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Inactivation of Adenoviruses, Enteroviruses, and Murine Norovirus in Water by Free Chlorine and Monochloramine▽

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Inactivation of infectious viruses during drinking water treatment is usually achieved with free chlorine. Many drinking water utilities in the United States now use monochloramine as a secondary disinfectant to minimize disinfectant by-product formation and biofilm growth. The inactivation of human adenoviruses 2, 40, and 41 (HAdV2, HAdV40, and HAdV41), coxsackieviruses B3 and B5 (CVB3 and CVB5), echoviruses 1 and 11 (E1 and E11), and murine norovirus (MNV) are compared in this study. Experiments were performed with 0.2 mg of free chlorine or 1 mg of monochloramine/liter at pH 7 and 8 in buffered reagent-grade water at 5°C. CT values (disinfectant concentration × time) for 2- to 4-$\log_{10}$ (99 to 99.99%) reductions in virus titers were calculated by using the efficiency factor Hom model. The enteroviruses required the longest times for chlorine inactivation and MNV the least time. CVB5 required the longest exposure time, with CT values of 7.4 and 10 mg · min/liter (pH 7 and 8) for 4-$\log_{10}$ inactivation. Monochloramine disinfection was most effective for E1 (CT values ranged from 8 to 18 mg · min/liter for 2- and 3-$\log_{10}$ reductions, respectively). E11 and HAdV2 were the least susceptible to monochloramine disinfection (CT values of 1,300 and 1,600 mg-min/liter for 3-$\log_{10}$ reductions, respectively). Monochloramine inactivation was most successful for the adenoviruses, CVB5, and E1 at pH 7. A greater variation in inactivation rates between viruses was observed during monochloramine disinfection than during chlorine disinfection. These data will be useful in drinking water risk assessment studies and disinfection system planning.

Disinfection is a critical step in the drinking water treatment process to inactivate infectious viruses because primary treatment is less effective for the removal of viruses. Chlorine and monochloramine are the most widely used disinfectants in the United States (2). Free chlorine is widely used as a primary disinfectant following filtration and also as a secondary disinfectant in distribution systems. Under the Long Term 2 Enhanced Surface Water Treatment Rule (38), monochloramine can also be used as a primary disinfectant, but because it requires longer contact times to achieve the same level of disinfection as free chlorine it is primarily used as a secondary disinfectant to maintain a stable disinfectant residual in the distribution system and minimize disinfection by-product formation and biofilm growth.

The efficacy of chlorine disinfection for viruses has been evaluated in numerous studies over the years. Many early studies focused on the disinfection of polioviruses by chlorine (14, 17, 26, 28, 30, 39, 40, 43). Early investigators suggested a number of variables that must be controlled in the disinfection of viruses: contact time, temperature, ionic strength, pH, chlorine concentration, and virus aggregation (29, 30). These researchers concluded that comparisons and general trends of disinfection efficacy can only be discerned for viruses when the same disinfection parameters are applied.

Fewer studies have investigated the disinfection efficacy of monochloramine, but monochloramine disinfection has been found to be less effective than free chlorine for viruses. In comparative studies of chlorine and monochloramine disinfection, coxsackievirus B5, adenovirus 2, and adenovirus 41 were found to be inactivated far more readily by chlorine than monochloramine (4, 5, 32). For drinking water treatment systems where monochloramine is used as a secondary disinfectant, it is important to know its efficacy for a wide range of viruses, as infectious viruses may be introduced in the distribution system where only monochloramine is present. In addition, relatively few studies have investigated the efficacy of monochloramine as systematically as free chlorine; frequently only one concentration, pH, or temperature has been investigated. Two notable exceptions were investigations that examined monochloramine disinfection of human adenovirus 2 (HAdV2) and coxsackievirus B5 (CVB5) at multiple pH levels (21, 31).

In 2005, the U.S. Environmental Protection Agency (USEPA) published its second candidate contaminant list (CCL2). The CCL2 is comprised of unregulated microbial and chemical contaminants of potential public health concern that are known or anticipated to occur in drinking water systems and includes: echovirus, coxsackievirus, adenovirus, and calicivirus (36). A number of researchers have reported the disinfection efficacy of free chlorine for representatives of the CCL2 viruses (4, 5, 7, 11, 13, 18, 20, 22, 27, 33, 34, 35), but fewer studies have investigated the disinfection efficacy of monochloramine on these viruses (4, 5, 21, 31). In addition, comparison between existing studies of chlorine or monochloramine disinfection is difficult because of differences in the viruses examined, experimental parameters investigated, and analytical methods used.

