

Comparative Efficacy of Potential Chemical Disinfectants for Treating Unballasted Vessels

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ABSTRACT. *The release of ballast water from transoceanic vessels is a major vector for the introduction of nonindigenous species into the Laurentian Great Lakes. This study assessed the effectiveness of treating unballasted transoceanic vessels using three different biocides: glutaraldehyde plus a surfactant adjuvant (Disinfekt 1000®), sodium hypochlorite (NaOCl), and SeaKleen™ (menadione and menadione metabisulfite 2:8). Efficacy against several classes of aquatic organisms was evaluated using 24 h acute toxicity experiments and 11 day ballast tank simulation experiments. The results indicate substantial, compound-specific variations in organism sensitivity. For water-only exposures, NaOCl and SeaKleen™ were most effective: NaOCl had the lowest LC₉₀ (90% lethal concentration value) for the oligochaete Lumbriculus variegatus (1.0 mg L⁻¹), while SeaKleen™ had the lowest LC₉₀ for the amphipod Hyalella azteca (2.5 mg L⁻¹). Sediments profoundly affected efficacy, particularly for NaOCl: At a 1:4 sediment-water ratio, the estimated LC₉₀ for L. variegatus was > 2,000 mg L⁻¹. Sediment quality also impacted efficacy: Sediments with higher organic carbon content typically required greater biocide concentrations to achieve comparable toxicity. Efficacy was further evaluated with 11 day bioassays using sediments from unballasted vessels. Results indicated that NaOCl and Disinfekt 1000® were more effective than predicted based on small scale sediment-water exposures. Overall, the data suggest that although NaOCl may be effective under water-only conditions, the higher concentrations required in the presence of sediments may cause corrosion problems for ballast tanks. Because of this, less reactive, non-oxidizing biocides such as SeaKleen™ and Disinfekt 1000® may be better candidates for treating sedimented tanks.*

INDEX WORDS: *Ballast water, invasive nonindigenous species, biocides, Laurentian Great Lakes, glutaraldehyde, hypochlorite, SeaKleen.*

INTRODUCTION

The problem of aquatic nonindigenous species (NIS) in the Laurentian Great Lakes has received increasing scientific and public attention over the past two decades. For unintentional introductions, invasion theory predicts that the likelihood of a successful invasion is generally determined by factors such as propagule supply (e.g., the total number of arriving invaders, the condition of the arriving in-

vaders, etc.), the ability of the first arrivals to find food, shelter, and mates, and the ability of the initial population to grow and spread (Elton 1958, Mollison 1986, Williamson 1996, Lonsdale 1999). Although all of these factors are relevant to the Great Lakes, propagule supply has particular importance for helping reduce the risk of future invasions: If the total number of NIS released into the Great Lakes is reduced, then the likelihood of future introductions (and establishments) should decrease.

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In the Laurentian Great Lakes, one of the major vectors for the release of NIS is the ballast water of transoceanic vessels. Releases can be from either ballasted or unballasted vessels. The largest volume of water release is from vessels carrying ballast (ballast-on-board, or BOBs). These vessels enter the Great Lakes without cargo and instead carry a large amount of ballast water in order to maintain trim and stability. When these ships take on cargo at a Great Lakes port, they release their ballast water and any viable organisms into receiving waters. Ballasted vessels comprise the minority of vessels entering the Great Lakes (e.g., 11.5% in 2000; Eakins 2001) and are currently subjected to high-seas ballast water exchange. In contrast, unballasted vessels (those declaring no-ballast-on-board or NOBOBs) arrive into the Great Lakes carrying cargo. Despite their classification, these vessels contain residual ballast water and sediments due to the configuration of the ballast pumps, which prevents complete discharge of the tanks. The amount and distribution of water and sediments in these vessels is highly variable, with the total amount of residuals (water and sediments) ranging from negligible to 200 metric tones, of which sediments constitute up to 30% of total residual volume (T. Johengen, personal communication, Cooperative Institute for Limnology and Ecosystems Research, University of Michigan, Ann Arbor, MI). In terms of trafficking patterns, unballasted vessels generally travel to multiple Great Lakes ports and fill their tanks with Great Lakes water as they discharge cargo. This water mixes with the residual foreign water and sediment and then is usually released prior to exiting the lakes, when the vessels load outbound cargo. NOBOBs constitute the majority of oceanic vessels entering the Great Lakes (e.g., 88.5% in 2000; Eakins 2001) and are known to contain viable organisms (van Overdijk *et al.* 2003) and resting stages (Bailey *et al.* 2003). In addition, these vessels are not currently required to engage in ballast water management. Because they constitute the majority of oceanic vessels entering the Great Lakes, NOBOBs may pose the greatest risk for NIS introductions (MacIsaac *et al.* 2002).

One potential management approach for NOBOB vessels is to treat tanks with a biocide in order to reduce the number of viable organisms released (NRC 1996). Although there are a few published studies assessing the efficacy of different chemicals for treating ballasted vessels (Bolch and Hallegraeff 1993, Kuzirian *et al.* 2001, Tamburri *et al.* 2002), biocide treatment may be a more feasible option for

NOBOB vessels since a smaller amount of biocide is required for treatment (because these vessels carry substantially less water than ballasted ones) and the subsequent dilution with lake water may facilitate biocide degradation prior to release into receiving waters (Lubomudrov *et al.* 1997). Despite this potential, several critical issues regarding biocide use must be addressed before this approach can be considered viable. These issues include (1) determining concentrations required to achieve high mortality rates of representative aquatic organisms, (2) establishing the efficacy of biocide treatment in the presence of sediments, (3) establishing efficacy under operating conditions, and 4) evaluating risk for the discharge of residuals into receiving waters.

This project evaluated the comparative effectiveness of three different biocides for treating NOBOB vessels: (1) Disinfekt 1000[®], which is a combination of glutaraldehyde and a proprietary nonionic surfactant and has been formulated for use in poultry hatcheries and farm animal housing facilities; (2) Sodium hypochlorite (NaOCl), a form of active chlorine that has been used as a disinfectant for many decades and is particularly effective against bacteria and viruses; and (3) SeaKleen[™], a menadione (vitamin K3) salt mixture, which is a product developed specifically for ballast water treatment. This biocide became available late in the study and the number of experiments employing SeaKleen[™] is limited.

The primary objectives of this study were to establish the relative potency of the biocides, to determine a concentration that is effective in the presence of sediments, and to demonstrate effectiveness of Disinfekt 1000[®] and NaOCl in treating water and sediment from NOBOB vessels.

METHODS AND MATERIALS

Chemicals and Analytical Methods

The Disinfekt 1000[®] was supplied by Diversified Nutri-Agri Technologies, Inc. (Gainesville, GA, USA) and is a mixture (by volume) of 20% glutaraldehyde (CAS 111-30-8), approximately 79% of a proprietary nonionic surfactant, and approximately 0.2% of methanol. Concentrations of the active ingredient, glutaraldehyde, were measured using a spectrophotometric assay employing 3-methyl-2-benzothiazolinone hydrazone hydrochloride as the color-developing agent (Sawicki *et al.* 1962, Pakulski and Benner 1992). Actual glutaraldehyde concentrations were determined from a standard curve consisting of three concentrations

(0.5 mg L⁻¹, 1 mg L⁻¹, and 8 mg L⁻¹). The range of this method was 0.5–8.0 mg glutaraldehyde L⁻¹, and many solutions had to be diluted to fall within this sensitivity range. All Disinfekt 1000[®] concentrations are presented as mg glutaraldehyde L⁻¹.

