Evaluation of a New Disinfection Approach: Efficacy of Chlorine and Bromine Halogenated Contact Disinfection for Reduction of Viruses and Microcystin Toxin

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Abstract. Contaminated drinking water is responsible for causing diarrheal diseases that kill millions of people a year. Additionally, toxin-producing blue-green algae associated with diarrhea and neurologic effects continues to be an issue for many drinking water supplies. Disinfection has been used to reduce these risks. A novel gravity-fed household drinking water system with canisters containing N-halamine bromine or chlorine media was challenged with MS2 bacteriophage and microcystin. Chlorine and bromine systems were effective against this virus, with an mean $\pm$ SE reduction of $2.98 \pm 0.26 \log_{10}$ and $5.02 \pm 0.19 \log_{10}$, respectively. Microcystin toxin was reduced by 27.5% and 88.5% to overall mean $\pm$ SE concentrations of $1,600 \pm 98$ ng/L and 259 ± 50 ng/L for the chlorine and bromine canisters, respectively. Only the bromine units consistently produced microcystin effluent $< 1,000$ ng/L (the World Health Organization recommended level) when challenged with 2,500 ng/L and consistently surpassed the U.S. Environmental Protection Agency virus reduction goal of 99.99%.

INTRODUCTION

The World Health Organization (WHO) stated that as of 2002 approximately 1.1 billion people in developing countries lacked access to adequate sources of drinking water and 2.6 billion people lacked access to improved sanitation.¹ Kosek and others² reported that 2.5 million annual deaths occur from diarrheal disease and 88% of the cases¹ are caused by the combination of unsafe water supply, poor sanitation, and hygiene. Diarrheal diseases caused by poor water quality are also responsible for other burdens in the developing world, such as missed school or the inability to work while sick, and substantial economic losses while seeking care for sick family members.³ Lack of sanitation and protection of water sources enables human wastes and pollution to contaminate drinking water, thus leading to the staggering numbers described above.

Enteric viruses are excreted on the order of millions in human feces.^{4,5} These viruses are nanoscale in size and tend to be more resistant to common disinfection than bacteria,⁶ and have high potency (low doses have high probability of causing an infection) where even one exposure can lead to diarrheal disease.⁷ Despite having more advanced water treatment than developing countries, during 1996–2006, the United States continued to experience drinking water outbreaks attributable to enteric viruses, at the rate of approximately two per year.^{8–13} Globally, specific enteric viruses such as poliovirus and hepatitis viruses remain of significant concern. Diarrheal diseases caused by enteric viruses can be deadly in the young, elderly, or those who are immunocompromised because of chronic conditions such as human immunodeficiency virus/acquired immunodeficiency syndrome and malnutrition.¹⁴

Another global water quality problem is associated with cyanobacterial toxins, which are capable of poisoning humans. Codd and others¹⁵ stated that at least 54 countries, including those within Europe, Africa, and Asia, had documented incidences of cyanobacterial-attributable animal poisonings and human health problems. These toxins can cause diarrhea and liver, kidney, and lung failure, and can also act as tumor pro-

motors.¹⁶ Cyanotoxins, although not of the fecal–oral route, benefit from the nutrients in human sewage and other pollution sources (e.g., agricultural runoff) that impact drinking water supplies (i.e., lakes and rivers). Microcystin toxin, one of the most widespread cyanotoxins, is formed by blue-green algae, which can proliferate as a result of heavy nutrients, intense sunlight, and high temperatures.¹⁷ Because microcystin toxins are not constitutively produced by the blue-green algae *Microcystis*, it has been suggested that the probability of exposure to a lethal dose of microcystin from drinking water is unlikely.¹⁸ However, chronic exposure to microcystin, exacerbated by the lysis of microcystis cells during water treatment, is a major concern.^{17,18}

