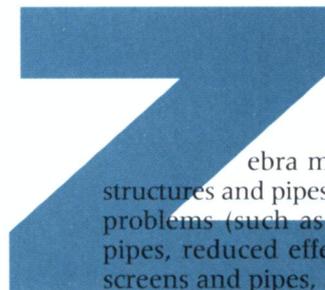




Toxicity of chlorine dioxide to adult zebra mussels

*Effective kills depend on the right combination
of concentration and exposure time.*

**Gerald Matisoff,
Gary Brooks,
and Brent I. Bourland**



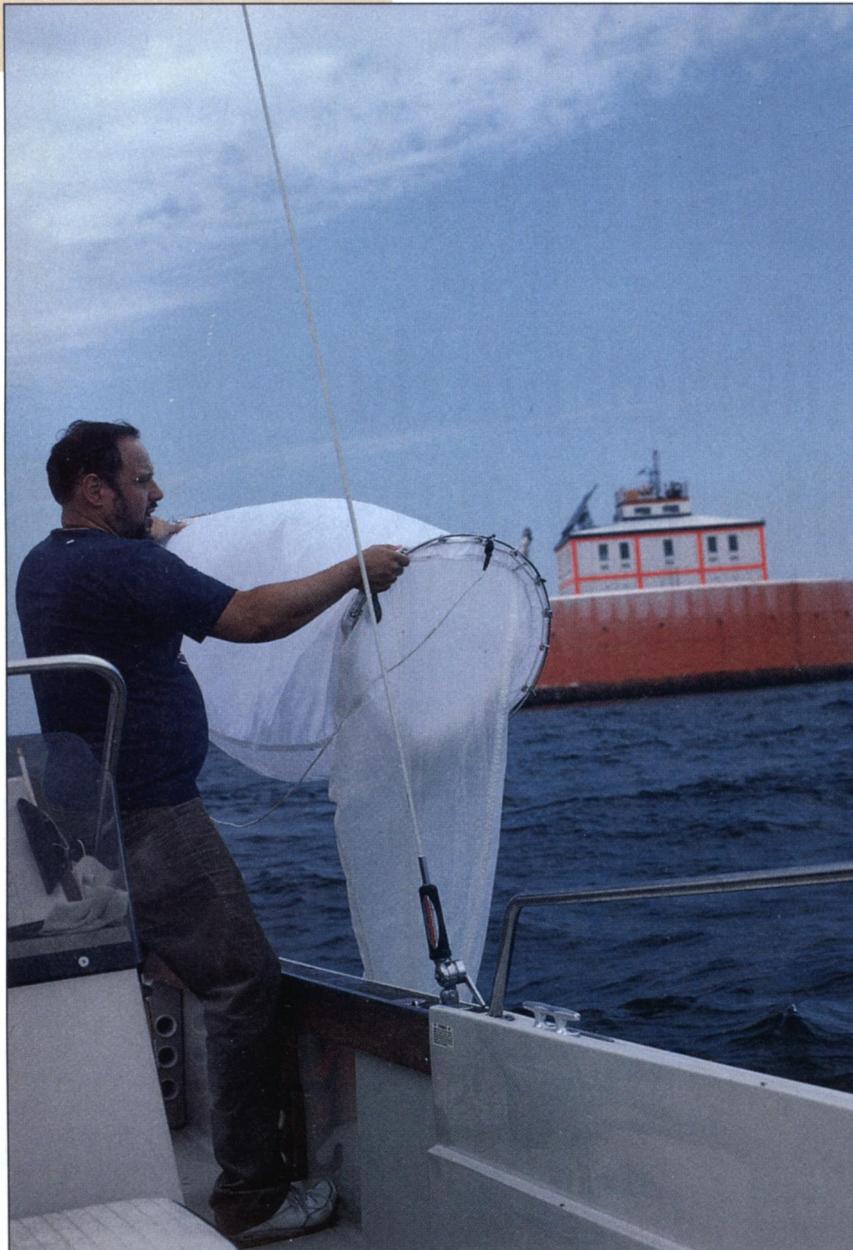
ebra mussel infestation of water intake structures and pipes has caused biofouling and related problems (such as increased frictional drag within pipes, reduced effective pipe diameter, clogging of screens and pipes, and waste disposal of zebra mussel debris^{1,2}) and has prompted the search for effective,

economical, and safe chemical control agents and treatment methodologies. Several potential strategies may be implemented to control biofouling by zebra mussels (*Dreissena polymorpha*). It can be prevented if zebra mussel veligers are either killed before they settle or kept from settling by the creation of an environment in which settlement is postponed.³⁻⁷

Chlorine dioxide (ClO₂) was evaluated as a control agent for adult zebra mussels using single, intermittent, and continuous exposures. A single 30-min exposure to 20 mg/L ClO₂ or higher concentration induced at least 50 percent mortality. NaOCl, KMnO₄, and H₂O₂ were ineffective under the same conditions. Intermittent chemical exposure for 30 min each day indicated that ClO₂ at concentrations of 10–20 mg/L can induce significant mortality over three to seven consecutive days (LC₅₀ = 13.0 mg/L), but 1–5 mg/L ClO₂ applied over 28 consecutive daily exposures are ineffective (<30 percent mortality). Identical intermittent exposure to NaOCl was ineffective at all tested concentrations up to 30 mg/L. Continuous exposure to ClO₂ for four days was effective at concentrations >0.5 mg/L (LC₅₀ = 0.35 mg/L), and 100 percent mortality at ClO₂ concentrations >1 mg/L was achieved. NaOCl and KMnO₄ were less effective. Pooled data yield median lethal dosages at which 50 percent mortality occurred of 14.5 and 82.5 mg h/L for ClO₂ and NaOCl, respectively.

*An earlier version of this article was published in *Proceedings: Third International Zebra Conference* (EPRI TR-102077), copyrighted by the Electric Power Research Institute in 1993.

Gerry Gubanich of Cleveland's Division of Water collects a plankton net sample near the water intake crib in Lake Erie for zebra mussel veliger monitoring.



Alternatively, control can be accomplished by periodically killing adult mussels with either chemical^{1,8-16} or physical¹⁷⁻²⁰ measures. Which strategy is optimal for a particular situation depends on the relative effectiveness of the different antifoulants on veliger survival and settlement, on the survival of attached mussels, on water quality effects caused by treatment, and on the costs of installing antifouling equipment and buying chemicals.

Physical control measures are not necessarily suitable for drinking water intakes because long, large-diameter pipes cannot be easily physically cleaned. Even if cleaning is possible, the procedures can be time-consuming and expensive. Chemical control

measures for nonpotable waters have used nonoxidants^{13,14,21,22} but often employ oxidants because of the requirement to supply a nontoxic end product. Traditionally, chlorine (Cl_2) has been the oxidant of choice, and its effectiveness against adult zebra mussels is well established.^{1,4,6,9-11,15,16,23-25} However, regulatory concerns about the ecological effects of Cl_2 and public health effects of chlorination-derived trihalomethanes (THMs) have spurred interest in the use of alternatives to Cl_2 .²⁴⁻²⁷ Moreover, intermittent treatment with Cl_2 is ineffective as a control methodology;^{1,8} Cl_2 must be continuously applied. Thus, Cl_2 might not be the best antifoulant for use against zebra mussels.