Purified Grade NaOCl (4–6%) was obtained from Fisher Scientific (Fairfield, NJ, USA). Dilutions of the concentrated product were based on the manufacturer's estimate that the solution contained 5.65% NaOCl. A 1% or 0.1% NaOCl stock solution was made by measuring the appropriate amount of concentrated solution and diluting with deionized water (DW). Stock solutions were kept at 4°C in the dark. Prior to use, the chlorine concentration was measured for a 2 mg L⁻¹ solution made from the stock to ensure solution integrity. If the chlorine measurement dropped below 1.70 mg L⁻¹, the stock solution was discarded and a fresh one made. Chlorine content in mg L⁻¹ was measured using the VVR Water Analysis System from CHEMetrics, Inc. (Calverton, VA, USA). This photometric method measures the color intensity of N, N-diethyl-p-phenylenediamine when oxidized by free chlorine. Because the system measures chlorine in the range of 0–6 mg L⁻¹, it was often necessary to dilute samples with DW prior to measurement. The spectrophotometer was zeroed using the same water comprising the majority of the sample, e.g., DW, filtered well water (WW; Ann Arbor, MI, USA), filtered Huron River water (HRW; Dexter, MI, USA), or artificial culture media (as described below). If turbidity was present, the sample was centrifuged for 5 minutes at a setting of 100 on a DYNAC centrifuge (Parsippany, NJ, USA) in order to remove particles prior to chlorine measurement. All concentrations of hypochlorite are expressed as free chlorine.

The SeaKleen[™] was obtained from Vitamar Inc. (Memphis, TN, USA). The material tested was the wettable powder, consisting of menadione and menadione metabisulfite in a 2 to 8 ratio (Lot # 042602). SeaKleen[™] has been formulated specifically for ballast tank treatment, and no analytical technique was available for measuring active concentrations, therefore all reported concentrations are nominal, not measured. For bioassays, concentrations of SeaKleen[™] were made immediately prior to experimental use by creating a concentrated stock solution and diluting as appropriate for testing.

Test Organisms

Four different freshwater macroinvertebrates were tested: the amphipod *Hyalella azteca*, the

oligochaete *Lumbriculus variegatus*, the cladoceran *Daphnia magna*, and the zebra mussel *Dreissena polymorpha*. For SeaKleen[™], only *L. variegatus* and *H. azteca* were tested. In addition, the algae *Pseudokirchneriella subcapitata* (formerly *Selenastrum capricornutum*, Hindák 1990) and *Artemia* spp. cysts (a representative resting stage) were tested with NaOCl and Disinfekt 1000[®].

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 kept 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 at the Hudson Mills Metro Park, Dexter, Michigan, USA (total hardness: 165 mg L⁻¹ as CaCO₃; 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 1 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 2000).

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 (> 500 nm). Well water (total hardness: 500 mg L⁻¹ as CaCO₃; total alkalinity: 5.00 meq L⁻¹; pH: 7.2–7.8) was used for the culture and acquired from the USGS, Great Lakes Science Center (Ann Arbor, MI, USA). 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.

Daphnia magna sub-adults were obtained from Aquatic Biosystems, Inc. (Fort Collins, CO, USA). The organisms were maintained in an aerated 1-gallon aquarium of HRW and fed a combination of *P. subcapitata* and a yeast, Cerophyll[®], Trout Chow

mixture (U.S. EPA 1993). Organisms were kept at 24°C on a 16:8 light:dark photoperiod.

Dreissena polymorpha were obtained from Lake Michigan with a benthic sled trolling along the 30 m depth contour west of Muskegon, MI. The organisms were maintained for less than 1 week in an aerated 50-gallon aquarium and fed approximately 1 g daily of dried *Chlorella* (obtained from a local aquarium supply store). Prior to use, the mussels were separated into petri dishes (10–13 each) and submerged back into water for 24 h to attach to the dishes. Only organisms that attached to the petri dishes were used for testing.

Artemia spp. embryos were obtained from Fisher Scientific (Pittsburgh, PA, USA). The embryos are provided as an encysted metabolically inactive gastrula stage (i.e., in a diapause state), and possess an outer shell that provides protection against environmental insults. The cysts were maintained in their original packaging prior to use in the bioassays.

Test Sediments

Three different sediment types were used for these experiments: Terwilliger's Pond (TP) sediment was collected from Terwilliger's Pond on South Bass Island, Lake Erie (41.6567° N and 82.8276° W); Lake Michigan (LM) sediment was obtained west of Muskegon, MI, USA at a depth of 110 meters (43.1914° N and 86.5378° W); and Gallup Park (GP) sediment was collected from the northeast area of Geddes Pond, just east of Huron Parkway, Ann Arbor, MI, USA (42.2973° N and 83.7220° W) 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). These sediments varied in organic carbon content: the amount of organic carbon was highest for Terwilliger's Pond sediment (6.5 ± 0.3 % OC on a dry weight basis). Gallup Park sediment contained the second highest organic carbon content (2.6 ± 0.4%), while the Lake Michigan sediment had the lowest organic carbon content (0.49 ± 0.05%). Other physical characteristics (such as particle size distribution) were not assessed.

TEST METHODS

Water-only Exposures

The efficacy of the three biocides under water-only exposures was assessed using 24 h static

bioassays (except for experiments using *D. polymorpha*, which extended to 48 h). A summary of the treatment conditions and associated number of experiments is provided in Tables 1–3. Experiments were conducted in accordance with ASTM (1998a) and U.S. EPA (1993) protocols. Organisms were added to test 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 usually contained five organisms (for a total of 25 organisms per treatment per experiment), although some experiments used ten organisms per replicate (while maintaining the same water:organism ratio). Experiments were usually repeated, depending on data quality. Organisms were tested in their respective culturing water, with controls consisting of the same dilution water as the treatments: *H. azteca*, *D. polymorpha*, and *D. magna* were tested in Huron River water (HRW) that was filtered with a 0.45 µm and a 0.22 µm Millipore filter (Bedford, MA, USA). *L. variegatus* was tested in well water (WW) that was filtered through 0.45 µm and 0.22 µm Millipore filters (Bedford, MA, USA).

All water-only experiments were conducted in the dark in an incubator held at ±1°C of the temperature of the cultures. Samples to determine biocide concentration were collected at the start of the experiment. For hypochlorite, these samples were taken immediately after addition of the organisms to the experimental vessels. At the end of the experiment, the temperature, pH, DO, and biocide concentration were measured in all of the test chambers except for hypochlorite experiments, in which biocide concentration was measured in one chamber only. No samples were taken for SeaKleen™ because no method was available for measuring actual concentrations. Organism condition was assessed in terms of alive/dead or mobile/immobile.