Because most communities in developing countries do not have sufficient funds or infrastructure to adequately treat water for drinking purposes or provide safe water at the tap, point of use (POU) or household water treatment (HWT) devices have been suggested as intermediate and/or alternative solutions. These devices use physical (e.g., biosand filter, ceramic filter) or chemical (e.g., chlorine, flocculation/coagulation) means of reducing microbial threats. POU and HWT approaches, such as the biosand filter (BSF) and ceramic filter (CF), have shown reductions in fecal indicator bacteria and diarrheal illnesses in rural areas of the Dominican Republic (BSF),¹⁹ Bolivia (CF),²⁰ Cambodia (CF),²¹ and Columbia (CF).²² Souter and others²³ illustrated reductions for pathogenic viruses (poliovirus and rotavirus), protozoa (*Cryptosporidium parvum*, *Giardia lamblia*), and bacteria (*Campylobacter* spp., enteropathogenic/toxigenic *Escherichia coli*, *Salmonella* spp., *Vibrio cholerae*, and *Enterococcus*) in laboratory challenges and drinking water sources from developing countries using a flocculation and disinfection product (1 packet/10 liters). The study of Souter and others²³ appears to be the only published research that evaluated virus reduction, where rotavirus and poliovirus were reduced by at least 5.81 and 4.59 $\log_{10}$, respectively, by the flocculation/disinfection product in model and stressed waters.

Disinfection remains a key approach for providing microbiologically safe water. A novel system has been developed that delivers disinfection, as the water passes through the device, by halogen oxidants that act as a contact biocide. Therefore, the end user does not have the responsibility of adding chemicals

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or waiting the appropriate amount of time before the water can be consumed. The HaloPure® halogenated N-halamine media is part of a larger water purifier called AquaSure™, which is commercially available and sold throughout India. AquaSure™ is a gravity-fed system in which water passes through a cartridge containing HaloPure® media, after which the treated water is collected. Low levels of disinfectant are released as the water flows through the canister, thereby meeting the disinfectant demand, of the influent water (i.e., as demand increases in the influent, more halogens are released from the HaloPure® media). In previous studies, the disinfection canisters reduced *Staphylococcus aureus* and *E. coli* O157:H7 by greater than 6.6 log₁₀.²⁴ The specific objectives of this study were to evaluate the HaloPure® chlorine and bromine media for their ability to reduce microcystin toxins, and the bacteriophage MS2 (a coliphage), a proxy for enteric viruses.

METHODS

Disinfection units. Dichlorinated HaloPure® halogenated canisters and mono-brominated HaloPure® halogenated canisters, designed by Eureka Forbes (HaloSource, Seattle, WA), were housed in AquaSure™ Purifier Units. The upper reservoir holds 11 liters of water, which flows by gravity through the disinfectant HaloPure® canister to a lower reservoir for retention of the treated water, and empties by a tap or spigot (Figure 1).

The disinfectant canisters are filled with an insoluble, solid-state halogen-polystyrene matrix. The chlorine-polystyrene combination, poly[1,3-dichloro-5-methyl-5-(4'-vinylphenyl)hydantion] or poly1-Cl, and the bromine-polystyrene combination, poly[1,3-dibromo-5-methyl-5-(4'-vinylphenyl)hydantion] or poly1-Br, are uniform in size (0.25–0.80 mm). The poly1-Cl and poly1-Br have electron-donating alkyl groups on the heterocyclic rings that enable the regulated release of free halogens.²⁵ The bead chemistry has been previously described by Chen and others,²⁵ and has an approximate void space $\geq 100 \mu\text{m}$ based on the theoretical packing of the spherical particles (HaloSource, unpublished data).

Preparation and assay for bacteriophage MS2. To create a viral stock, cultures of bacteriophage MS2 (#15597-B1; American Type Culture Collection, Manassas, VA) were grown to high titer using the host *E. coli* C3000 (#15597; American Type Culture Collection) and a double agar overlay method with trypticase soy agar (Difco, Sparks, MD) and 1.5% agar overlays according to United States Environmental Protection Agency (EPA) Method 1602.²⁶ Agar overlay plates were prepared with a count of approximately 10^4 plaque-forming units (PFU)/plate, then flooded with 20 mL of BBL™ trypticase soy broth (TSB; Difco) and placed on an automatic shaker for 30 minutes at room temperature. The TSB solution was then vacuum-filtered by using a Stericup filtration device with a 0.22 μm GP Express Plus membrane (Millipore, Billerica, MA). The resulting filtrate solution consisted of TSB and high-titered viral stock (3×10^{10} to 1×10^{12} PFU/mL), which was then stored at 4°C until used. The night before testing, an overnight culture of the phage host (*E. coli* C3000) was prepared by mixing 9 mL of TSB and 1 mL of concentrated bacteria stock. This culture was incubated at 37°C for 18–24 hours. The next morning, a mid-log culture was prepared by mixing a 1:10 dilution of the overnight culture into fresh TSB. This culture was incubated in a shaker incubator at 37°C for 4–6 hours to