Ozone requires similar contact and lag times as Cl_2 , although it does have an advantage in not creating THMs, and it apparently effectively destroys the byssal threads.^{24,28} Cameron et al²⁶ determined the effectiveness of Cl_2 , potassium permanganate (KMnO_4), monochloramine, chlorine dioxide (ClO_2), and bromamine on the mortality of adult and juvenile Asiatic (*Corbicula fluminea*) clams. They report an LT_{50} (i.e., the median lethal time to 50 percent mortality) for juvenile clams of less than one day for 1 mg/L residual ClO_2 . Increased temperature also caused the LT_{50} for monochloramine to decrease.²⁹ Khalanski¹² reported that eight days of continuous ClO_2 at a mean residual oxidant concentration of 0.2 mg/L at 15°C successfully killed adult *Dreissena* at power plants on the Seine and

Moselle rivers. Preliminary work for this study also indicates that ClO_2 is an effective biocide,³⁰⁻³² successfully killing *Dreissena* at lower oxidant concentrations and exposure times than Cl_2 or KMnO_4 .

Successful facilities tests in the United States have used ClO_2 to kill adult zebra mussel infestations. At Evansville, Ind., on the Ohio River, the water plant is successfully controlling zebra mussel infestations with a subdemand ClO_2 feed of 0.6–0.7 mg/L. The Brockport, N.Y., water utility has used ClO_2 in the raw water as a bactericide. Before zebra mussels appeared in Lake Ontario, plant operators extended the point of introduction of ClO_2 2,800 ft (853.4 m) to the intake pipe. They feed continuously at <1 mg/L to maintain

In a batch exposure test of zebra mussels, author Gary Brooks injects ClO₂ into the sidestream experimental apparatus.

a 0.2–0.3-mg/L residual at the plant. They report that the treatment method successfully prevents infestation of the inside of their intake pipe, even though the outside has been extensively colonized. These results suggest that ClO₂ might be a more effective chemical for controlling zebra mussels than Cl₂ or KMnO₄.

Methods of applying oxidizers have included a single, end-of-season application; periodic single applications (similar to the end-of-season treatment); intermittent treatment (one or two low-dosage treatments once or twice a day); and continuous treatment (24 h a day for a specified length of time).²⁹ Selection of an appropriate application strategy requires knowledge about the effectiveness of the chemical; the animal's life cycle; physical and chemical factors affecting operations; and economic, safety, and regulatory concerns.³³

A single treatment at the end of the season is desirable because of cost-effectiveness, short treatment duration, chemical minimization, and less potential harm to the environment. However, this method allows mussels to accumulate within the system during a full growing season, generates considerable posttreatment shell debris and dead animal waste, and may use treatment dosages sufficiently high to require detoxification.

Intermittent treatment has all of the same benefits and none of the drawbacks. In addition, it is more likely to keep the system free of adult mussels throughout the season. However, as noted earlier, intermittent low-dosage treatment (with Cl₂) is generally considered ineffective for obtaining mortality of adult populations and marginally effective for con-



trolling settlement of veligers and juveniles. Tests using ClO₂ have not been performed on adults. Intermittent treatment with a higher dose (10–30 mg/L) has not been used in the past because of ineffectiveness and problems associated with detoxification, coloration, and handling of large volumes.

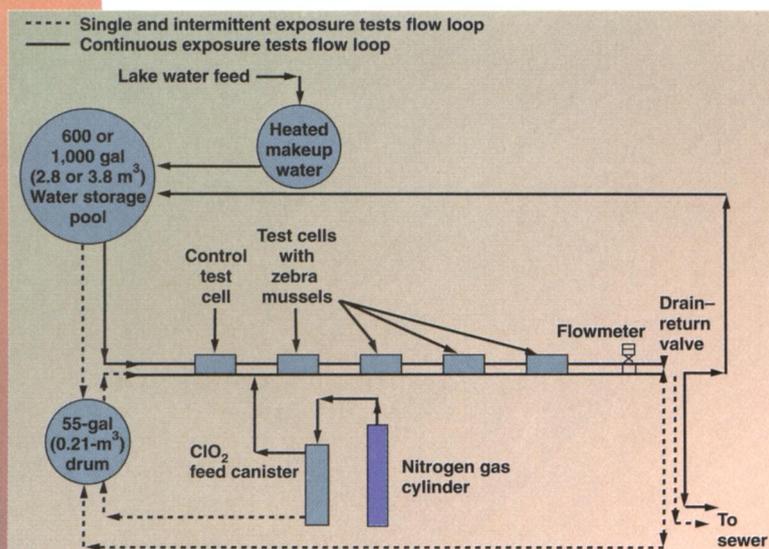
The approach used for this study was to determine the toxicity of ClO₂ to adult mussels in single-, intermittent-, and continuous-exposure tests. Several experiments were repeated using sodium hypochlorite (NaOCl), KMnO₄, or hydrogen peroxide (H₂O₂) to compare the relative effectiveness of the different oxidants and permit comparison of results using ClO₂ with those of other workers using other oxidants.

Materials and methods

ClO₂ generation. There are many ways of generating ClO₂, and the efficacy of the chemical as a molluscicide depends in part on the nature of the generating mechanism, especially if the generation method uses excess Cl₂. Most of the ClO₂ used during the experiment was generated on location with a patented* system. This system reacts sodium chlorite (NaClO₂) with Cl₂ gas under a vacuum before being diluted with water. Before this system was installed, ClO₂ was also obtained from a generator† at the water treatment plant in Ravenna, Ohio.

Collection of mussels. Adult mussels used in the first series of experiments from March to May 1992 were collected in February 1992 by a scuba diver who scraped large clumps of mussels from the Fairport Harbor, Ohio, crib. The animals were stored in an ice chest

FIGURE 1 Schematic of the experimental system



*WM Series, Rio Linda Chemical Co., Sacramento, Calif.

†WM Series, Rio Linda Chemical Co., Sacramento, Calif.

with Lake Erie water and returned to the laboratory, where they were separated into two groups. One group remained in the ice chest and received continuous flushing of untreated lake water (3°C); the other group was transferred to trays and brought to room temperature over seven days at the rate of 2–3°C a day. These animals were then placed in a 600-gal (2.3-m³) pool until needed.

Animals collected for the second series of experiments in October and November 1992 were collected from rocks along the Lake Erie shoreline near the experimental setup. In the second series of experiments the pool was larger (~1,000 gal [~3.8 m³]), and

because the water was warmer at the time of collection, the animals were placed directly in the large pool. The natural food supply in the water was supplemented with 0.15 g/d per individual of the marine algae *Chlorella* (Sun Chlorella A) by homogenizing 0.3 g of the dried algae with lake water and freezing the result into ice cubes for daily feeding (one ice cube per 10 individuals). Individual adult mussels in the size range from 10–20 mm were separated from clumps using a razor and were placed in a fish breeding basket until selected for use in a test cell.

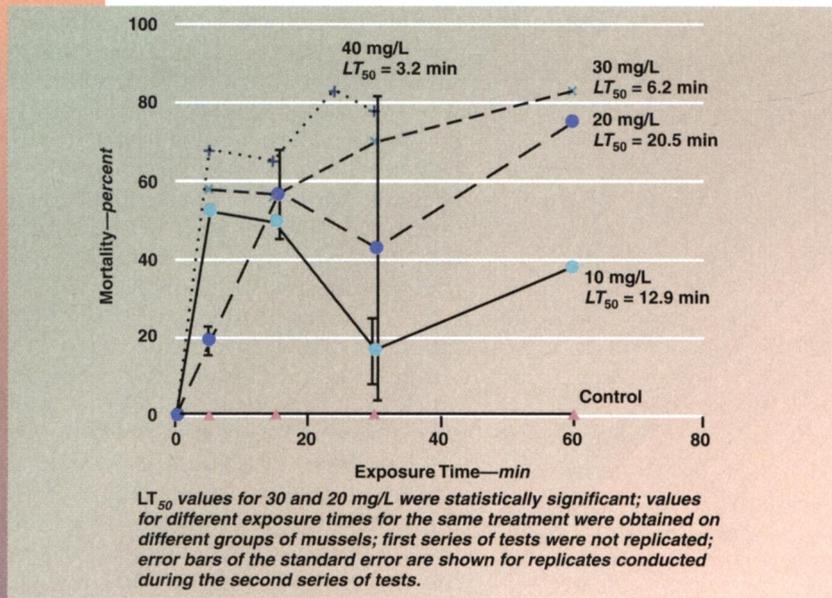
Experimental setup The experiments were conducted at the Cleveland, Ohio, Crown Water Filtration