The experimental protocol for *D. polymorpha* differed slightly. On the day prior to the experiment, zebra mussels were removed from culture and separated into 9-cm glass petri dishes, with 10–13 individuals per dish. The petri dishes were submerged in untreated filtered HRW, and the organisms were allowed to attach to the substrate. To initiate the experiment, the petri dishes containing the organisms were randomly distributed to test vessels and the dosing solution added. These experiments consisted of three replicates of each of five concentrations and a control. At 24 and 48 h, organism status (alive/dead) was assessed based on response of the mussels to the touch of a blunt probe.

TABLE 1. Hypochlorite data from water-only and sediment-water experiments. Concentration-response data for individual bioassays of the same species have been grouped to generate LC values and associated confidence intervals. The LC data reflect organism status after 24 hr of exposure, except for *D. polymorpha* (which reflects cumulative mortality at 48 hr) and *Artemia* spp. cysts (which reflects cumulative mortality at 72 hr). Values greater than 10 have been rounded to the nearest whole number. GP: Gallup Park sediment; LM: Lake Michigan sediment; TP: Terwilliger's Pond sediment.

Organism	# of Exps	Temp (°C)	Sed:H ₂ O Ratio	Sed. Type	LC50 (mg L ⁻¹)	95% CI (mg L ⁻¹)	LC90 (mg L ⁻¹)	95% CI (mg L ⁻¹)
<i>H.azteca</i>	2	24	0:1	none	3.7	3.5–3.9	4.7	4.4–5.1
<i>H.azteca</i>	2	24	1:8	GP	43	40–50	55	48–81
<i>H.azteca</i>	1	24	1:4	GP	67	61–72	106	94–129
<i>L.variegatus</i>	2	19	0:1	none	0.70	0.68–0.73	1.0	0.94–1.06
<i>L.variegatus</i>	2	19	1:8	GP	42	34–49	85	71–115
<i>L.variegatus</i>	2	19	1:4	GP	110	66–178	2,904	1,178–16,827
<i>L.variegatus</i>	2	19	1:4	LM	75	35–138	11,561	2,642–379,315
<i>L.variegatus</i>	1	19	1:4	TP	1,014	637–1,977	25,586	8,790–187,500
<i>L.variegatus</i>	2	10	0:1	none	1.0	0.9–1.3	1.6	1.4–1.9
<i>L.variegatus</i>	2	10	1:4	GP	71	30–113	653	406–1,581
<i>D.magna</i>	2	23	0:1	none	0.4	0.4–0.5	0.7	0.6–0.9
<i>D.polymorpha</i>	2	20	0:1	none	23	17–29	130	96–203
<i>Artemia</i> cysts	2	27	0:1	none	0.3	0.1–0.7	53	28–147

If mussels were closed, the probe was gently inserted in the gape and if no adductor muscle activity was noted, the organism was considered dead (Fisher *et al.* 1999).

An additional set of water-only exposures was conducted at 10°C using *L. variegatus*. Organisms were acclimated by decreasing the temperature of the culture water by 2°C every 48 hours. After reaching 10°C, the organisms were maintained at this temperature for 60 hours prior to the initiation of the experiment. The experimental set-up and organism isolation were conducted in a 10°C cold room in order to avoid significant temperature fluctuations. The rest of the protocol was identical to the 24 h bioassays described above.

Algal Growth Experiments

Ninety-six-hour growth experiments using the green alga species, *Pseudokirchneriella subcapitata* (formerly *Selenastrum capricornutum*) were conducted according to ASTM (1998b) and U.S. EPA (1994) standards. These exposures were conducted in 250-mL Erlenmeyer flasks using artificial algal media (U.S. EPA 1994). Each flask contained 100

mL of test media per replicate, with four replicates per concentration and six concentrations (including control) per experiment. The flasks were inoculated with approximately 10,000 cells mL⁻¹ of algae that were in an exponential growth phase. Test flasks were then placed on an orbital shaker operating at 100 rotations per minute (rpm). The flasks were maintained at 25°C with a 16h:8h light:dark cycle. Full spectrum lights (American Environmental Products, Fort Collins, CO) were used, which provided an approximate uniform illumination of 86 $\mu\text{E m}^{-2} \text{ s}^{-1}$. Samples for estimating algal density were collected at 0, 72, and 96 hours after test initiation. Cell density was estimated both by spectrophotometric analysis (Geis *et al.* 2000) and confirmed with manual cell counts using a hemacytometer (Bright-Line counting chamber, Hausser Scientific, Horsham, PA, USA).

Artemia Exposures

The design of the *Artemia* bioassays was based on the methods outlined in U.S. EPA (1993). All bioassays were conducted in synthetic salt water prepared using deionized water (DW) and Instant

TABLE 2. *Disinfekt 1000*[®] data from water-only and sediment-water experiments. Concentration-response data for individual bioassays of the same species have been grouped to generate LC values and associated confidence intervals. The LC data are for mortality assessed at 24 hr for most organisms except for *D. polymorpha* (which reflects cumulative mortality at 48 hr) and *Artemia* spp. cysts (which reflects cumulative mortality at 72 hr). Values greater than 10 have been rounded to the nearest whole number. GP: Gallup Park sediment; LM: Lake Michigan sediment; TP: Terwilliger's Pond sediment.

Organism	# of Exps	Temp (°C)	Sed:H ₂ O Ratio	Sed. Type	LC50 (mg L ⁻¹)	95% CI (mg L ⁻¹)	LC90 (mg L ⁻¹)	95% CI (mg L ⁻¹)
<i>H. azteca</i>	2	24	0:1	none	189	174–205	272	243–329
<i>H. azteca</i>	2	24	1:8	GP	166	145–183	277	246–336
<i>H. azteca</i>	2	24	1:4	GP	230	203–256	496	419–641
<i>H. azteca</i>	2	24	1:2	GP	284	250–318	575	494–721
<i>L. variegatus</i>	2	19	0:1	none	6.3	5.2–7.2	7.6	6.8–13
<i>L. variegatus</i>	2	19	1:8	GP	39	34–45	76	63–104
<i>L. variegatus</i>	2	19	1:4	GP	118	102–137	248	200–361
<i>L. variegatus</i>	2	19	1:2	GP	236	201–272	551	447–767
<i>L. variegatus</i>	2	10	0:1	none	16	15–16	18	18–20
<i>L. variegatus</i>	2	10	1:4	GP	21	15–28	84	62–131
<i>L. variegatus</i>	2	19	1:4	LM	59	53–63	75	70–87
<i>L. variegatus</i>	2	19	1:4	TP	157	134–185	443	340–682
<i>D. magna</i>	2	23	0:1	none	21	19–23	33	30–40
<i>D. polymorpha</i>	2	20	0:1	none	21	9.6–28	50	39–85
<i>Artemia</i> cysts	3	27	0:1	none	27	19–37	353	224–671

TABLE 3. *SeaKleen*[™] data from water-only and sediment-water experiments. Concentration-response data for individual bioassays of the same species have been grouped to generate LC values and associated confidence intervals. The LC data are for mortality assessed after 24 hr of exposure. Values greater than 10 have been rounded to the nearest whole number. GP: Gallup Park sediment.