The present study compared the inactivation kinetics for

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representative CCL2 viruses with levels of free chlorine and monochloramine recommended for drinking water disinfection. Duplicate experiments with both disinfectants were carried out in pH 7 and 8 buffered chlorine-demand-free (CDF) water at 5°C, with eight viruses chosen to represent the CCL2 virus types. Coxsackieviruses B5 and B3 (CVB5 and CVB3) and echoviruses 1 and 11 (E1 and E11) were chosen based on existing data suggesting resistance to free chlorine, disease implications, and likelihood of presence in higher numbers in natural water. Three representative human adenoviruses were studied, including both serotypes of species F HAdV (40 and 41) that cause gastroenteritis and HAdV2, a representative of respiratory HAdV that may be found in water because they are present in fecal excretions (9). Murine norovirus (MNV), phylogenetically similar to human norovirus and the only norovirus that can be propagated in cell culture, was used as a surrogate for human norovirus. Kinetic inactivation curves are presented, and CT values (disinfectant concentration × time, reported in mg · min/liter) were calculated by using the efficiency factor Hom (EFH) model (16).

MATERIALS AND METHODS

Virus propagation and infectivity assays. CVB5 (Faulkner strain), CVB3 (Nancy strain), E1 (Farouk strain), and E11 (Gregory strain) were obtained from the American Type Culture Collection (ATCC; Manassas, VA) and propagated in Buffalo green monkey (BGM) cells (Scientific Resources Program, Centers for Disease Control and Prevention (CDC; Atlanta, GA). Clones of CVB5 and E1 selected from larger plaques were propagated and used in experiments, in order to shorten assays to 2 days. HAdV2 (strain 6), HAdV40 (Dugan strain), and HAdV41 (a clinical isolate) were obtained from the CDC (Respiratory Virology Diagnostics, GRVLB, DVD, CDC, Atlanta, GA) and propagated in A549 (human epithelial lung carcinoma) cells obtained from the CDC (Scientific Resources Program, CDC, Atlanta, GA). HAdV40 and HAdV41 were selected and propagated as described previously (10). MNV was obtained from H. W. Virgin and C. E. Wobus (19) and propagated in RAW 264.7 (murine monocyte/macrophage) cells obtained from the ATCC.

All virus titers were determined by plaque assay. Appropriate cell monolayers were infected nearly or completely confluent at 2 days after seeding or at 1 day for RAW 264.7 cells. Tenfold dilutions of virus or experimental samples (0.25- or 0.7-ml MNV samples) were added to each 60-mm^2 cell monolayer; two or more dilutions of each sample were assayed in duplicate. After 1 h adsorption at 37°C, the infected cells were overlaid with 5 ml of the cell-appropriate medium containing 0.5% SeaKem ME agarose. Plaque assays for adenoviruses and enteroviruses also contained an additional 30 mM $MgCl_2$. After a 2-day incubation of MNV and enterovirus assays, a second agarose overlay containing 2% neutral red was added to visualize plaques within 4 to 6 h. HAdV2 was incubated 5 days; HAdV40 and HAdV41 were incubated 12 and 9 days, respectively, as previously described (10). Adenovirus plaque assays were stained by the addition of a 1/10 volume of 0.5% thiazolyl blue tetrazolium bromine (Sigma-Aldrich) in phosphate-buffered saline (PBS) for 2 h.

CAV preparations. Cell monolayers were infected at a multiplicity of infection of ca. 0.5 to 1.0 and cultured in serum free medium. At predetermined times postinfection for maximum virus titer (based on kinetic studies of each virus), the supernatant was removed, and 10 ml of CDF Dulbecco's PBS (DPBS) was added to the flask. These cell-associated virus (CAV) preparations were frozen at −70°C and stored for days or up to 2 months before use. After thawing, the CAV preparation was incubated overnight at 4°C with 8% polyethylene glycol and 0.3 M NaCl. After centrifugation at 10,000 × *g*, the pellet was suspended in CDF DPBS and extracted by vigorous shaking with an equal volume of chloroform for 2 min. After centrifugation at 10,000 × *g*, the upper aqueous layer (purified CAV [pCAV]) was taken for use in disinfection experiments on the same day. Electron microscopy indicated that all pCAV preparations did not contain virus aggregates, and aggregates were not formed upon dilution in CDF water. Only single particles were observed, with rare to occasional doublets.