TABLE 1 Target and measured residual oxidant concentrations during the single, intermittent, and continuous exposures of adult zebra mussels to ClO₂, NaOCl, KMnO₄, and NaClO₂

Treatment			Oxidant Dosage	Oxidant Concentration			
Type	Duration	Frequency		Mean	± Standard Error	Range	n
Single		15 min	10.0 mg/L ClO ₂	8.4			1
Single		30 min	10.0 mg/L ClO ₂	6.8			1
Single		60 min	10.0 mg/L ClO ₂	8.0	± 0.3	7.8–8.4	3
Single		15 min	20.0 mg/L ClO ₂	16.4	± 2.0	15.0–17.8	2
Single		15 min	20.0 mg/L ClO ₂	11.4			1
Single		15 min	20.0 mg/L ClO ₂	19.2			1
Single		30 min	20.0 mg/L ClO ₂	18.0	± 1.1	17.2–18.7	2
Single		15 min	30.0 mg/L ClO ₂	26.9	± 0.5	26.5–27.2	2
Single		30 min	30.0 mg/L Cl ₂ *	30.2	± 2.5	28.4–33.1	3
Single		30 min	30.0 mg/L Cl ₂ *	31.0	± 0.0	31.0–31.0	2
Single		30 min	30.0 mg/L KMnO ₄	27.3	± 0.8	26.7–27.9	2
Single		30 min	30.0 mg/L H ₂ O ₂	29.6	± 0.4	29.3–29.8	2
Single		30 min	30.0 mg/L Cl ₂ †	32.5	± 0.7	32.0–33.0	2
Single		15 min	40.0 mg/L ClO ₂				
Intermittent	28 days	30 min per day	1.00 mg/L ClO ₂	0.76	± 0.2	0.5–1.1	15
Intermittent	28 days	30 min per day	1.00 mg/L Cl ₂ *	0.88	± 0.2	0.5–1.1	16
Intermittent	21 days	30 min per day	3.00 mg/L ClO ₂	2.7	± 0.4	2.1–3.25	16
Intermittent	21 days	30 min per day	3.00 mg/L Cl ₂ *	2.8	± 0.3	1.92–3.1	14
Intermittent	28 days	30 min per day	5.00 mg/L ClO ₂	4.4	± 0.5	3.4–5.6	15
Intermittent	28 days	30 min per day	5.00 mg/L Cl ₂ *	4.5	± 0.3	4.0–5.0	13
Intermittent	3 days	15 min per day	10.0 mg/L ClO ₂	10.5	± 0.5	9.7–11.0	5
Intermittent	3 days	30 min per day	10.0 mg/L ClO ₂	10.6	± 0.8	9.6–11.6	8
Intermittent	3 days	30 min per day	10.0 mg/L ClO ₂	8.2	± 1.3	7.0–9.5	4
Intermittent	3 days	30 min per day	10.0 mg/L ClO ₂				
Intermittent	3 days	30 min per day	10.0 mg/L ClO ₂				
Intermittent	7 days	30 min per day	10.0 mg/L ClO ₂	9.1	± 0.6	8.2–9.7	8
Intermittent	7 days	30 min per day	10.0 mg/L Cl ₂ *	9.7	± 1.4	8.3–12.2	6
Intermittent	3 days	15 min per day	20.0 mg/L ClO ₂	14.2	± 2.9	11.2–16.9	3
Intermittent	3 days	15 min per day	20.0 mg/L ClO ₂	18.5	± 2.6	16.3–21.3	3
Intermittent	3 days	30 min per day	20.0 mg/L ClO ₂	16.1	± 1.5	14.6–18.6	6
Intermittent	3 days	30 min per day	20.0 mg/L ClO ₂	17.6	± 5.6	9.3–21.6	4
Intermittent	3 days	15 min per day	30.0 mg/L ClO ₂				
Intermittent	3 days	30 min per day	30.0 mg/L Cl ₂ *	27.4	± 4.0	22.0–33.1	7
Continuous			0.25 mg/L ClO ₂	0.27	± 0.1	0.08–0.67	51
Continuous			0.25 mg/L ClO ₂	0.28	± 0.04	0.24–0.36	13
Continuous			0.50 mg/L ClO ₂	0.51	± 0.08	0.36–0.69	25
Continuous			0.50 mg/L ClO ₂	0.48	± 0.08	0.25–0.95	15
Continuous			1.00 mg/L ClO ₂	1.03	± 0.19	0.50–1.64	49
Continuous			1.00 mg/L ClO ₂	1.10	± 0.24	0.80–2.00	33
Continuous			2.00 mg/L ClO ₂	1.71	± 0.7	0.68–2.62	13
Continuous			5.00 mg/L ClO ₂	4.59	± 0.7	3.22–5.5	13
Continuous			0.25 mg/L Cl ₂ *	0.26	± 0.08	0.13–0.64	58
Continuous			0.50 mg/L Cl ₂ *	0.76	± 1.08	0.02–5.13	23
Continuous			1.00 mg/L Cl ₂ *	1.04	± 0.34	0.48–1.88	59
Continuous			2.00 mg/L Cl ₂ *	1.83	± 0.32	1.26–2.24	59
Continuous			5.00 mg/L Cl ₂ *	4.68	+1.21	1.10–6.7	21
Continuous			1.00 mg/L KMnO ₄	1.04	± 0.29	0.63–1.85	59
Continuous			5.00 mg/L Cl ₂ †	5.36	+1.53	3.22–8.2	18

*NaOCl
† NaClO₂

FIGURE 2 Effect of exposure duration for adult zebra mussels to single doses of 0–40 mg/L ClO_2



Plant. Two series of experiments were run in 1992—the first from March to May and the second in October and November. Water for the holding tanks and experiments was taken from a raw water feed line at the water plant. Water for the plant comes from Lake Erie through a crib on the bottom of the lake 13,000 ft (3,962 m) from shore (16,000 ft [4,877 m] from the water plant). At the crib, water enters the 8-ft- (2.44-m-) diameter pipe to the plant at a depth of 35 ft (10.7 m), 15 ft [4.6 m] from the bottom of the lake.

Figure 1 illustrates the experimental system. Water was obtained from the raw water supply line in the plant and pumped to a makeup pool (125 gal [0.473 m³] in the first tests and 1,000 gal [3.8 m³] in the second tests), where it was brought to room temperature (~20°C) with aquarium heaters. Warmed water from the makeup pool was pumped into a water storage pool (600 gal [2.3 m³] for the first tests and 1,000 gal [3.8 m³] for the second tests) that served as a water source and a holding tank for mussels. For the single- and intermittent-exposure tests, water was pumped from the storage pool into a tubular (1.5-in. [3.81-cm] inside diameter) pipe system (flow loop) with clear acrylic sections 10 in. (25.4 cm) long with a 2-in. (5.1-cm) inside diameter and spaced 15 ft (4.57 m) apart, which contained the experi-

*Model 5-MSP, Little Giant, Oklahoma City, Okla.

†Model MC-11, Halliburton, Houston, Texas

mental mussels (Figure 1, solid lines).