FIGURE 1. Schematic of the AquaSure® system used for this study. Arrows represent the flow of water in the system and the sections are designated as the upper reservoir (A), HaloPure canister (B) within the lower reservoir (C), and halogenated beads (D).

create log phase growth optimal for plating. Enumeration of bacteriophages was completed using the single agar overlay method according to U.S. EPA Method 1602.²⁶ The limit of detection was 1 PFU/mL.

Preparation and assay for microcystin. To prepare the microcystin stock, a 0.5-mg sample of microcystin LR (from the algae *Microcystis aeruginosa*) was obtained from Sigma-Aldrich (St. Louis, MO) and dissolved in 10 mL of ethanol. This stock was used to seed the water for the trials. The seeded influent and effluent samples were analyzed using the Envirologix Quantiplate™ Kit for Microcystins according to the manufacturer protocol (Rev. 10-26-05), where the detection limit was < 50 ng/L. A spectrophotometer using wavelengths with 450 nm and 620 nm was used to probe the samples. Lab-systems Genesis version 3.03 software (Genesis Laboratory Systems Inc., Grand Junction, CO) was used to compute the standard curve for the assay and to calculate the concentrations of the analyzed samples.

Experimental procedure. Three chlorine and three bromine AquaSure™ Water Purifier Units were set up with new HaloPure® halogenated canisters according to manufacturer's directions with the exception of the cloth and granulated activated carbon filters, which were removed for this study. This was done to test the effectiveness of HaloPure® disinfectants without interference from other sources of virus/toxin removal.

A set volume of non-chlorinated well water consisting of 10 liters was used for bacteriophage experiments; 5 liters was used for microcystin experiments. The well water quality was evaluated by the State of Michigan's Drinking Water Laboratory, Department of Environmental Quality and had a pH of 7.8, specific conductance of 580 $\mu\text{mhos/cm}$, heterotrophic plate count on average of 717 colony-forming units/mL, total organic carbon below detection limit levels (detection limit = 0.5 mg/L, Standard Method 5310C), hardness as CaCO_3 of 58 mg/L, alkalinity as CaCO_3 311 mg/L, and with occasional detection of total coliforms $\leq 5/100$ mL. The well water was seeded with bacteriophage or microcystin toxin for their respective challenges. The seeded well water was shaken thoroughly and then poured into the upper (unfiltered) reservoir of the water purifier. The time of water addition was noted. An influent sample was taken immediately from the upper reservoir and dispensed into a centrifuge tube. All tubes contained sodium thiosulfate (to a 0.1% final concentration) to neutralize any remaining halogen. However, no residual was observed in the influent. The spigot was left open throughout the entire experiment to determine the time of first available water and flow rate. Effluent samples were collected on a time course of every 15 minutes (time points = 15, 30, 45, 60, 75, and 90 minutes) for the bacteriophage experiments and at 5, 10, 20, and 30 minutes for the microcystin experiments. Fewer time points were collected for evaluation of the chemical residuals for the microbial challenge. Samples were diluted as necessary with phosphate-buffered water for the bacteriophage overlay analysis. In the bacteriophage trials, three canisters each for chlorine (C1, C2, and C3) and bromine (B1, B2, and B3) system were tested, and samples were run in triplicate.

To analyze the purifiers' capability to remove microcystin, 2 mL of the microcystin solution was then added to 20 liters of well water, and mixed by shaking the carboy. Five liters of this challenge water were then added to each unit in side-by-side chlorine-based and bromine-based AquaSure™ filter units. Sample collection procedures were identical to those described above with the exception that sodium thiosulfate was added to the bottom reservoirs to quench residuals. Two canisters for each chlorine (C1, C2) and bromine (B1, B2) system were tested and samples were assayed in duplicate.