Reagents and glassware. CDF DPBS and CDF reagent-grade water were prepared according to standard method 4500-Cl C (1). CDF water was buffered to 0.01 M and brought to pH 7 and 8 by dissolving 0.83 g of Na_2HPO_4 (anhydrous) and 0.58 g of NaH_2PO_4 (monohydrate) per liter and 1.3 g of Na_2HPO_4 and 0.1 g of NaH_2PO_4 per liter, respectively. A free chlorine stock solution was prepared by diluting 5.65 to 6% sodium hypochlorite (Fisher Scientific, Fair Lawn, NJ) in CDF water. Prior to each experiment, this stock was added to the experimental waters to achieve the desired free chlorine concentration. The starting concentration of the water was adjusted so that the addition of the inoculum did not cause a >0.05 mg/liter decrease in the free chlorine concentration in order to achieve a final starting value of 0.2 mg/liter. Free and total chlorine were measured by the DPD method using colorimetric methodology with a DR/850 colorimeter (Hach Company, Loveland, CO). A monochloramine stock solution was made by mixing equal volumes of 200 mg of free chlorine/liter and 800 mg of ammonium chloride/liter in pH 8 CDF water and stored at 4°C for 2 weeks. Before each experiment, a 1-mg/liter monochloramine solution was prepared by diluting the stock solution in pH 7 or 8 CDF water. Monochloramine was measured by using a Hach DR/850 colorimeter. CDF glassware was prepared by soaking in ≥5 mg of free chlorine/liter overnight. The glassware was rinsed five times with CDF water, covered with clean foil, and baked at 200°C for 2 h. All glassware and water were prechilled at 5°C before each experiment.

Experimental protocol. All experiments were conducted at 5 ± 0.2°C in a recirculating water bath inside a biological safety cabinet. A multiplace stir plate placed under the water bath allowed for continual mixing during an experiment. For monochloramine experiments lasting longer than several hours, samples were moved to a refrigerator maintained at 5 ± 0.5°C. For each pH, four 50-ml Erlenmeyer flasks were used, each containing 40 ml of CDF water with 1 mg of monochloramine/liter or, for free chlorine experiments, 20 to 40 ml of CDF water with >0.2 mg of free chlorine/liter. Two flasks were used for the experimental replicates; one flask was used to monitor free and total chlorine residual or monochloramine residual, and one flask was used to monitor virus titer at selected points during the experiment. At time zero, 0.2 to 1 ml of a pCAV stock was inoculated into each flask. At selected time points, a 5-ml sample was removed and added to a tube containing sodium thiosulfate to achieve a final concentration of 50 mg/liter.

Free and total chlorine residuals were measured immediately before an experiment, immediately after virus inoculation, at the midpoint when possible, and at the end of an experiment. Monochloramine residual was measured immediately before an experiment, immediately after virus inoculation, at the midpoint, and at the end of an experiment, at a minimum. These values were incorporated into the kinetic modeling and CT calculations. Prior to virus inoculation into the virus control flask, 50 mg of sodium thiosulfate/liter was added to quench the free chlorine or monochloramine residual. This flask was sampled immediately after virus inoculation and at the end of the experiment to ensure that virus infectivity was stable without disinfectant present. Only HAdV40 and HAdV41 were found to lose infectivity during the time frame of the monochloramine experiments; therefore, control samples were taken at every sampling point. After sampling at the indicated time points, 10× PBS containing 10% fetal bovine serum was added to each sample in order to have isotonic samples for assay. Samples were assayed on the same day as the experiment when possible or, in the case of lengthy monochloramine experiments, held at 4°C until the final samples were taken.

Kinetic modeling and CT calculations. Viral inactivation was determined by calculating the survival ratio (N/N_0; infectious viruses at time *t* divided by infectious viruses at time zero) for each experimental sample. The EFH model was used to calculate predicted survival ratios based on experimental conditions, including disinfectant decay over time using a first-order kinetic equation (16). Samples were included in the EFH modeling and CT calculations only if the plaque assay counts averaged ≥10 PFU/plate. Inactivation curves were created by using Microsoft Excel to compare observed versus predicted inactivation values. CT values were calculated for 2-, 3-, and 4-$\log_{10}$ inactivation for each virus and condition through application of the EFH model. Linear regression using a quadratic response function was used to compare viral inactivation between different viruses and pH levels using SAS version 9.0. Statistical significance was set at $\alpha = 0.05$.