Water was pumped at rates up to 0.5 fps (1.52 m/s) with submersible pumps* controlled by throttling valves and monitored with an inline flow analyzer.† Each acrylic section housed 10 mussels (first tests) to 30–40 mussels (second tests), which were acclimated about 24 h before the first chemical exposure. One section 5 ft (1.52 m) upstream of the chemical injection port served as the control. Diluted stock solutions of ClO_2 and other test chemicals were injected into the pipe at 100–500 mL/min at pressures of 2 psi (13.8 kPa) more than the system pressure to achieve the desired test concentration. A valve at the end of the flow loop permitted disposing treated water

to the sewer or recirculating untreated water back to the storage pool. Intermittent test concentrations were target dosages; ClO_2 demand in the raw water varied but typically averaged about 0.3–0.4 mg/L. Target and measured residual oxidant concentrations, numbers of mussels, and mortalities for each experiment are summarized in Table 1.

Water for the first continuous tests was pumped into four 55-gal (0.2-m³) drums from the 600-gal (2.3-m³) pool (Figure 1, dashed lines). From each drum, water partially recirculated through the flow loops, but about 300 mL/min was overflowed to the

FIGURE 3 Effect of 30-min exposures of 30-mg/L oxidant concentration on zebra mussel mortality

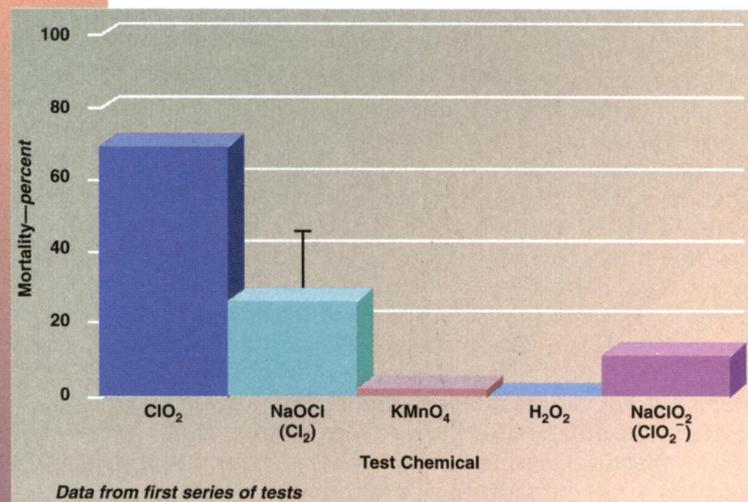
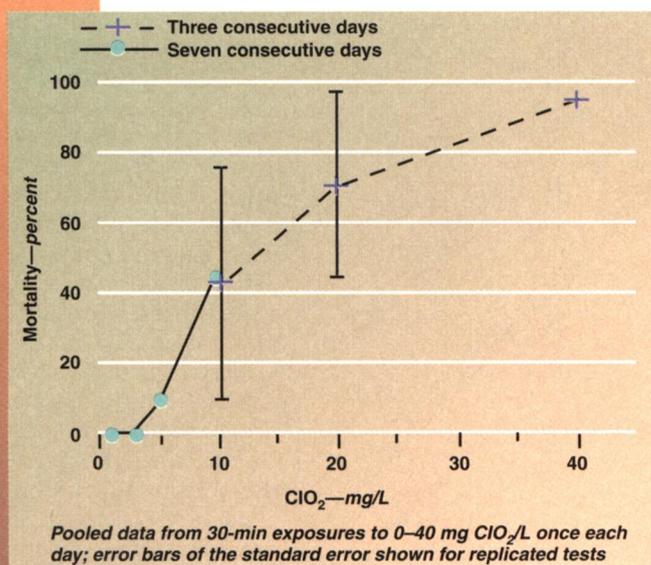


FIGURE 4 Mortalities of adult zebra mussels from intermittent 30-min exposure to ClO_2



sewer and replaced with water continuously pumped from the 600-gal (2.3-m³) pool. Four of the 10-in.- (25.4-cm-) long acrylic sections in each flow loop housed the experimental mussels in these tests.

One section was removed each day during the four-day tests. Diluted stock solutions of the chemicals were pumped into the drums at known rates of about 1–3 mL/min to achieve the target residual concentration. One flow loop received no chemical addition during each experiment and served as the control. In the second series of continuous tests a similar flow system was used, except that the number of acrylic sections in each flow loop housing mussels was increased to six, and the length between them was shortened to 5 ft (1.52 m). The larger water storage and makeup water tanks in the second series of continuous tests permitted once-through flow at flow rates of about 90 gph (0.341 m³/h). Diluted stock solutions of ClO_2 were injected into the drums at rates up to 20 mL/min to achieve the desired test concentrations.

After exposure to the test chemical for the desired length of time, the test animals were rinsed for 30 min by allowing flow to continue through the cell without chemical addition. The water was disposed to the sewer. The mussels were removed from each flow cell and placed in fish breeding baskets suspended in the holding pool, and each animal was monitored for seven days. Mussels were assumed to be alive if they were closed or if, in response to gentle probing, they moved or closed their valves. The animals were counted as dead if the shells were empty, if they were open but not filtering, if they exhibited no response to external stimulation, or if they were decomposing.

Monitoring continued for a week after testing to account for latent mortality or for animals that might appear dead initially upon exposure but recover later.

A few animals did recover, but significant latent mortality occurred in almost all experiments—most of it in the first four days. Latent mortality was also greater for ClO_2 compared with the other tested oxidants and higher at higher test concentrations. Cumulative mortalities more than doubled from day 1 to day 7, increasing by an average 27.4 percent. Similar latent mortality did not occur in the control cells, where the average mortality was 1.35 percent in the single- and multiple-exposure tests and 0.00 percent in the continuous-flow cells. Less latent mortality occurred in the second set of experiments, in which lower ClO_2 concentrations were used. The mortalities presented in this article are those recorded after chemical exposure and seven days of monitoring.

Chemical analyses. If possible, waters were analyzed by immersing electrodes in the water storage pool. For other chemical tests, a beaker of water was collected from the end of the flow loop, and the water was analyzed immediately. pH and oxidation reduction potential (ORP) measurements

were made with a portable pH meter.* A combination probe† with built-in temperature sensor was used for pH measurements and was calibrated at least daily using buffer standards supplied by the manufacturer. The ORP measurements were made with a combination ORP probe.‡ Temperature and dissolved oxygen were monitored using an oxygen meter§ and Clark-style (gold) oxygen electrode. Titrations for Cl_2 , ClO_2 , and chlorite (ClO_2^-) were done with an amperometric titrator** following procedures given in *Standard Methods*³⁴ (methods 4500- ClO_2 C and 4500-Cl D).

Speciation measurements determined that ClO_2 measured in this way contained no ClO_2^- or chlorate (ClO_3^-). Cl_2 residual contained only the hypochlorite ion and hypochlorous acid, and the reported values are total residual chlorine (TRC). Additionally, a DPD method (4500-Cl G) was used on some samples for Cl_2 residuals using reagents†† and a portable spectrometer.‡‡ Results given by the spectrometer were in good agreement with results obtained by amperometric titration. KMnO_4 residuals were determined using the DPD spectrophotometric method (4500-Cl G). Raw water quality characteristics during the testing periods are summarized in Table 2.

Statistical analyses. For each tested concentration of ClO_2 and NaOCl , LT_{50} was computed by probit analysis with log-transformed exposure times.³⁴ Similarly, for ClO_2 the median lethal concentration (LC) at which 50 percent mortality occurred (LC_{50}) because of intermittent daily exposures was com-

*Model 43800-00, Hach Co., Loveland, Colo.

†Model 44200-21, Hach Co., Loveland, Colo.

‡Model 44480-21, Hach Co., Loveland, Colo.

§Model 58, YSI Inc., Yellow Springs, Ohio

**Fischer and Porter, Warminster, Pa.

††Hach Co., Loveland, Colo.

‡‡Model DR2000, Hach Co., Loveland, Colo.

puted by probit analysis with log-transformed concentrations. Probit analysis was also used to calculate the median lethal dosage (LD ; $C \times T$), at which 50 percent mortality occurred (LD_{50}) on pooled samples of all the ClO_2 and $NaOCl$ tests. Differences in mortality between treatment and control tests, between concentrations of a particular biocide, and between biocides were determined by F -values from analysis of variance (ANOVA) and were considered statistically significant when p was <0.05 .

