 80 mg L^{-1} upon initial dilution (i.e., the first day of the grow-out period) and were undetectable by the final day of the grow-out period (day 10).

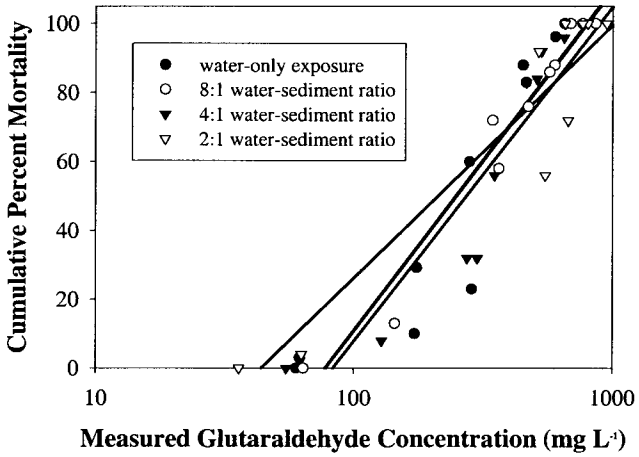

FIG. 3. Comparison of concentration-response data for* H. azteca *under water-only and water-sediment exposure conditions. Lines represent best fit of log-transformed data based on a simple linear regression model. Note that data from the water-only and 8:1 water-sediment ratio are similar and the lines overlap.

The organic carbon content of the sediment from the second ballast tank collection was similar to

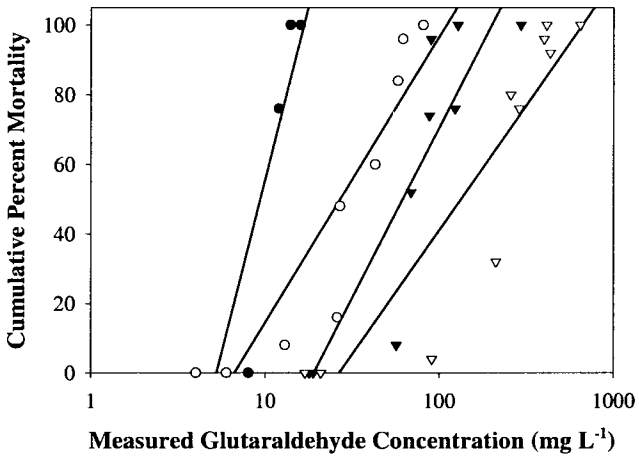

FIG. 4. Comparison of concentration-response data for* L. variegatus *under water-only and water-sediment exposures. Lines represent best fit of the data using a simple linear regression model. ● water-only exposure, ○ 8:1 water-sediment ratio, ▼ 4:1 water-sediment ratio, ∇ 2:1 water-sediment ratio.

that selected for the bioassays to determine the influence of sediment on glutaraldehyde toxicity. Thus, the selection of an organic rich sediment for examining the influence of sediment appeared to be an appropriate selection.

DISCUSSION

To assess the potential efficacy of glutaraldehyde in treating NOBOB vessels, we employed 24 h acute toxicity bioassays in water-only and water-sediment environments. Although most standard toxicity bioassays range in duration from 48 to 96 h, we selected a relatively short exposure duration for three reasons: 1) it provides a more conservative (maximum) estimate of required biocidal concentrations; 2) it may represent a time period during which high biocide concentrations can be maintained without significant degradation; and 3) it allows for potential "emergency" treatment of vessels at the St. Lawrence Seaway prior to entrance into the Great Lakes. For the mortality endpoint, we utilized the LC_{90} (90% lethal concentration). The majority of acute data are reported as LC_{50} values, since the LC_{50} is the most robust (least variable) statistical value. We selected the LC_{90}, instead, because it provides an estimate of concentrations required to achieve relatively high mortality rates, while still providing a statistical endpoint with reasonable bounds (e.g., compared to an LC_{99}). Using the LC_{90} to estimate treatment concentrations will not guarantee that no viable organisms will be released from the ballast tanks of vessels; however, removing up to 90% of viable organisms may significantly reduce propagule pressure and thereby decrease the overall risk of nonindigenous species introductions into the Great Lakes.

Although the ballast water and sediments of NOBOB vessels may contain a range of organisms, it was possible to test only a handful of organisms for the laboratory bioassays. The species selected for this study are standard testing organisms. They have been adopted as such because they meet several important criteria for toxicity testing including being readily available (since they can be cultured and maintained under laboratory conditions), exhibiting broad tolerance of testing conditions (especially the physico-chemical characteristics of sediment), having broad geographic distribution, and having a large toxicological database (U.S. EPA 1999). In addition, these organisms provide a wide range of sensitivity: *L. variegatus* is used in sediment toxicity bioassays because it is known to be relatively resistant to many chemical toxicants, particularly heavy metals (Schubauer-Berigan *et al.* 1993, Phipps *et al.* 1995, U.S. EPA 1999). In contrast, *H. azteca* has generally been found to be more sensitive to many compounds relative to other organisms (Ankley *et al.* 1991, West *et al.* 1993, Moore *et al.* 1998). This trend, however, does not apply across all test compounds. Most studies have found that organism sensitivity is largely chemical specific and that interspecific variation in response is due mainly to differences in chemical toxicity and not to intrinsic sensitivity differences between species (Vaal *et al.* 1997, Vaal *et al.* 2000). To compensate for this variability, we chose aquatic test organisms that span different phyla in order to generate a range of responses. The goal of this approach was to generate a range of LC values for glutaraldehyde, not to assess efficacy against any single species.