Organism	# of Exps	Temp (°C)	Sed:H ₂ O Ratio	Sed. Type	LC50 (mg L ⁻¹)	95% CI (mg L ⁻¹)	LC90 (mg L ⁻¹)	95% CI (mg L ⁻¹)
<i>H. azteca</i>	1	24	0:1	none	1.7	1.4–1.9	2.5	2.1–3.3
<i>H. azteca</i>	1	24	1:4	GP	1.9	1.6–2.2	3.5	2.9–4.8
<i>L. variegatus</i>	1	19	0:1	none	1.2	1.1–1.3	1.8	1.6–2.3
<i>L. variegatus</i>	1	19	1:4	GP	25	16–35	88	59–171

Ocean[®] (Aquarium Systems, Mentor, OH, USA), a commercially available synthetic sea salt mixture. Water quality parameters including alkalinity, pH, and dissolved oxygen were determined for quality control and consistency between stocks. To initiate the experiment, forty *Artemia* cysts were isolated into 30-mL polystyrene cups, and the dosing solutions then added to each cup (including the controls). The cysts were exposed for 24 h in the dark,

at 27°C. At the end of this period, cysts were transferred to replacement vessels containing untreated synthetic seawater and maintained for an additional 48 h grow-out period. During the grow-out period, the water was replaced after 24 h, and the test vessels were maintained at 27°C with a 16h:8h light:dark photoperiod. Organisms were observed daily for hatch success.

Preliminary studies were conducted to assess the

hatching success and viability of organisms under control conditions. These tests followed the bioassay protocol as outlined above. After 48 h, hatch rate success ranged from 45% to 65% (which is comparable to results from other studies; MacRae and Pandey 1991, Sarabia *et al.* 1998). Because hatching percentage fell well below 100%, Abbott's Correction (Abbott 1925) was used to normalize the toxicant exposure data to control values.

Sediment-water Exposures

Sediment-water bioassays were conducted in a similar manner to water-only exposures. Treatment conditions are summarized in Tables 1–3. Biomass loading was kept the same as in the water-only exposures, and experiments were conducted for 24 h in unlit incubators held at the temperature of the cultures ($\pm 1^\circ\text{C}$). In contrast to water-only bioassays, sediment-water 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 sediment:water (vol/vol) ratio. For experiments using *L. variegatus*, the oligochaetes and approximately 10 mL of water were added to the sediment and the worms were allowed to burrow for approximately 20 minutes. The appropriate concentration of biocide was then added to the test chamber, accounting for dilution due to sediment volume. Final concentrations were therefore set for the total volume of sediment plus water of the exposure system. For *H. azteca*, the organisms were added to the test chambers after the addition of the dosing solution. Once the experiment was initiated, test flasks were placed on an orbital shaker set at 66 rpm to simulate mixing as might occur in a ballast tank.

At the end of the experiment, the contents of the beakers 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 HRW. Organism status was then assessed and recorded. Recovery rate for *L. variegatus* (based on controls) was approximately 98%. For *H. azteca*, organisms were also removed from the filter and placed in deionized water. In some cases, *H. azteca* were directly removed from the chambers and placed in a separate container to facilitate recovery. Organism status was assessed in terms of mobility (either

mobile/immobile). Recovery rates averaged 97% for controls.

Ballast Tank Simulation Experiments

Toxicity tests employing ballast tank sediments were performed twice using both Disinfekt 1000[®] and hypochlorite. Ballast tank sediments were collected using sterile scoops and spatulas and generally taken from the longitudinal shell frames of the tanks that tend to trap sediments (Bailey *et al.* 2003). The ballast sediments used for these experiments were obtained from the following source ships: For experiments using Disinfekt 1000[®], the sediments for the first experiment came from the port tanks of a bulk carrier originating from Holland, and the sediments for the second experiment came from the port tank of a bulk carrier originating from Venezuela. These sediments had an organic carbon content of $2.65 \pm 0.09\%$ and $5.96\% \pm 0.44\%$, respectively. Salinity for these samples ranged from 22 parts per thousand for the first experiment and 6 parts per thousand for the second experiment. For toxicity tests using hypochlorite, sediments for the first experiment were collected from the starboard tank of a bulk carrier originating in Shanghai. Sediments for the second experiment were collected from the forepeak tank of a bulk carrier originating from Ghent. The sediments from the first experiment had an organic carbon content of $1.57 \pm 0.11\%$ and a salinity of 37 parts per thousand, and the sediments for the second experiment had an organic carbon content of $3.16 \pm 0.22\%$ and a salinity of 4 parts per thousand. The protocol for the simulation experiments differed from that for the sediment-water experiments because these bioassays were performed to assess efficacy at a single concentration and under more realistic exposure conditions. The simulation experiments were initiated by weighing out 1 L of ballast sediment into each of four 20-L carboys. Into each of the carboys, 2 L of prefiltered HRW was added, together with 30 *L. variegatus* and 30 *H. azteca*. The oligochaetes were allowed to burrow for approximately 20 minutes prior to disinfectant addition. Two liters of the appropriate concentration of biocide were then added to the test vessels, accounting for dilution due to the volume of sediments and water to yield 250 mg L⁻¹ of Disinfekt 1000[®] and two concentrations of hypochlorite, 1,000 and 2,000 mg L⁻¹. Thus, the concentrations were set for the total water plus sediment volume and yielded a 1:4 sediment-water ratio. Once the experiment was

initiated, the carboys were placed on an orbital shaker set at 45 rpm to simulate ship movement. Exposures were conducted for 24 h in the dark, at 21°C. After 24 h, the toxicant solutions were drained from the carboys, and the remaining sediment mixture was diluted with approximately 20 L of prefiltered HRW. A 10-day grow-out period was then initiated at 21°C, with a 16h:8h light regimen and with aeration, to prevent oxygen depletion. Organism mortality was assessed at the end of the grow-out period. The contents of the carboys were sieved (using a 90 µm dia. sieve and unfiltered HRW) to retain the organisms and their status assessed.

Data Analysis

Acute data were analyzed by estimating the 24 h (or 48 h) LC₅₀ and LC₉₀ values and associated 95% confidence intervals with logit analysis using SYSTAT Version 8.0 (SPSS, Chicago, IL, USA, 1998). The mortality plots for the bioassays were made using Sigma Plot 4.0 (SPSS, Chicago, IL, USA, 1986–1997). As mentioned previously, for the *Artemia* bioassays, Abbott's Correction (Abbott 1925) was used to normalize the toxicant exposure data to control values prior to logit analysis.