Results

Single exposures.

The experimental results of a single exposure to ClO_2 are shown in Figure 2, in which the percentage of mortalities is expressed as a function of exposure concentrations and exposure durations. In general, mortality to a single exposure increased with increasing exposure time and increasing ClO_2 concentration. Mortality was significantly higher in treated flow cells than in the controls ($F = 48.9$, $p < 0.001$), although differences among ClO_2 concentrations were not all statistically significant nor were all the LT_{50} values as shown in Figure 2. Exposure times as short as 5 min produced mortalities ranging from 20 percent to as high as 70 percent. This is reflected in the short LT_{50} values. Little additional mortality occurred at exposure concentrations greater than about 10–20 mg/L. These results indicate that a single exposure to 10–20 mg/L ClO_2 for about 15–30 min can produce about 50 percent mortality of adult zebra mussels. Thus, in the right



Multiple exposures to ClO_2 increased mortality. Brent Bourland injects ClO_2 during a batch exposure test.

situation a short, single-dose administration can be effective.

A single exposure is likely to lead to highly variable mortalities (Figure 2). This may be because of temperature differences or differences in experimental animals. Most data were collected during the first series of experiments. The first tests used larger animals collected from deeper water in the winter, and the second test used smaller mussels collected the following autumn from shallow waters near the shore. The 15-min exposures were conducted at a temperature of $19.8 \pm 0.5^\circ C$. The 20-mg/L dose was repeated at a lower temperature ($13.1^\circ C$) because mortalities of zebra mus-

sels, Asiatic clams, and other aquatic pests are known to increase with increasing temperature.^{8,10,12,29,35}

In agreement with these previously reported results, a lower mortality (37.5 percent) was obtained at the lower temperature than at the higher temperature (56.9 ± 12.4 percent). Exposures of 30 min at 10 and 20 mg/L were also repeated during the second series of tests. Mortalities were 16.8 ± 12.2 percent at 10 mg/L ClO_2 and 43 ± 49.5 percent at 20 mg/L ClO_2 compared with 25 and 78 percent, respectively, during the first series of tests. These differences in mortality between test temperatures are not statistically significant ($F = 0.36$, $p = 0.59$ for 10 mg/L; $F = 0.33$, $p = 0.67$ for 20 mg/L). Therefore, these limited data cannot be used to calculate the temperature dependency of mortality from a single exposure to ClO_2 .

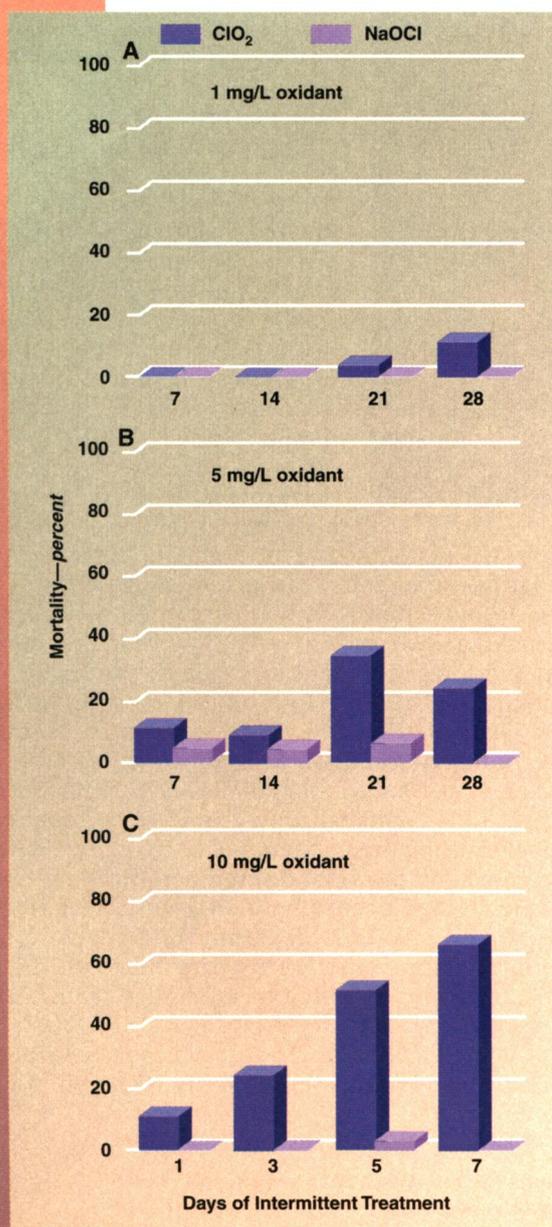
The effect of a single dosage of several other oxidants on zebra mussel mortality is shown in Figure

TABLE 2 Raw water quality characteristics during first tests (March–May 1992) and second tests (November–December 1992) of adult zebra mussel exposures

Parameter	First Tests			Second Tests		
	Mean \pm Standard Error	Range	<i>n</i>	Mean \pm Standard Error	Range	<i>n</i>
Raw water temperature— $^\circ C^*$	7.44 \pm 4.45	1.11–15.6	92	8.04 \pm 2.40	2.22–12.2	61
Experimental temperature— $^\circ C$	21.44 \pm 0.95	18.6–25.4	625	18.15 \pm 1.62	15.4–21.3	53
Turbidity— <i>ntu</i>	10.2 \pm 10.5	1.00–49.0	92	19.0 \pm 14.9	1.00–79.0	61
Conductivity— $\mu S/cm$	254.9 \pm 16.1	219–290	63	253.4 \pm 13.6	230–283	42
Dissolved oxygen— <i>mg O₂/L</i>	12.0 \pm 1.42	9.00–14.0	63	11.2 \pm 1.07	9.20–13.2	42
pH	8.13 \pm 0.07	8.00–8.30	63	8.09 \pm 0.07	8.00–8.20	42
Hardness— <i>mg/L CaCO₃</i>	122.2 \pm 1.49	120–124	63	121.4 \pm 1.43	120–124	42
Alkalinity— <i>mg/L CaCO₃</i>	85.8 \pm 1.46	80.0–86.0	63	81.2 \pm 1.35	80.0–84.0	42
Calcium— <i>mg/L Ca</i>	31.2 \pm 0.00	31.2–31.2	3	31.6 \pm 0.06	31.2–32.1	2

*Water was warmed to experimental temperature for experiments.

FIGURE 5 Mortalities of adult zebra mussels from daily 30-min exposures to 1 or 5 mg/L ClO_2 or NaOCl for up to 28 consecutive days (parts A and B) or 10 mg/L for seven consecutive days (part C)



3. These data were all obtained in experiments of a single 30-min exposure of a 30-mg/L concentration of each test chemical (Table 1). It can be clearly seen that ClO_2 produced the greatest mortality of all the tested chemicals, inducing 70 percent mortality after a single 30-min exposure to 30 mg/L. NaOCl was also somewhat effective: 26 ± 16 percent mortality. These results are in contrast with previous reports that single-batch or intermittent exposure is an ineffective control protocol.^{8,29} The most likely explanation is that the chemical concentrations in the sin-

gle-exposure tests conducted for this study were higher than those previously used and would be expected to yield greater mortalities. It is also possible that the experimental procedure reported in this article produced greater mortality because higher temperatures were used in this experiment than in others. Another possibility is that the flow of water in this experiment (~ 0.5 fps [~ 15 cm/s]) stimulates the mussels, causing them to open and filter and thus become exposed when the chemical is added to the system.