RESULTS

A summary of CT values (mg · min/liter) obtained for free chlorine and monochloramine disinfection of all study viruses at pH 7 and 8 are shown in Tables 1 and 2, respectively. Each CT value is an average of replicate experiments with the variation between replicates less than 25%. CT values were obtained directly from experimental data by using the EFH model. Calculation of extrapolated 4-$\log_{10}$ CT values using the EFH model was possible for many of the viruses that did not

TABLE 1. CT values for free chlorine inactivation of study viruses with 0.2 mg of free chlorine/liter at 5°C

Virus	CT value (mg · min/liter)					
	2-log$_{10}$ CT		3-log$_{10}$ CT		4-log$_{10}$ CT	
	pH 7	pH 8	pH 7	pH 8	pH 7	pH 8
HAdV2	0.02	0.04	0.06	0.12	0.15	0.27
HAdV40	<0.02	<0.02	<0.02	<0.02	<0.04	<0.04
HAdV41	0.005	<0.02	0.01	<0.02	ND[c]	<0.03
CVB3	0.97	0.65	1.4	1.1	2.9	1.7
CVB5	3.6	4.7	5.5	7.6	7.4[b]	10[b]
E1	0.96	0.99	1.3	1.3	1.5[a]	1.6
E11	0.82	0.54	1.0[a]	0.97	1.1[a]	1.4[a]
MNV	<0.02	<0.02	<0.02	<0.02	<0.07	<0.08

[a] CT value for this level of inactivation extrapolated using the EFH model.
[b] Only one replicate achieved the desired inactivation (the CT value for the second replicate was extrapolated).
[c] ND, no data (the CT value could not be extrapolated due to asymptotic inactivation curves).

achieve 4-log$_{10}$ inactivation experimentally. However, extrapolated CT values for monochloramine disinfection of HAdV40, HAdV41, and E1 could not be calculated due to limitations in the EFH model at predicting inactivation reaching asymptotic levels (see Fig. 3 and 4, note the scales).

HAdV40, HAdV41, and MNV were the most readily inactivated study viruses by free chlorine, with at least 3-log$_{10}$ inactivation within 5 s. The rapid rate of inactivation did not allow for statistical comparisons to be performed. CVB5 required the longest time for inactivation, significantly more than CVB3 ($P < 0.0001$) at pH 7 and at pH 8. CVB5, E1, and HAdV2 were each inactivated more rapidly at pH 7 than at pH 8 ($P < 0.0001$). In contrast, E11 was inactivated more rapidly at pH 8 ($P < 0.0001$), and CVB3 showed no difference in inactivation rates at the different pH values.

Chlorine inactivation curves for the four enteroviruses (Fig. 1 and 2, note scales) indicate that these viruses were inactivated according to first-order reaction kinetics. Inactivation curves for HAdV2 (Fig. 2) exhibited a second-order tailing effect.

All of the viruses were significantly different from each other in their relative resistance to monochloramine disinfection,

TABLE 2. CT values for 2-, 3-, and 4-log$_{10}$ inactivation of study viruses with 1 mg of monochloramine/liter at 5°C

Virus	CT value (mg · min/liter)					
	2-log$_{10}$ CT		3-log$_{10}$ CT		4-log$_{10}$ CT	
	pH 7	pH 8	pH 7	pH 8	pH 7	pH 8
HAdV2	600	990	1,000	1,600	1,500[b]	2,300
HAdV40	90	360	ND[d]	ND	ND	ND
HAdV41	58	190	190	ND	ND	ND
CVB3	270	240	390	330	500[a]	420
CVB5	510	670	710	900	900	1,100
E1	8	8	15	18	42[a,c]	ND
E11	1,000	880	1,300	1,200	1,500[a]	1,400[b]
MNV	26	36	70	78	150	170

[a] CT value for this level of inactivation extrapolated using the EFH model.
[b] Only one replicate achieved the desired inactivation (the CT value for the second replicate was extrapolated).
[c] Data from one replicate only.
[d] ND, no data. The CT value could not be extrapolated due to asymptotic inactivation curves.