RESULTS

Water-Only Exposures

Sodium Hypochlorite

The organisms tested demonstrated varying sensitivity to hypochlorite based on the LC₅₀ and LC₉₀ values (Table 1). The lowest LC₅₀ value of 0.47 mg L⁻¹ was for *D. magna* (95% C.I. 0.42–0.54), while *D. polymorpha* demonstrated the greatest resistance to hypochlorite, with an LC₅₀ value of 23 mg L⁻¹ (95% C.I. 17.1–29.4). The discrepancies between the organisms were even more pronounced for the LC₉₀ values: *D. magna* had an LC₉₀ value of 0.7 mg L⁻¹ (95% C.I. 0.6–0.9) and *D. polymorpha* had a value of 129.7 mg L⁻¹ (95% C.I. 96.2–202.8). The difference in LC values indicate that the concentration-response curve for *D. magna* is much steeper (greater) than that for *D. polymorpha*, signifying that lower incremental concentrations of hypochlorite result in larger increases in mortality rates for *D. magna* compared to *D. polymorpha*.

The *Artemia* ephippia were moderately sensitive to hypochlorite as measured by the concentration to yield 50% reduction in hatch (0.3 mg L⁻¹; 95% C.I.

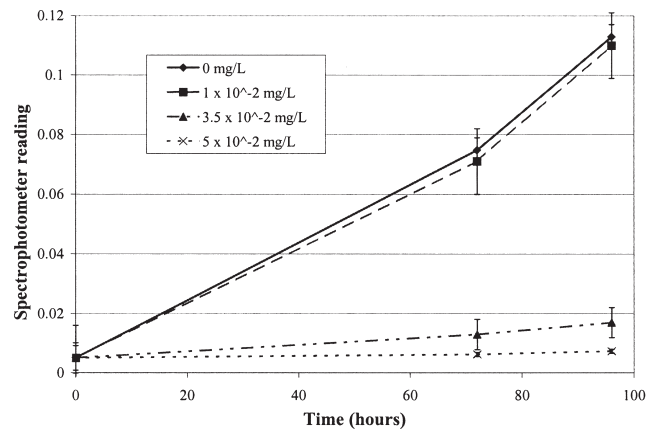


FIG. 1. Hypochlorite concentration-response data for 96 h bioassays using the alga, *Pseudokirchneriella subcapitata*.

0.1–0.7); however, the slope of the concentration response curve is shallow, and the concentration required for 90% reduction was much higher (53.0 mg L⁻¹; 95% C.I. 27.5–146.6).

Results from the algal growth experiments indicated decreased growth in *P. subcapitata* occurring at concentrations between 3.5×10^{-2} mg L⁻¹ and 5×10^{-2} mg L⁻¹ (Fig. 1). Data indicated a decrease in growth at all concentrations greater than 1×10^{-2} mg L⁻¹, while all concentrations greater than 5×10^{-2} mg L⁻¹ effectively eliminated all the algae (resulting in no growth).

Disinfekt 1000®

The freshwater organisms tested with Disinfekt 1000® also demonstrated substantial differences in sensitivity (Table 2). The lowest estimated LC₅₀ value was for *L. variegatus* (6.3 mg L⁻¹; 95% C.I. 5.2–7.2), while the highest estimated LC₅₀ was for *H. azteca* (189 mg L⁻¹; 95% C.I. 174–205). The zebra mussel was intermediate in its response to Disinfekt 1000®, which was substantially different from that observed for the hypochlorite. The *Artemia* cysts were also intermediate in their response.

The LC₉₀ values generally followed the same trend as the LC₅₀ values (Table 2), with the notable exception of the *Artemia* cysts, which had a higher LC₉₀ value compared to *H. azteca* (LC₉₀ 353 mg L⁻¹; 95% C.I. 224–671 and 272 mg L⁻¹; 95% C.I. 243–329, respectively). As with the hypochlorite, the *Artemia* ephippia exhibited a shallow dose response curve such that the LC₅₀ value is moderate

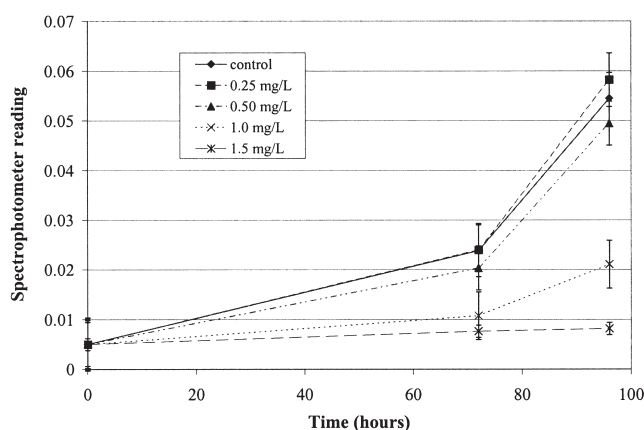


FIG. 2. Disinfekt 1000[®] concentration-response data for 96 h bioassays for the alga, *Pseudokirchneriella subcapitata*.

compared to other organisms, while the LC₉₀ is in the upper range.

Algal growth experiments using Disinfekt 1000[®] indicated no decrease in growth up to a concentration of 0.5 mg L⁻¹, however, concentrations greater than, and equal to 1 mg L⁻¹, demonstrated a significant decrease in growth compared to controls (Fig. 2).

SeaKleen[™]

Toxicity tests using SeaKleen[™] were performed to provide preliminary information for comparison with the other disinfectants, however only two organisms were employed (Table 3). As with the other disinfectants, the LC₅₀ value observed for *L. variegatus* (1.2 mg L⁻¹; 95% C.I. 1.1–1.3) was somewhat lower than that for *H. azteca* (1.7 mg L⁻¹; 95% C.I. 1.4–1.9), with the LC₉₀ values exhibiting the same trend. For both organisms, the slope of the toxicity curve is steep, such that only small incremental increases in concentration were required to achieve 90% mortality (1.8 mg L⁻¹ for *L. variegatus* and 2.5 mg L⁻¹ for *H. azteca*).

Temperature Effects

Temperature affected the toxicity of both hypochlorite and Disinfekt 1000[®] to *L. variegatus* (Tables 1 and 2). The LC₅₀ and LC₉₀ values increased with a decrease in temperature, indicating the compounds were less toxic at the lower temperature. For hypochlorite, the decrease in temperature caused the LC₅₀ value to increase by a factor of 1.4 and the LC₉₀ value to increase by a factor of 1.6. For Disinfekt 1000[®], the LC₅₀ value increased by a

factor of 2.5 at the lower temperature and the LC₉₀ value increased by a factor of 2.4.

Water-sediment Exposures

Sodium Hypochlorite

Adding sediment to exposure chambers dramatically altered lethal concentrations for both *H. azteca* and *L. variegatus* (Table 1). For *H. azteca*, the LC₅₀ value was 43 mg L⁻¹ (95% C.I. 40–50) for a 1:8 sediment-water ratio and increased to 67 mg L⁻¹ (95% C.I. 61–72) for a 1:4 sediment-water ratio. The magnitude of the increase in the LC₉₀ values was slightly larger. Similarly, the LC₅₀ value for *L. variegatus* in a 1:8 sediment-water exposure using GP sediments rose to 42 mg L⁻¹ (95% C.I. 33.7–48.6), and the LC₉₀ rose to 85 mg L⁻¹ (95% C.I. 70.8–114.6). These estimates changed dramatically when the sediment-water ratio was elevated to 1:4, with the LC₉₀ value increasing to 2,904 mg L⁻¹ (95% C.I. 1,178–16,827).