In this study, a few minutes after the mussels were loaded into the experimental cells, about 80 percent of them were open and filtering; they remained so until they closed in response to chemical addition. Even so, other oxidants were ineffective; KMnO_4 produced only 2.5 percent mortality, and H_2O_2 did not kill any mussels (0.0 percent). NaClO_2 is both a precursor chemical for generating ClO_2 and a breakdown chemical of the ClO_2 produced. During the reduction of ClO_2 , about 50 percent of the ClO_2 goes to ClO_2^- and 50 percent goes to the chloride ion (Cl^-). The 7.5 percent mortality NaClO_2 causes at the 30-mg/L level is shown in Figure 3 for comparison with the other chemicals.

Intermittent exposures. Multiple batch exposure tests are summarized in Figure 4, in which percentage of mortalities is shown as a function of the exposure concentrations for either three or seven 30-min exposures. The mortalities in all 30-min multiple exposures increased with increasing exposure concentration. At concentrations lower than about 5–10 mg/L, three to seven intermittent treatments for 30 min a day is not effective. However, the same treatment strategy is likely to be successful if oxidant concentrations greater than about 20 mg/L are employed. There is no a priori reason to assume that the three- and seven-day tests should yield the same results, but the agreement in mortalities obtained at 10 mg/L ClO_2 hint that the data may be pooled. Probit analysis on the pooled data yields an LC_{50} of 13.0 mg/L ClO_2 .

Comparison of the data in Figures 2 and 4 indicates that multiple exposures to ClO_2 on three or more successive days usually increased mortality compared with a single exposure. For example, the highest mortality achieved with a single dose of 10 mg/L ClO_2 was 52.5 percent, but identical multiple exposures resulted in mortalities up to 78 percent. This is also true for shorter exposure times (not shown); the average mortality of the single 15-min 20-mg/L ClO_2 tests was 56.9 ± 12.4 percent, statistically less than the 83.5 ± 6.6 percent in the multiple exposures ($F = 5.18$; $p = 0.08$).

Protocols that used multiple exposures more than once a day did not produce mortalities statistically greater than single-dose administrations. For example, a 5-min, 35-mg/L exposure repeated hourly for 6 h achieved a 63 percent mortality similar to the 30-min single-dose mortalities obtained at 30 mg/L (57.5 percent) and 40 mg/L (67.5 percent). Similarly,

duplicate exposures at 4-h intervals did not significantly increase mortalities. Two 15-min exposures of 20 mg/L ClO_2 4 h apart produced a mortality of 50 percent, a result similar to the single-dose administration of 56.9 ± 12.4 percent.

It is not known why mortality is enhanced with a 24-h delay between exposures but not enhanced with 1- and 4-h delays between oxidant additions. However, it may be because a long delay between multiple oxidant additions enticed many healthy mussels to open and permit chemical exposure again. These findings are in agreement with those reported elsewhere that short (0–4 h) daily chemical treatments can be an effective protocol,⁶ but more frequent multiple exposures may not be more effective.^{8,10}

In the first series of experiments, only one high-concentration multiple-exposure test was performed with an oxidant other than ClO_2 to determine whether multiple exposures to other oxidants also enhanced mortality. A 30-min dose of 30 mg/L of NaOCl was repeated on three sequential days and yielded a mortality of 35 percent. This is similar to

Single exposures are highly cost-effective because they require so little chemical, although mortality is highly variable.

the 26 ± 16 percent observed in the single-exposure tests with OCl^- . These results are in agreement with previous studies using OCl^- that show that low-concentration, intermittent treatment is not effective.⁸

Because of the potential cost savings of intermittent treatment over continuous treatment at similar concentrations, the low-concentration, intermittent treatment was repeated and continued for up to 28 days (second series of tests; Figure 5). ClO_2 is a more effective biocide, according to pooled data from all concentrations at all exposure times ($F = 9.53$, $p = 0.005$). The data show that intermittently applied 1-mg/L ClO_2 induces little mortality and that hypochlorite is ineffective at all tested concentrations and treatment times. Only about one third of the mussels were

killed by intermittent treatment with 5 mg/L ClO_2 , but 10-mg/L intermittent treatment for seven days induced up to 65 percent mortality. These results also indicate that low-concentration intermittent treatment is likely to be ineffective but that intermittent treatment with ClO_2 at higher concentrations may be successful. It must be emphasized that these treatment protocols are based on mortalities to adult mussels. Different methods, lower concentrations, or both, may be suitable for prevention of settling.⁷

Continuous exposures. Continuous-exposure experiments were conducted during both testing periods. Mortalities from continuous 0.25–5-

mg/L ClO_2 exposures are shown in Figure 6. Continuous exposure to concentrations higher than 5 mg/L produced almost total mortality within 24 h and is not shown on the graph. Mortality differences between treated and untreated mussels is statistically significant ($F = 29.4$, $p < 0.001$). Two general trends can be observed in the data. First, mortality increases with increasing exposure time. Second, higher concentrations produced higher mortalities sooner than did lower concentrations. Mortality differences between pairs of concentrations were statistically significant for all concentration pairs except for the 0.25 and 0.5 mg/L. This trend of increased mortality with increased concentration is also reflected in the LT_{50} values. For example, LT_{50} values decrease from 118 h at 0.25 mg/L to 15 h at 5 mg/L.

FIGURE 6 Mean mortality of adult zebra mussels plus or minus standard error from zero to six days' continuous exposure to 0–5 mg/L ClO_2

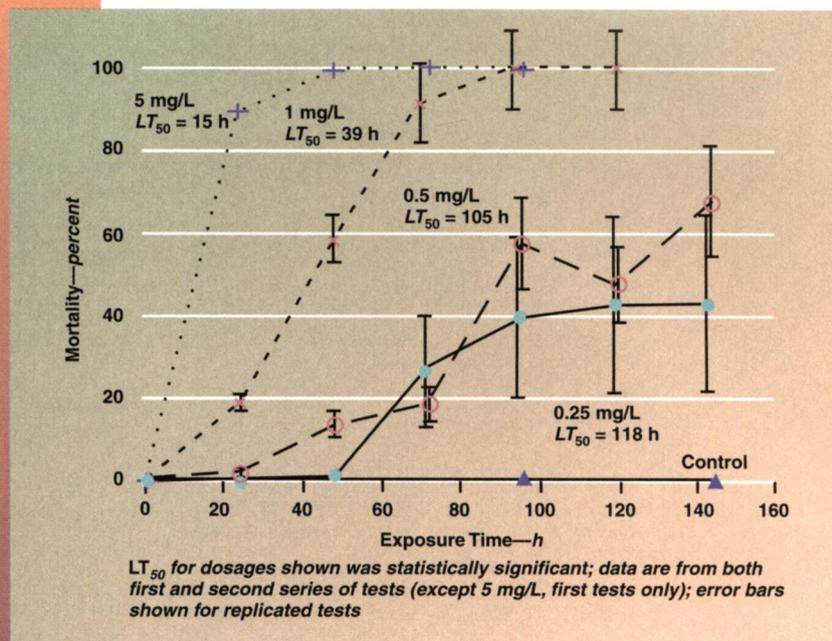
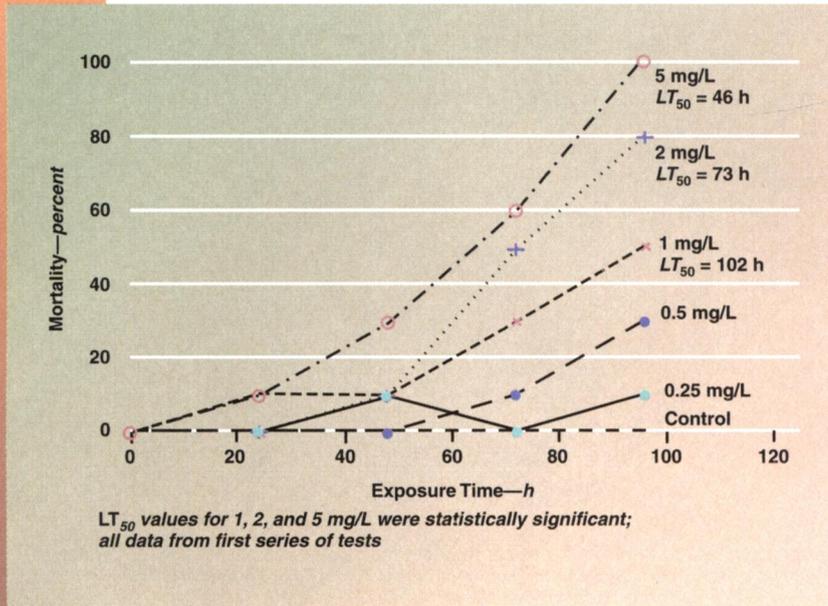