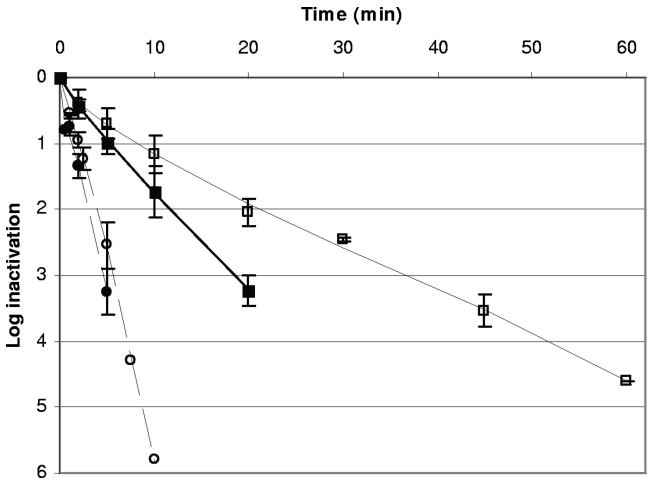

FIG. 1. Free chlorine inactivation of CVB5 at pH 7 (■) and pH 8 (□) and E1 at pH 7 (●) and pH 8 (○).

including viruses of the same type. Inactivation of CVB3 was two to three times faster than CVB5, depending on pH ($P < 0.0001$). At both pH levels, inactivation of E1 was more than 100 times faster than E11 ($P < 0.0001$). Inactivation of HAdV41 was slightly faster than HAdV40 ($P = 0.0002$ at pH 7, $P = 0.0006$ at pH 8); however, inactivation of HAdV40 was five to seven times faster than HAdV2, depending on pH ($P < 0.0001$). Overall, monochloramine disinfection was most effective for E1 ($P < 0.0001$) and least effective for E11 and HAdV2 ($P < 0.0001$).

Monochloramine disinfection was more effective at pH 7 than pH 8 for HAdV2 ($P = 0.006$), HAdV40 ($P < 0.0001$), HAdV41 ($P = 0.001$), CVB5 ($P < 0.0001$), and E1 ($P = 0.029$). There was no significant difference between the efficacy of disinfection at pH 7 and pH 8 for CVB3 ($P = 0.18$), E11 ($P = 0.37$), and MNV ($P = 0.07$).

For each virus, monochloramine inactivation kinetic curves were similar at pH 7 and pH 8 (Fig. 3 and 4). HAdV2, CVB3,

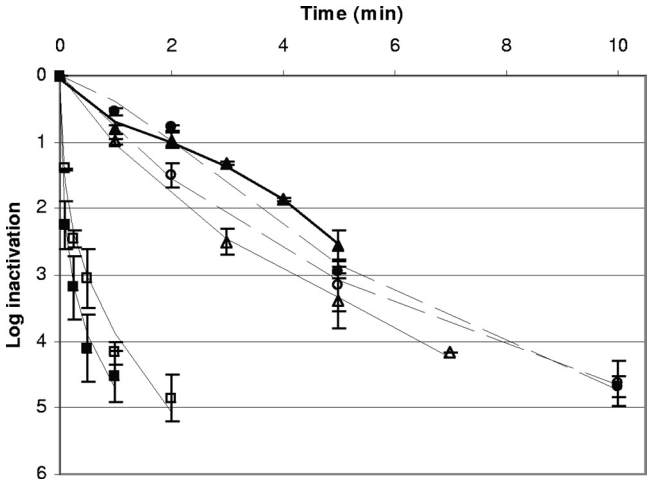

FIG. 2. Free chlorine inactivation of HAdV2 at pH 7 (■) and pH 8 (□), CVB3 at pH 7 (●) and pH 8 (○), and E11 at pH 7 (▲) and pH 8 (△).