In contrast to the water-only exposures (in which toxicity decreased with decreasing temperature), the toxicity of hypochlorite to *L. variegatus* in the presence of sediments at the lower temperature was greater by a factor of about 5 for the LC₉₀ values; however, the LC₅₀ values were not statistically different (110 mg L⁻¹; 95% C.I. 66–178 at 19°C compared to 71 mg L⁻¹; 95% C.I. 31–113 at 10°C).

The source of sediments also altered hypochlorite toxicity (Table 1). The LC₅₀ values for *L. variegatus* bioassays in GP and LM sediment were similar (110 mg L⁻¹ and 75 mg L⁻¹, respectively). However, the LC₅₀ value for *L. variegatus* using TP sediment was substantially higher at 1,014 mg L⁻¹ (95% C.I. 637–1,977). The organic carbon content of the TP sediment is higher than for the other two sediments suggesting that hypochlorite reacted with the organic material in the sediments. In addition, there was considerable variability in the *L. variegatus* LC₉₀ values for the different types of sediment (2,904, 11,561, and 25,586 mg L⁻¹ for the GP, LM, and TP sediments respectively). In all cases, 100% mortality was not obtained at the tested concentrations, and the LC₉₀ estimates therefore have a large error value. *H. azteca* was not tested in the different sediments.

Disinfekt 1000[®]

As with the hypochlorite, both sediment source and sediment-water ratio affected Disinfekt 1000[®] toxicity (Table 2); however this effect was largely

limited to *L. variegatus*. For *H. azteca*, LC values using GP sediment at a 1:8 sediment-water ratio were similar to those from water-only exposures. In contrast, the LC values at a 1:4 and 1:2 sediment-water ratio were higher than the value at a 1:8 ratio, showing minimal effect of sediments on toxicity to *H. azteca*. For *L. variegatus*, the impact of sediment was more profound, but not as dramatic as with hypochlorite. The LC₅₀ value at a 1:8 sediment-water ratio was 39 mg L⁻¹ (95% C.I. 34–45), while the value at a 1:2 sediment-water ratio was 236 mg L⁻¹ (95% C.I. 201–272). This added amount of sediment resulted in a 6-fold increase in the LC₅₀ values. The increase in LC₉₀ values was of a similar magnitude: The value for the 1:2 ratio was 7 times higher than the value for the 1:8 ratio.

The quality (i.e., organic content) of the sediments further impacted Disinfekt 1000® efficacy. The sediment with the lowest organic content (LM sediment) was associated with the lowest LC₅₀ and LC₉₀ values (59 mg L⁻¹ and 75 mg L⁻¹, respectively). The LC values for the sediment with the highest organic carbon content (TP sediments) were substantially larger: The LC₅₀ value was 157 mg L⁻¹ (95% C.I. 134–185), and the LC₉₀ value was 443 mg L⁻¹ (95% C.I. 340–682).

SeaKleen™

As with the other two compounds, the presence of sediment affected the toxicity of the SeaKleen™, although this effect was limited to *L. variegatus* (Table 3). At a 1:4 sediment-water ratio (the only ratio tested for this compound), the LC₅₀ value for *L. variegatus* was 21 times higher than the value for water-only exposures, and the LC₉₀ value was 49 times higher. The presence of sediment had less of an impact on the LC₉₀ value for *H. azteca* (with a difference of a factor of less than 2 between the water-only value and the 1:4 sediment-water value). The efficacy of SeaKleen™ was not tested in different sediment types.

Ballast Tank Simulation Experiments

Sodium Hypochlorite

The selected concentrations for experiments using hypochlorite were based on the toxicity test results from the 1:4 sediment-water exposures. At both of the hypochlorite concentrations tested, the *H. azteca* and *L. variegatus* suffered 100% mortality (Table 4). In addition, there was mortality of *H. azteca* in the control that was likely due to the

TABLE 4. Summary of ballast tank simulation experiments employing Disinfekt 1000® and sodium hypochlorite. Each concentration had two replicates, denoted by the A, B label. The experiments were performed twice, using different sediment sources.

Experiment	Nominal Conc. (mg L ⁻¹)	Mortality (%)		
		<i>H. azteca</i>	<i>L. variegatus</i>	
Disinfekt 1000®				
1 ¹	0 A	23.3	0	
	0 B	30	0	
	250 A	100	100	
	250 B	100	100	
2 ²	0 A	3.3	0	
	0 B	13.3	0	
	250 A	100	100	
	250 B	100	100	
Hypochlorite				
3 ³	0 A	33.3	0	
	0 B	20	0	
	2,000 A	100	100	
	2,000 B	100	100	
	4 ⁴	0 A	20	0
		0 B	10	0
1,000 A		100	100	
1,000 B		100	100	

¹Port of origin: Holland (Organic carbon content: 2.65 ± 0.09%; Salinity: 22 parts per thousand)

²Port of origin: Venezuela (Organic carbon content: 5.96 ± 0.44%; Salinity: 6 parts per thousand)

³Port of origin: Shanghai (Organic carbon content: 1.57 ± 0.11%; Salinity: 37 parts per thousand)

⁴Port of origin: Ghent (Organic carbon content: 3.16 ± 0.22%; Salinity: 4 parts per thousand)

physical abrasion of the rotating sediments. Control survival for *L. variegatus* was 100%. The lowest concentration of hypochlorite (1,000 mg L⁻¹) used in this experiment was much lower than the concentration expected to cause toxicity based on the sediment-water experiments. It is unknown from these results whether a lower concentration of hypochlorite would be as effective as the 1,000 mg L⁻¹ concentration employed in this experiment.

Disinfekt 1000®

In each of the two ballast tank simulation experiments, 100% mortality was observed for the organisms in the dosed carboys after the 10 day grow-out

period. Organism recovery for control vessels varied, and was essentially 100% for *L. variegatus*, while losses were recorded for *H. azteca* (Table 4). The size of the simulations, together with the relatively large volume of sediment used, made the recovery of organisms difficult. While mortality was not expected for the controls, the motion of the vessels may have impacted *H. azteca* survival. In contrast, for the controls, the number of *L. variegatus* recovered after the grow-out period exceeded the number originally incorporated (due to propagation).

DISCUSSION

The use of a 24 h exposure period to estimate biocide efficacy represents a potential minimum time frame required for ballast water treatment. For vessels that are treated prior to a transoceanic transit, the actual biocide exposure period might be much longer, depending on the length of time the biocide persists in a ballast tank environment. Shorter exposure times (such as the 24 h time frame employed here) may be appropriate both for biocides that decay rapidly and for vessels that arrive at the St. Lawrence Seaway without any prior ballast water treatment. Calculation of the LC₅₀ and LC₉₀ values used for this study provides a general estimate of the concentration required to achieve 50% mortality and 90% mortality, respectively. The former endpoint provides the most stable statistical value for estimating a lethal concentration. In comparison, the LC₉₀ provides a more desirable mortality rate in terms of ballast water treatment, but is also characterized by much higher variability. The selection of an LC₉₀ value as opposed to an LC₉₉ value provides an endpoint that can reasonably be estimated and return a calculable error estimate without requiring the use of a substantially larger number of organisms.