FIGURE 7 Mortalities of adult zebra mussels from zero to four days' continuous exposure to 0–5 mg NaOCl/L



Results of continuous exposure to 0.25–5-mg/L OCl⁻ are shown in Figure 7. Mortality differences between treated and untreated mussels are statistically significant ($F = 3.57$, $p = 0.075$) at the 90 percent confidence level. As with ClO₂, mortality increases with increasing exposure time and increasing concentration of OCl⁻.

LT₅₀ values for OCl⁻ calculated for the various concentrations are shown in Figure 7. The LT₅₀ values for ClO₂ are about a third to half those of NaOCl. These results are similar to those reported by Gautier³⁶ for the marine mussel *Mytilus galloprovincialis*, which showed an LT₅₀ of 15 days at 0.05–0.1 mg/L residual ClO₂ (0.2 mg/L applied ClO₂) and an LT₅₀ of >28 days at 0.6–0.7 mg/L residual Cl₂ (2–2.5 mg/L applied NaOCl). The LT₅₀ values reported here for NaOCl are also similar to those reported elsewhere in static exposures (3.2 days at 2.5 mg/L, 4.5 days at 1 mg/L, and 6.9 days at 0.5 mg/L)⁸ at similar temperatures. However,

Periodic continuous exposure to 1 mg/L chlorine dioxide for four days should be effective in controlling adult zebra mussel populations.

they are significantly shorter than those in flow-through exposures at lower temperatures (16.3 days at 2.5 mg/L, 31.9 days at 1 mg/L, and 53.7

days at 0.5 mg/L at 7–18°C⁸ and 13 days at 1 mg/L at 9–15°C¹¹). The significantly higher mortalities obtained with OCl⁻ in this study may be attributed to the higher temperatures at which the experiments were conducted. This indicates that one way to improve any treatment protocol is to apply the chemicals when the water is warmer.

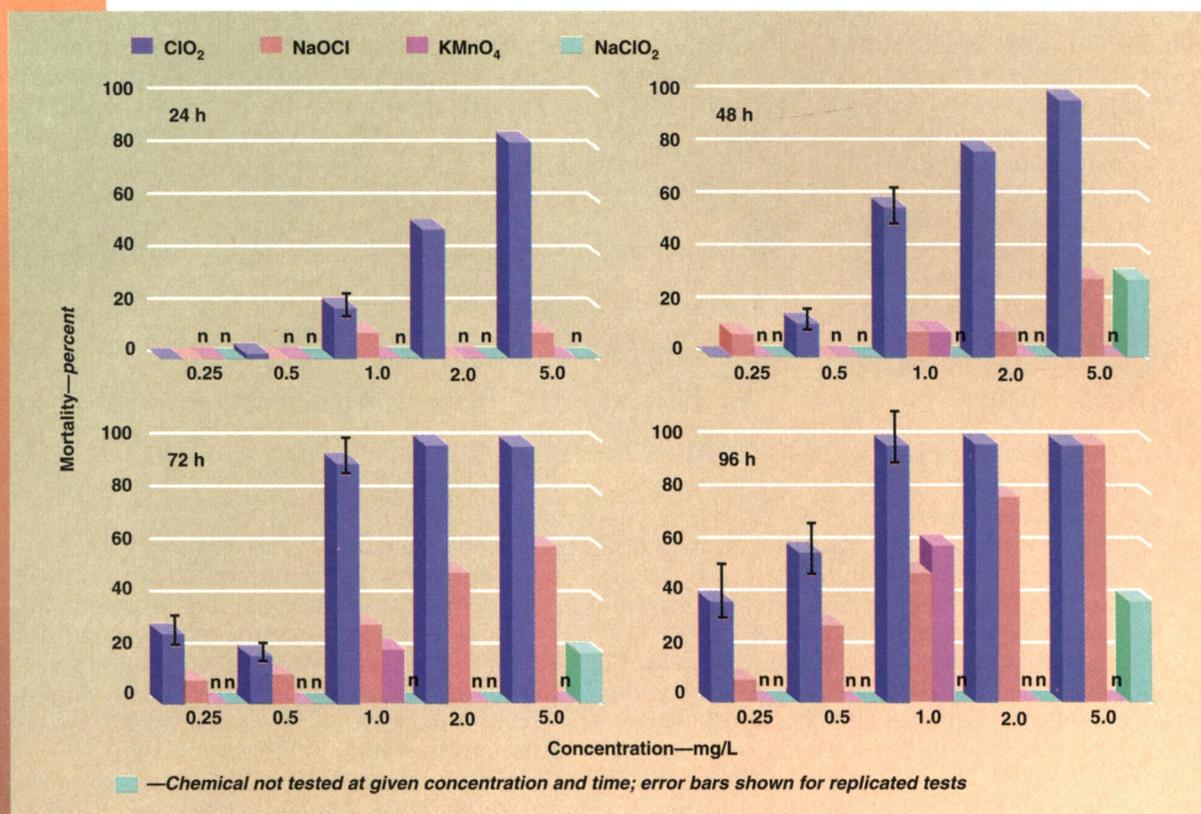
The effect of continuous exposure to different oxidants on zebra mussel mortality is shown in Figure 8 for ClO₂, NaOCl, KMnO₄, and NaClO₂ for concentrations ranging from 0.25 to 5 mg/L. ClO₂ produced the greatest mortality at all applied concentrations and had the shortest exposure time of all the tested chemicals. At 0.25 mg/L residual

concentration, ClO₂ induced a modest 40 percent mortality within four days, whereas NaOCl appeared to have little effect. At higher residual concentrations, both ClO₂ and NaOCl were more effective for these short exposure times. Of interest is that 100 percent mortality was achieved after 96 h at all ClO₂ concentrations >1 mg/L, but 100 percent mortality was achieved only at the highest concentration (5 mg/L) using NaOCl and was never attained after four days' exposure of 1 mg/L KMnO₄ or 5 mg/L NaClO₂. However, the data for 1 mg/L indicate that KMnO₄ has a mortality-inducing effect similar to that of NaOCl; therefore, KMnO₄ might be expected to also cause complete mortality at higher concentrations. Of course, higher concentrations of KMnO₄ are generally not used because of the intense color created by that chemical at higher concentrations.