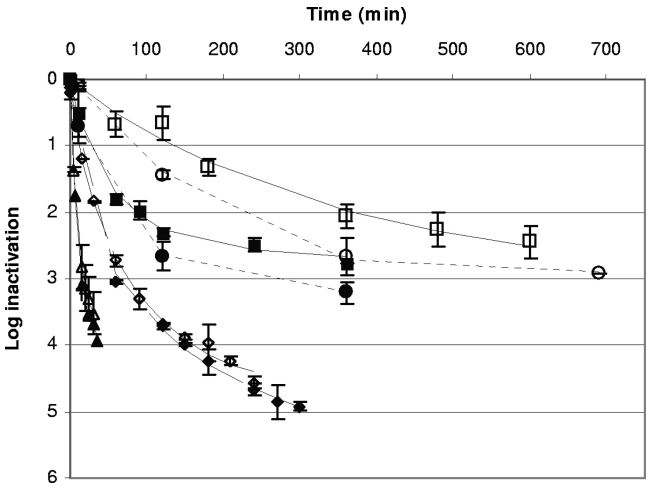

FIG. 3. Monochloramine inactivation of HAdV40 at pH 7 (■) and pH 8 (□), HAdV41 at pH 7 (●) and pH 8 (○), E1 at pH 7 (▲) and pH 8 (△), and MNV at pH 7 (◆) and pH 8 (◇).

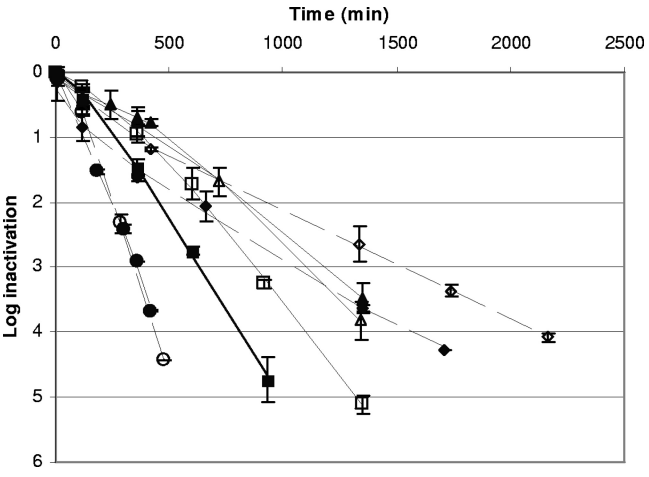

FIG. 4. Monochloramine inactivation of CVB5 at pH 7 (■) and pH 8 (□), CVB3 at pH 7 (●) and pH 8 (○), E11 at pH 7 (▲) and pH 8 (△), and HAdV2 at pH 7 (◆) and pH 8 (◇).

CVB5, and E11 exhibited first-order inactivation curves, whereas inactivation curves for HAdV40, HAdV41, E1, and MNV exhibited a second-order tailing effect.

DISCUSSION

Few studies have examined both chlorine and monochloramine disinfection of the same viruses under the same experimental conditions. No studies have examined representatives of all types of CCL2 viruses for disinfection efficacy of chlorine or monochloramine. Because chlorine and monochloramine are the most widely used disinfectants in U.S. drinking water systems, an understanding of the efficacy of each of these disinfectants for a range of viruses is important (2). The CCL2 and the 2009 CCL3 indicate that adenoviruses, coxsackieviruses, echoviruses, and caliciviruses are potentially important microbiological contaminants for public drinking water systems. Although disinfection data are needed for each of the virus groups, it is also important to understand the range of disinfection resistance for different viral strains within each virus group.

Previous research on chlorine inactivation of multiple enteric viruses in river water identified CVB5 as more resistant than CVB3, E1, and E11 (25). In the present study, CVB5 also required more time for 2-, 3-, and 4-$\log_{10}$ inactivation than CVB3 and the two echovirus strains. More recently, other investigators have reported inactivation of CVB5 in demand-free water. At pH 7.5 and 5°C, investigators reported CT values of 5.4, 8.4, and 11.5 for 2-, 3-, and 4-$\log_{10}$ inactivations, respectively (7), which is somewhat consistent with results in the present study for pH 7 and 8 (see Table 1). If the CT values are estimated based on reported data, other studies using CVB5 have reported somewhat higher values (~2-fold) for 4-$\log_{10}$ inactivation (32) or ~2-fold lower for 2-$\log_{10}$ inactivation (13) than those found in the present study. In one study, the inactivation rates of CVB3 and CVB5 were the same, and the investigators suggested that this was a result of CVB5 aggregation (18). No aggregation was found in the virus preparations used in the current study, and other investigators have also found different inactivation rates for CVB3 and CVB5 in river water (25) (estimated CT for 2 $\log_{10}$ = 8.1 and 19.8, respectively, by another author [15]). The collective data in the literature indicates that the rate of inactivation of CVB5 with free chlorine is consistently lower than for other enteric viruses, including hepatitis A virus (HAV) (32). More research is needed to understand mechanisms contributing to the lower rate of inactivation of CVB5 in comparison with other viruses, including other enteroviruses that have been tested.