Based on the water-only exposures, the two most effective biocides were sodium hypochlorite and SeaKleen™. The hypochlorite was most effective against *L. variegatus* and *D. magna*. For these two organisms, the LC₉₀ was less than 5 mg L⁻¹. SeaKleen™ also proved very effective against *H. azteca* and *L. variegatus*, but was not tested on any other freshwater organism. The estimated LC₉₀ for these two organisms was less than 2.5 mg L⁻¹. Although there are currently no published data to compare for SeaKleen™, the results for hypochlorite are generally comparable to those reported elsewhere: In water-only exposures, free chlorine (measured as

hypochlorous acid and hypochlorite ion) has been found to be quite toxic to many freshwater organisms at very low concentrations (with 24 h LC₅₀ values generally less than 1 mg L⁻¹, depending on the organism; see Mattice and Zittel 1976 for a review).

The least effective biocide under water-only conditions was Disinfekt 1000®. For all of the organisms tested, except one, the LC₅₀ and LC₉₀ values were considerably higher than those for either hypochlorite or SeaKleen™. The single exception was for *D. polymorpha*: For this organism, Disinfekt 1000® was more effective than hypochlorite, indicated by a lower LC₉₀ value (50 mg L⁻¹ for Disinfekt 1000® versus 130 mg L⁻¹ for hypochlorite, with no overlap of the 95% C.I.). The relatively low efficacy of hypochlorite against adult zebra mussels is consistent with findings from other studies that have demonstrated that *D. polymorpha* is able to avoid chlorine exposure by keeping their shell valves closed for an extended period of time (Rajagopal *et al.* 2002, Rajagopal *et al.* 2003), which is a common reaction of bivalves to chemical exposure (Doherty *et al.* 1987, Kramer *et al.* 1989, Tran *et al.* 2003). In order to achieve 100% mortality in adults, for example, Rajagopal *et al.* (2003) needed to continuously expose adult mussels to chlorine for approximately 25 days. This effect is also borne out in our bioassay results: The 48 h exposure period used for zebra mussels was not long enough to generate sufficient mortality rates largely due to avoidance of the hypochlorite by the mussels.

In order to assess potential efficacy against resting stages, bioassays were conducted using *Artemia* cysts. For the two compounds tested, hypochlorite was more effective than Disinfekt 1000®. The 24 h LC₉₀ value for hypochlorite was 53 mg L⁻¹, although the error term is large, signifying a high degree of uncertainty. In addition, the concentration-response curves for both hypochlorite and Disinfekt 1000® were relatively shallow, indicating that substantially higher concentrations of the biocides are required in order to achieve proportionally small increases in mortality rates. Because these bioassays were conducted in a water-only exposure, the LC values represent the lowest concentration of biocide required to eliminate *Artemia* cysts. In a ballast tank environment, resting stages are more likely to be located either on the surface of, or buried within, the sediments. Under these conditions, the actual concentrations required to eliminate this life history stage will likely be higher.

Several aquatic test organisms, spanning numer-

ous phyla, were selected for the water-only exposures. The only consistent trend in sensitivity was that *P. subcapitata* (phylum Chlorophyta) was the most sensitive organism to both hypochlorite and Disinfekt 1000®. The other organisms exhibited no obvious trend with respect to sensitivity. This finding is consistent with results from other experiments, which indicate both that organism sensitivity to most chemicals is compound specific and that interspecific variation is attributed primarily to differences in chemical toxicity, not to intrinsic differences in species sensitivity (Vaal *et al.* 1997, Vaal *et al.* 2000). It may be therefore difficult to predict whether a given biocide will be more or less effective against organisms from a certain class, order, or phyla.

Although the water-only exposures were useful for estimating the relative potency of the different biocides, they would not likely represent NOBOB ballast tank conditions, which often contain sediments. To address this factor, efficacy studies in the presence of sediments were conducted at different sediment-water ratios and using different source sediments (with varying organic carbon content) to better anticipate performance in NOBOB tanks.

The presence of sediments had a large impact on the efficacy of all three biocides; however, the magnitude and nature of this effect varied for the different compounds. Hypochlorite efficacy was most affected by sediments, with both the amount and quality of the sediments altering LC values, particularly for *L. variegatus*. This impact appeared to be two-fold: the sediments served as protective refugia for *L. variegatus*, and the organic material in the sediment reacted with hypochlorite, dramatically decreasing biocidal concentrations. For *L. variegatus*, both factors are likely important. In terms of refugia, for all sediment-water exposures, there was at least 2–3 cm of compacted sediments that was not affected by overlying water motion. This factor combined with degradation effects resulted in a dramatic increase in the LC₉₀ for hypochlorite, which was approximately 25,000 times the concentration required in water-only exposures. In contrast, the difference in efficacy was not as pronounced for *H. azteca* (an epibenthic organism) and appeared to be due mainly to the latter effect (i.e., the reactivity of hypochlorite with organic matter). The rapid degradation of hypochlorite observed in these studies is corroborated by results from Landrum *et al.* (2003): At 25°C, the estimated half-life of a 10 mg L⁻¹ hypochlorite solution is approximately 24 h (although this decay rate is not linear, and much of the

initial decay occurs within the first few hours). In addition, it is likely that lower concentrations of hypochlorite (< 2 mg L⁻¹) will disappear even more rapidly in the presence of organic material (Taylor 1993).

For Disinfekt 1000®, the presence of sediments minimally affected LC values for *H. azteca* but substantially increased LC values for *L. variegatus*. This difference is consistent with the ecology of the two organisms, since amphipods are epibenthic (residing primarily on surface sediments), while oligochaetes are benthic (burrowing into the sediments). Unlike hypochlorite, Disinfekt 1000® did not appear to react appreciably with organic material, and biocidal concentrations were maintained throughout the 24 h period. This is consistent with degradation results reported by Landrum *et al.* (2003) in which the half-life of a 100 mg L⁻¹ Disinfekt 1000® solution was 100 h at 25°C (much longer than the 24 h treatment period employed in the acute toxicity bioassays).

The impact of sediments on the toxicity of SeaKleen™ was similar to that for Disinfekt 1000®: there was minimal difference in the concentration required to treat *H. azteca*, but a large effect on concentrations required to treat *L. variegatus*. Again, this implies that the main effect of sediments was to provide a refugia for the oligochaete. As with Disinfekt 1000®, it is likely that the SeaKleen™ did not react strongly with organic material; however, because we did not measure actual concentrations, it is impossible to verify this.