Calculated 72- and 96-h LC₅₀ values are 0.49 and 0.35 mg/L for ClO₂ and 2.57 and 0.65 mg/L for NaOCl, respectively. These results are similar to the six-day exposure at 0.15–0.2 mg/L continuous residual ClO₂ exposure needed to achieve 50 percent mortality,¹² to the 0.07-mg/L LC₅₀ reported elsewhere for residual ClO₂ in preliminary experiments,^{30,31} and to reports from Italy that uninterrupted treatments with

ClO₂ injections of 0.1–0.4 mg/L have replaced chlorination (2.0–2.5 mg/L active Cl₂) for control of marine mussels.³⁶

FIGURE 8 Mortalities of adult zebra mussels from zero to four days' continuous exposure to 0.25–5 mg/L ClO_2 , NaOCl , KMnO_4 , or NaClO_2



One way to compare the results of single-exposure, intermittent-exposure, and continuous-exposure results is to examine mortality as a function of the product of the chemical concentration and the

differ in that LT_{50} values for this study are significantly less. This is probably because experimental temperatures were lower in their experiments than in this study (5–18°C versus ~20°C). This is also in agreement with flow-through OCl^- mortality tests conducted by Claudi,³⁷ who observed a time to 80 percent mortality of 80 days at 0.5 mg/L TRC and 21 days at 2.0 mg/L TRC at 4–10°C. Because mussel mortality increases with increasing temperature,^{8,11,35,38} the

One way to improve any treatment protocol is to apply the chemicals when the water is warmer.

higher temperatures in the experiments described here probably result in shorter kill times.

exposure times (dosage). Pooling all data, LD_{50} values of 14.5 and 82.5 mg/h/L for ClO_2 and NaOCl , respectively, can be calculated. The LD_{50} ratio is about a factor of 6 and is slightly greater than the factor of 2–5 LC_{50} ratios from the 72- and 96-h continuous tests. This most likely reflects the much greater effectiveness of ClO_2 than NaOCl in the single and intermittent tests.

Although the data are limited, KMnO_4 appears to be as effective as NaOCl , suggesting that KMnO_4 can also be an effective oxidant. Klerks et al.⁴ conducted continuous flow-through exposures of OCl^- and KMnO_4 and also concluded that both could be effective oxidants. However, results from the current study

higher temperatures in the experiments described here probably result in shorter kill times.

NaClO_2 is a chemical precursor in the generation of ClO_2 , and it was modestly effective at zebra mussel control by itself at the 5-mg/L concentration. However, because ClO_2 was so effective at concentrations 10–20 times less than that, its effectiveness is not caused by the presence of excess ClO_2^- from the ClO_2 generation process. Therefore, NaClO_2 is unlikely to be suitable as a mortality-inducing agent.

Both ClO_2 and NaOCl are oxidants and therefore might be expected to induce corrosion. Details of the corrosion evaluation are given elsewhere;³² neither chemical has much effect on corrosion of

carbon steel in an oxygenated environment. Linear polarization resistance (LPR) measurements of carbon steel electrodes after 28 days of intermittent exposure to 3.0 mg/L of oxidant show an increase of 0.4 mil/yr for ClO_2 (treated at 1.6 mil/yr minus control at 1.2 mil/yr = 0.4 mil/yr) and 0.6 mil/yr for Cl_2 (treated at 1.9 mil/yr minus control at 1.3 mil/yr = 0.6 mil/yr). LPR readings taken during the five-day continuous treatment of 1 mg/L of ClO_2 indicate no change in corrosion rate (control = 2.9 mil/yr; treated = 2.9 mil/yr). Corrosion rates calculated from strip coupons used in the 28-day intermittent evaluation of 1 and 5 mg/L of oxidant indicated a corrosion rate that was less in the chemical treatment flow system than in the controls. For example, at 1 mg/L ClO_2 , the untreated coupon had a corrosion rate of 7.21 mil/yr and the treated coupon corrosion rate was 6.34 mil/yr.

It is useful to compare the relative costs of NaOCl and ClO_2 for control of zebra mussels. Assuming the costs of chemical feed pumps and generators are similar, cost differences will depend only on price differences of the chemicals. The cost per gallon for raw materials is about \$0.75 for commodity 12.5 percent NaOCl , \$7.50 for retail 25 percent NaClO_2 , and \$0.75 for commodity 15 percent HCl . This yields a cost for the two chemicals (assuming 1 lb Cl_2 equivalent per gallon of NaOCl) of \$0.75/lb for NaOCl and \$4.80/lb for ClO_2 generated using the three-chemical system $\text{NaClO}_2 + \text{NaOCl} + \text{HCl}$. If applicable, Cl_2 gas can be substituted for NaOCl and HCl . ClO_2 clearly costs more per pound than Cl_2 . However, more NaOCl than ClO_2 is required, because NaOCl is a less effective toxicant. A better relative comparison can be made by considering a continuous application of the two oxidants at a treatment rate of 1 mg/L to LT_{50} (102 h NaOCl ; 39 h ClO_2). This yields a relative cost-per-pound ratio of $\$1.97/\$4.80 = 0.41$ for $\text{NaOCl}:\text{ClO}_2$. Cl_2 is less expensive. If detoxification is required, additional detoxification time and chemical expense will be required for Cl_2 , making the two chemical treatments comparable in cost.

Discussion

It is difficult to compare the results of single, intermittent, and continuous chemical exposures with one another and with results of other workers who used different experimental protocols. The selection of a treatment protocol is likely to be case-specific and will be based on factors such as engineering design of the proposed treatment protocol; water quality criteria, especially THMs and Cl_2 and ClO_2^- residuals; water usage after treatment, i.e., cooling water or drinking water; corrosion consid-

erations; waste disposal considerations; and costs. Although it is clear that continuous treatment will be the application method of choice for some facilities, it is also possible that other plants will have the option to choose among different treatment protocols. Also, a higher oxidant demand would be expected in any system containing organic debris

These findings are in agreement with those reported elsewhere that short daily chemical treatments can be an effective protocol, but more frequent multiple exposures may not be more effective.

or dissolved organics. Thus, the TRC values reported here represent a minimum of oxidant needed; dirty systems may need considerably more oxidant.

A single 30-min exposure to ClO_2 concentrations 20 mg/L and higher produced mortalities averaging about 50 percent. Single exposures are highly cost-effective because they require so little chemical, although mortality is highly variable. Thus, in the right situation, a short, single-dose administration can be effective. Implementation of such a control protocol, however, requires either (1) dilution of the treated water at the other end of the pipe by blending with other water, such as at facilities with multiple raw water feeds or large holding tanks or (2) detoxification of the residual ClO_2 and chlorite because the applied 20 mg/L (~10 mg/L chlorite residual) may be too high to permit direct use of the water after treatment for some applications.

Multiple exposures for three to seven consecutive days can induce greater mortalities with somewhat less variability than single exposures. At concentrations lower than about 5–10 mg/L, multiple intermittent treatments for 30 min a day are not an effective treatment strategy. However, this strategy is likely to be successful if oxidant concentrations greater than about 20 mg/L are employed. If low application rates and low residuals are necessary, continuous exposure to 0.25 mg/L and 0.5 mg/L residual ClO_2 will provide 40–60 percent mortality within three days.

Treatment mortalities are highly dependent on temperature; higher mortalities are obtained at lower concentrations when water is warmer.^{8,10,12,29,35} This indicates that the success of any treatment protocol depends not only on the chemical chosen and its concentration and exposure time but also on the water temperature at the time of application. It therefore seems prudent to implement a treatment schedule that incorporates the greater effectiveness of chemical exposure in warmer water. Because veliger

settling appears to occur primarily in late July and August,^{5,39,40} it seems reasonable to employ chemical control at the end of August while water is still warm and the zebra mussel population is at its largest. However, such a control policy will not prevent mussels from colonizing the pipes after treatment, overwintering, and growing all summer. A second treatment in June to kill the overwintered population may also be necessary to prevent major problems from occurring during periods of high water demand in summer.

Similarly, the implementation of continuous treatment may not be cost-effective in April–May and October–November, because the cooler water is likely to make the chemicals less effective. Continuous treatment from June to September should be sufficient to keep the intake pipes clear of almost all adult mussels. Periodic continuous exposure for four days to 1 mg/L chlorine dioxide should be effective in controlling adult zebra mussel populations.

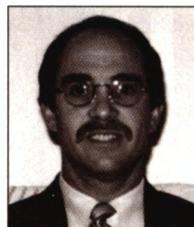
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