The results from the water-only bioassays indicate that glutaraldehyde can be effective against a range of freshwater organisms. The concentration of glutaraldehyde required to eliminate tested organisms was less than 20 mg L^{-1} for 70% of the test organisms (5 out of 7 organisms and life stages). However, glutaraldehyde was relatively ineffective at eradicating the amphipod, *H. azteca*. The LC_{90} for this species was 5 times higher than that for the next most resistant organism (*D. magna* adults). Both of these organisms were of the adult stage and presumably more resistant to glutaraldehyde than younger life stages. The observed discrepancies in LC values from these experiments indicate that some ballast water organisms may be quite resistant to glutaraldehyde treatment: Based on these data, eliminating most organisms may require concentrations of glutaraldehyde up to 500 mg L^{-1}.

Results using Geddes Pond sediments (organic carbon 3%) indicate that the amount of sediments in ballast tanks may substantially impact glutaraldehyde efficacy. Although the LC values for epibenthic *H. azteca* did not differ substantially between the water-only and water-sediment exposures, there was a large difference in LC values for *L. variegatus*. The LC_{90} values for the 2:1 water-sediment exposure using *L. variegatus* was 27 times higher than that for the water-only treatment. Further, the LC values for *L. variegatus* in sediment demonstrated a consistent increase with decreasing water-sediment ratios (i.e., less water, more sediment). In contrast, the LC_{90} values for *H. azteca* in different exposure environments were almost identical across water-sediment ratios. The differences between these two

species can be attributed to their ecology: *H. azteca* is an epibenthic organism that resides on the surface of sediments, while *L. variegatus* is wholly benthic and exists almost entirely within the sediment matrix (except to defecate, in which case part of the oligochaete emerges from the sediment). The increase in LC_{90} values demonstrated by *L. variegatus* indicates that a higher amount of sediment (in relation to water) may afford benthic organisms greater protection against glutaraldehyde. Although only one sediment source was employed for these bioassays, this effect will likely pertain to other sediment types as well: If there is sufficient sediment refugia for benthic organisms (such as oligochaetes), then higher amounts of biocide will be needed to eliminate these organisms. This effect may be particularly pronounced in ballast tanks that contain several inches of sediment and only a small amount of water. In these situations, the ability of glutaraldehyde (and perhaps any biocide) to penetrate compacted sediment and kill viable organisms may be limited.

Experiments using material taken from NOBOB vessels were conducted to provide both an assessment of glutaraldehyde efficacy at a single concentration and an indication of the potential for degradation upon dilution with natural waters. The results from these simulation experiments indicate that glutaraldehyde treatment of up to 500 mg L^{-1} was effective in eliminating all transplanted *H. azteca* and most *L. variegatus* from sediments after 24 h of exposure. Because only one concentration was tested, there is no method for estimating an LC_{90} for direct comparison with water-sediment bioassays; however, these results indicate that this concentration was effective against tested organisms using NOBOB sediments. Ballast tank sediments, however, also contain a variety of other biota and the efficacy of glutaraldehyde against these organisms was not assessed in this study. Although no macrobiota were observed in the treatment vessels after the 10-d growth period, there is no way to specifically determine whether glutaraldehyde was effective against any of the organisms already present in the sediments because the quantity and type of pre-existing organisms in the sediment could not be determined prior to testing. The results from these ballast tank simulations corroborate the findings from the water-sediment bioassays and indicate that a treatment concentration of 500 mg L^{-1} of glutaraldehyde may be effective in eliminating a large percentage of ballast tank organisms. In addition, the large amount of degradation that occurred after dilution of the test sediments indicates that glutaraldehyde treatment may pose a minimal risk for release into receiving waters.