To corroborate results from the sediment-water bioassays, we conducted several experiments using sediments from actual NOBOB vessels. The first experiment using hypochlorite indicated that the selected concentration (2,000 mg L⁻¹) may have been higher than required because it caused complete mortality of all transplanted organisms, and based on the sediment-water tests, some survival was expected. To compensate for this, the hypochlorite concentration for the second experiment was reduced by half: Even at this lower concentration, there was 100% mortality in transplanted organisms. These results are not consistent with data from the sediment-water experiments, which indicated an LC₉₀ value of approximately 3,000 mg L⁻¹ for *L. variegatus* at a 1:4 sediment-water ratio in the GP sediments (with an OC content of 2.6% which was roughly equivalent to that of the NOBOB sediments). This discrepancy may be explained, in part, by the larger surface area in the ballast tank simulation experiments: This resulted

in a thinner layer of sediments at the same sediment-water ratio used in the Erlenmeyer flasks and may have caused greater exposure of the oligochaetes to the biocide. In addition, the mixing action of the carboys was different than that of the flasks and may have caused greater agitation and thus higher biocide exposure, resulting in greater mortality. This increased agitation may have also resulted in some abrasion of the organisms, which could explain the relatively high mortality rates (approximately 20%) of *H. azteca* used in the controls. This mortality effect was consistent across experiments and using different ballast tank sediments with a range of physico-chemical characteristics.

The concentration of Disinfekt 1000[®] used for the ballast tank simulation experiments was 250 mg L⁻¹, and was effective in eliminating *L. variegatus* and *H. azteca*. These results are consistent with data from the sediment-water exposures; however, other concentrations were not tested, and thus it is unknown whether lower amounts of Disinfekt 1000[®] may have been equally effective against transplanted organisms (as was seen with the hypochlorite).

One of the motivations for using Disinfekt 1000[®] was to compare its efficacy to that of glutaraldehyde only. Disinfekt 1000[®] is a 20% glutaraldehyde solution containing a proprietary nonionic surfactant adjuvant to augment toxicity against microbial populations. Nonionic surfactants are widely used in agricultural applications, to enhance the effectiveness of pesticides (see Krogh *et al.* 2003 for a review). Results from water-only bioassays employing Disinfekt 1000[®] suggest that it is more effective than glutaraldehyde against the organisms tested in this study, but that this enhanced efficacy does not completely carry over to a sediment-water environment. In the water-only exposures, Disinfekt 1000[®] was approximately 40% more effective against *L. variegatus* based on both the LC₅₀ and the LC₉₀ values (Sano *et al.* 2003). The results were similar for *H. azteca*. In some applications, nonionic surfactants enhance efficacy by increasing delivery of a chemical to target sites, by either enhancing cell membrane permeability or disrupting membrane function (Rouse *et al.* 1994). In addition, nonionic surfactants also exhibit toxicity to different aquatic organisms, although the effect varies depending on the compound. For example, the toxicity of alcohol ethoxylates (a common type of nonionic surfactant) to *D. magna* increases with increasing ethoxylate chain length and can range

from a 48 h LC₅₀ of 0.46 mg L⁻¹ (for Neodol 23-5) to 12 mg L⁻¹ (for Dobanol 91-8; Wong *et al.* 1997). Because the surfactant formulation in Disinfekt 1000[®] is proprietary, it is not possible to compare these values with the surfactant concentrations in these bioassays to determine whether they approached biocidal levels.

In contrast to water-only results, Disinfekt 1000[®] did not demonstrate improved efficacy in the presence of sediments. Although Disinfekt 1000[®] was more effective than glutaraldehyde against *H. azteca* at the 1:8 and 1:4 sediment-water ratios, it was not more effective against *L. variegatus*. For the oligochaete, glutaraldehyde alone was actually more effective, although this difference was significant only at the 1:2 sediment-water ratio. The inability of the nonionic surfactant adjuvant to enhance toxicity in the presence of sediments may have been due to two factors: the presence of sediment may have provided enough physical protection to prevent the surfactant from gaining access to the organisms (in particular, *L. variegatus*) and/or the surfactant may have reacted with the sediments, either through adsorption to clay particles (Cano and Dorn 1996a, b; Brownawell *et al.* 1997) or through binding to organic carbon (Urano *et al.* 1984, but see also Cano and Dorn 1996a,b; Brownawell *et al.* 1997).

In addition to establishing the efficacy of the different biocides under conditions similar to those found in ballast tanks, the results from these experiments give an indication to the potential for environmental effects. Of all the organisms tested, the algal population proved most sensitive to both hypochlorite and Disinfekt 1000[®]. For hypochlorite, concentrations less than 0.005 mg L⁻¹ produced significant mortality. For Disinfekt 1000[®], the effect concentration was less than 1 mg L⁻¹. Because of this sensitivity, algal populations in receiving waters may be at highest risk due to environmental release of the two biocides. The actual magnitude of this risk will depend, in part, on the concentration and amount of biocide released into receiving waters and the timing of this release with respect to important ecological processes such as phytoplankton blooms. Because only one species of phytoplankton was tested, it is important to consider the possibility that other algal species may be more, or less, sensitive.

Another important consideration in identifying a potential biocide for NOBOB treatment is chemical cost. The biocides used in this study vary signifi-

cantly in per unit prices: Current market estimates range from \$15.75 gal⁻¹ for Disinfekt 1000[®] to \$1.00 gal⁻¹ for NaOCl. These are preliminary estimates and the actual per unit cost will also depend on the total quantity ordered and delivery prices. Using these estimates and based on the data from the ballast tank simulation experiments, the hypothetical cost to treat a NOBOB carrying 200 metric tons of residual water and sediment would range from approximately \$1,040 for Disinfekt 1000[®] (to treat at 250 mg L⁻¹) to \$536 for NaOCl (to treat at 1,000 mg L⁻¹). These estimates do not include the additional expenses associated with hardware installation, administrative costs, or potential field sampling to monitor biocidal concentrations.

The results from this study provide an assessment of the relative toxicity of three biocides under a range of controlled laboratory exposures. It is impossible to predict effectiveness in a ballast tank environment because the conditions of the tanks are likely to be variable. Differences in sediment amount and type in addition to changes in temperature will impact efficacy. In addition, although the organisms employed in these bioassays demonstrate a wide degree of sensitivity to toxicants, ballast tanks could possibly contain species that will be less sensitive and therefore require higher biocide concentrations. This may be particularly true for resting stages that reside in the sediments. In light of this, the objective for biocide use may be to achieve a certain level of disinfection (e.g., 75%, 85%, or 90%), with the realization that true sterilization (100% mortality) will not be possible (particularly when considering resistant resting stages such as bacterial endospores).

Decreasing the risk of future introductions of NIS into the Great Lakes will require a reduction in the number of viable organisms released from vessels. The results from this study indicate that biocides could be an effective method for reducing propagule pressure in the Great Lakes; however the effectiveness of any of the biocides will depend on ballast tank conditions. For reactive, oxidizing compounds such as hypochlorite, the presence of sediment and organic material poses two problems: Sediment can provide an effective refugia for benthic organisms, and organic matter can react with hypochlorite, thereby reducing biocidal concentrations. Although extremely high concentrations of hypochlorite could theoretically eliminate all organisms, such concentrations would also likely be corrosive to ballast tank materials. In comparison, non-oxidizing, less reactive compounds such as

SeaKleen[™] and Disinfekt 1000[®] are limited in the presence of sediment primarily because it serves as an effective refugia for benthic organisms. Decreasing the amount of sediment contained in NOBOB vessels may be one method for helping improve the effectiveness of these biocides. Whether they will be effective under field conditions can only be assessed, however, with field trials.

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