Investigators have reported similar CT values for chlorine inactivation of HAdV5 and HAdV41 (5). The results obtained in the current study for HAdV2 are similar to those reported for these two viruses. However, in the current study, HAdV40 and HAdV41 were inactivated more rapidly than in the study by Baxter et al. (5) or in the study by Thurston-Enriquez et al. (33). A possible explanation for the different findings of Baxter et al. is the use of borate buffer, in contrast to the phosphate buffer used in the current study. The potential for varying results due to differences in experimental buffers and ionic concentrations has been suggested by others (6, 30). In addition, both of the previous studies with HAdV's used different types of infectivity assays, either end point analysis of viral antigen production or cytopathic effect endpoint analysis (5, 33). These approaches could also have produced differences in reported CT values. In the present study, it was not possible to statistically compare the level of inactivation of HAdV2 with HAdV40 and HAdV41, although the CT values for 3-$\log_{10}$ inactivation indicate that HAdV2 required more time for inactivation than HAdV40 and HAdV41.

The second-order inactivation kinetics observed for HAdV2 (Fig. 2) could have been due to several factors, including multiple virus populations exhibiting differing resistance to disinfection (15). Another possibility is that more than one mechanism of inactivation may be in effect for the inactivation of this DNA virus, which has a more complex capsid than the picornaviruses and the norovirus investigated in the present study. The mechanism of inactivation of viruses by chlorine is not known. Conformational changes in capsid structure during inactivation of E1 under certain conditions have been observed

and are suggested to play a role in inactivation (44). More recent studies have suggested that the mechanism of chlorine inactivation of another picornavirus, HAV, was associated with RNA degradation (24). In the present study, the relatively straight lines for inactivation of the four enteroviruses indicated first-order kinetics, which suggests a single mechanism for inactivation.

In the present study the kinetics of chlorine inactivation of MNV could not be evaluated due to the rapid inactivation of this virus. This is in contrast to previous research reporting the failure of a 3.75-mg/liter concentration of chorine to inactivate Norwalk virus in drinking water (22). Inactivation was measured by infectivity in human volunteers with an inoculum of a Norwalk virus suspension in broth. The method of evaluation and preparation of the virus could have contributed to the need for a larger dose of chlorine for inactivation. In the present study, use of a partially purified MNV preparation in buffered water could have contributed to the rapid inactivation. Although investigators have used feline calicivirus as a surrogate for studies of human norovirus (11, 33–35), more recently investigators have suggested that MNV may be a more relevant surrogate for studying the survival of human noroviruses (3, 8, 41). Recently, investigators have found that MNV was inactivated more rapidly than poliovirus 1 in treated water from a water treatment plant (23). Future studies of MNV under different conditions and in drinking water may identify different requirements for chlorine inaction than were found in the present study.

Fewer published data are available for comparing the disinfection efficacy of monochloramine for viruses. The enteroviruses examined in the present study each exhibited markedly different responses to monochloramine disinfection. E11, CVB3, and CVB5 exhibited first-order inactivation curves (Fig. 4), while the inactivation curves of E1 exhibited a second-order tailing effect (Fig. 3). Inactivation of CVB5 and E1 was less effective at pH 8, but inactivation of E11 and CVB3 was similar at pH 7 and 8. The most notable difference between the enteroviruses was the 100-fold difference between CT values for E1 and E11. The differences in the responses of the enteroviruses to monochloramine are not readily explained, but they could suggest that the mechanism of monochloramine disinfection is not the same for all enteroviruses. There has been no prior research to compare monochloramine disinfection on multiple enteroviruses under the same experimental conditions. However, previous research found that enteroviruses responded differently to free chlorine disinfection, both in the time required for disinfection and in the shape of the inactivation curves (13, 25).

Comparison of monochloramine data from the present study with previous findings is limited due to differences in experimental conditions, such as buffering capacity of the water, temperature, and pH. However, a few studies have investigated monochloramine disinfection of CVB5 under conditions similar to those used here. Using 10 mg of monochloramine/liter at pH 8, Sobsey et al. reported a 4-$\log_{10}$ inactivation by 104 min, which, if translated into a CT value (1,040) is similar to the 4-$\log_{10}$ CT value reported here (1,100) (32). In addition, the inactivation kinetics and pH trend (monochloramine less effective at pH 8) reported by Kelly et al. for CVB5 are similar to the results of the present study (21).