The data from this study can be compared to acute toxicity values for glutaraldehyde from other studies using different organisms and different experimental conditions (see Leung 2001). Most of the available data for glutaraldehyde toxicity to freshwater organisms come from water-only exposures using fish species. In 96 h water-only static exposures, the reported LC_{50} values for fish species ranged from 3 mg L^{-1} for coho salmon, *Oncorhynchus kisutch*, (SFU 1993) to 12 mg L^{-1} for the rainbow trout, *Oncorhynchus mykiss* (UCC 1978). Although not stated explicitly, these results are likely for juvenile fish, following standard ASTM (American Society for Testing and Materials) guidelines. These LC estimates are lower than many of the LC_{50} values reported in this study, and indicate that juvenile fish may be quite sensitive to glutaraldehyde, particularly with extended exposure times. There is one published report of an LC_{50} of 5 mg L^{-1} for a 48 h exposure using *D. magna* (UCC 1981). This estimate is for a stock solution of 100% glutaraldehyde, and an adjustment is customarily made to account for differences in the base stock solution (Leung 2001). Because most standard bioassays use 24 h neonates of daphnids for acute toxicity bioassays, this value compares to the 24 hr LC_{50} of 14 mg L^{-1} estimated from this study (and using a 50% glutaraldehyde solution). Based on this result, maintaining concentrations of glutaraldehyde for longer than 24 h may decrease the amount of biocide required to treat vessels; however, the length of time during which glutaraldehyde concentrations are maintained will likely vary depending on the presence of sediments and the temperature of the ballast tanks.

Although this study was limited to testing glutaraldehyde toxicity to freshwater organisms (with the exception of the marine bacterium, *V. fischeri*), other studies have reported acute toxicity values for a range of marine/estuarine species (again, see Leung 2001). Similar to the results reported here, marine/estuarine species exhibited a wide range of sensitivity to glutaraldehyde, going from a 48 h LC_{50} of 0.11 mg L^{-1} for the calanoid copepod, *Acartia tonsa*, (SPL 1997) to a 96 h LC_{50} of 465 mg L^{-1} for the green crab, *Carinus maenas* (UCC 1975). Thus, the marine/estuarine species tested did not appear overtly more or less sensitive to glutaraldehyde, but instead exhibited a range of sensi-

tivity similar to the freshwater organisms tested in this work.

The results from these experiments provide a general overview of the potential efficacy of glutaraldehyde in treating ballast tanks. Although several different types of freshwater organisms were tested, many important organisms were not, including different types of resting stages (such as diapausing eggs) and nonindigenous species that have already established in the Great Lakes (such as the zebra mussel, *D. polymorpha*). Data from NOBOB vessels indicate that resting stages, in particular, may form an important component of the nonindigenous fauna inhabiting NOBOB tanks (Bailey *et al.* 2003). Hatch-out experiments demonstrate that these dormant eggs can arrive in a viable condition in ballast tank sediments and can hatch in a short period of time, releasing larval planktonic stages. The efficacy of glutaraldehyde in deactivating these stages is an important consideration in its overall efficacy in treating NOBOB vessels.

Several other factors may impact glutaraldehyde efficacy and were not considered in this set of experiments. These include temperature effects (e.g., lower temperatures may decrease biocide efficacy, while higher temperatures may enhance biocide effects), and the composition of the water and sediments (e.g., higher organic content or different particle-size distributions) may alter biocide efficacy either by interacting with active biocide or by altering the ability of benthic organism to burrow into the sediments.

The relatively high concentrations of glutaraldehyde indicated from this study have two important implications with respect to ballast water treatment: The cost of glutaraldehyde treatment and the potential for adverse environmental impacts. A study by Lubomudrov *et al.* (1997) provided a preliminary estimate of costs associated with glutaraldehyde treatment of varying volumes of residual water and sediment. Using the concentration estimate of 500 mg L^{-1} and a treatment volume of 200 metric tons (see Farley 1996), the estimated cost of glutaraldehyde is on the order of $5,000 per treatment (assuming a cost of $8/lb of glutaraldehyde). This cost does not include auxiliary expenses associated with hardware installation, administrative costs, or field testing to demonstrate that biocidal concentrations were achieved.

Higher concentrations of glutaraldehyde are also associated with an increased risk of adverse environmental impacts. The magnitude of these impacts will be related to the rate at which glutaraldehyde degrades (it is degraded by organisms under aerobic conditions to CO_2) and the amount of dilution of the compound prior to release into the Great Lakes (since NOBOB vessels take on a substantial amount of lake water, and non-biocidal concentrations will be more readily degraded by microbes). The potential chronic effects of exposure to this biocide will need to be investigated before it can be accepted as a viable management alternative.

The results from this study have important implications for biocide treatment of ballast water in general. As with glutaraldehyde, it is likely that most other biocides will demonstrate a wide range of efficacy against possible ballast tank organisms. The variability in effectiveness will be particularly large if sediments are present that harbor benthic organisms and/or resting stages**.** Both the physical and chemical characteristics of these sediments may also impact biocide efficacy: sediments that are more compacted or that have higher organic carbon content may further decrease biocide efficacy. In cases where sediments are present, biocides that persist for a longer period of time (i.e., those that are less likely to react with organic material) may be more effective than rapidly degrading (or oxidizing) compounds. The trade-off with more persistent biocides, however, is an increased possibility of adverse environmental impacts when the ballast tank material is released into receiving waters. The efficacy of biocide treatment may be dramatically improved if sediments are regularly removed from ballast tanks either through frequent tank cleaning or filtration of water prior to treatment.

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