Like the enteroviruses, the adenoviruses were notably different in their responses to monochloramine disinfection. HAdV40 and HAdV41 produced similar inactivation curves that exhibited tailing, but the inactivation curves for HAdV2 exhibited first-order kinetics. In addition, the magnitude of the differences between CT values for HAdV2 and those for HAdV40 and HAdV41 were substantial. However, one similarity for the three adenoviruses was a decreased effectiveness of monochloramine at pH 8 versus pH 7.

The differences in CT values and monochloramine inactivation kinetics between HAdV2 and both HAdV40 and HAdV41 might be explained by the fact that HAdV2 is a species C adenovirus and HAdV40 and HAdV41 are species F adenoviruses. However, Baxter et al. examined monochloramine disinfection of HAdV41 and HAdV5 (a species C HAdV) and found similar CT values for both viruses (5). They also reported a CT value for 2.5-$\log_{10}$ inactivation of HAdV41 (300) that was similar to the 2.5-$\log_{10}$ CT value from the present study (320; data not shown). Inactivation kinetics and pH data for HAdV2 from the present study were also consistent with previous research (31).

For each of the study viruses, the CT values were consistently higher using monochloramine than free chlorine, although the magnitude of this difference varied by virus type. Monochloramine disinfection yielded a 2-$\log_{10}$ CT value of 8 for E1 at pH 7, whereas chlorine disinfection yielded a 2-$\log_{10}$ CT of 0.96. The greatest difference between chlorine and monochloramine efficacy reported in the present study was for HAdV2, for which monochloramine was over 37,000 times less effective than chlorine in achieving a 2-$\log_{10}$ reduction at pH 7. In addition, the relative inactivation rates of the study viruses to disinfection were dramatically different for chlorine and monochloramine. Whereas HAdV2 was one of the least resistant viruses to chlorine (2-$\log_{10}$ CT = 0.02, pH 7), it was one of the most resistant viruses to monochloramine. The 2-$\log_{10}$ free chlorine CT values for E1 and E11 were similar (E1 = 0.96 and E11 = 0.82, pH 7), but the monochloramine CT values for these viruses were different by >100-fold.

Both free chlorine and monochloramine were highly effective for inactivation of MNV. Although there are no reports demonstrating that human noroviruses are less susceptible to chlorine or monochloramine inactivation than reported in the present study for MNV, human noroviruses have been identified as the etiologic agents in numerous waterborne disease outbreaks, including outbreaks in which free chlorine residuals were reported (12, 42).

The susceptibility of the study viruses to monochloramine varied greatly, both between and within virus types. Monochloramine was least effective for inactivating HAdV2 (at pH 8) and E11 (at pH 7), whereas monochloramine disinfection was most effective for E1 (at both pH values). The HAdV2 results from the present study indicate that a CT value of 2,300 may be needed to achieve a 4-$\log_{10}$ inactivation of HAdV2 with monochloramine at 5°C and pH 8, which is above the CT value of 1,988 recommended in the USEPA *Guidance Manual for Compliance with the Filtration and Disinfection Requirements for Public Water Systems Using Surface Water Sources* to achieve a 4-$\log_{10}$ inactivation with chloramines at pH 8 (37). Within virus types, differences in monochloramine 2-$\log_{10}$ CT values were 2- to 3-fold between CVB5 and CVB3, 5- to 10-fold between

HAdV2 and HAdV41, and 110- to 130-fold between E11 and E1. These data indicate that monochloramine inactivation modeling and system design should incorporate monochloramine efficacy data for multiple viruses of concern.

Because of the increasing level of treated wastewater entering natural water sources and use of reclaimed water, a complete understanding of the disinfection of different types of viruses in drinking water sources is needed. These data are also needed as input for the USEPA's ongoing CCL process to evaluate pathogens for potential rulemaking. The comparative effectiveness of free chlorine and monochloramine for disinfection of eight different CCL viruses in water has been demonstrated in the present study. Similar comparative studies at different temperatures and pH values in typical sources of drinking water are needed to better understand the level of disinfectant needed for these types of water.

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