Determination of halogen residual, flow rate, and $\log_{10}$ reduction. Free and total chlorine and bromine residuals were tested for all trials in the bacteriophage challenges in samples

collected at one hour and at the end-of-flow time points using Free and Total Chlorine Test Kits (Hach Company, Loveland, CO). An additional experiment performed under similar conditions was used to gather residual data at first flush (high flow rate conditions). Samples for residual measurements were taken in separate collection tubes (with no sodium thiosulfate). Bromine residuals were measured by the Total Chlorine Test Kit (Hach Company). Thus, a conversion factor of 2.25 was applied to all bromine residual values (chlorine and bromine react with the colorant in equimolar amounts, and bromine has 2.25 times greater atomic weight than that of chlorine). Influent samples were also tested as negative controls. The flow rate (milliliters/minute) was measured and recorded.

Average bacteriophage $\log_{10}$ reduction for each trial and each replicate canister was determined by converting all values to $\log_{10}$ and subtracting the effluent concentration from the influent measurements ($\log_{10}$ reduction = influent $\log_{10}$ – average effluent $\log_{10}$). The average effluent concentration was determined by averaging all time points. Microcystin toxin percent reduction was determined by dividing the sampling effluent concentration by the influent concentration (percent reduction = $1 - [\text{effluent concentration}/\text{influent concentration}]$). The standard error of the reduction concentrations are shown as the $\pm$ values after each concentration value. These reductions were compared with the U.S. EPA Guide Standard and Protocol for Testing Microbiological Water Purifiers²⁷ and the WHO Guidelines for drinking water quality²⁸ for bacteriophage and microcystin, respectively.

Statistical analysis. Data management and analysis was conducted using Microsoft (Redmond, WA) Excel 2007 and SPSS version 11.0 (SPSS Inc., Chicago, IL). Normality tests were assessed for each dataset to determine which significance test to conduct.^{29,30} All datasets were normal after $\log_{10}$ transformation. Thus, one-way analysis of variance was used to assess significant differences.^{29,30} A significant relationship was determined with respect to an α value of 0.05. The sample sizes (n) refers to the total number of samples processed.

RESULTS

Bacteriophage reduction. Table 1 shows the influent concentrations, mean effluent concentrations, mean $\log_{10}$ reductions, time of first flow, and average free chlorine, and total chlorine and total bromine residuals for the bacteriophage

TABLE 1
Bacteriophage challenge and reduction by HaloPure® contact disinfectant units*

Canister	Mean influent concentration ($\log_{10}$ PFU/mL $\pm$ SE)	Mean effluent $\log_{10}$ concentration ($\log_{10}$ PFU/mL $\pm$ SE)	Mean $\log_{10}$ removal	First flow (minutes)	Mean $\pm$ SE residual, mg/L (minimum and maximum)
Chlorine					
C1 (n = 3)	7.11 $\pm$ 0.41	3.70 $\pm$ 0.29	3.41 $\pm$ 0.29	6.67	Total: 0.47 (0.38–0.60) Free: 0.15 (0.00–0.35)
C2 (n = 3)	6.15 $\pm$ 0.07	3.61 $\pm$ 0.38	2.54 $\pm$ 0.38	7.00	Total: 0.49 (0.45–0.50) Free: 0.36 (0.20–0.45)
C3 (n = 3)	7.95 $\pm$ 0.74	0.66 $\pm$ 0.63	8.61 $\pm$ 0.63	9.00	Total: 0.47 (0.35–0.55) Free: 0.21 (0.10–0.40)
Mean	7.07 $\pm$ 0.28	3.65 $\pm$ 0.04†	2.98 $\pm$ 0.26†		Total: 0.48 $\pm$ 0.01 Free: 0.24 $\pm$ 0.03
Bromine					
B1 (n = 3)	8.28 $\pm$ 0.77	3.10 $\pm$ 0.48	5.18 $\pm$ 0.48	8.00	1.14 (0.90–1.80)
B2 (n = 3)	7.81 $\pm$ 0.40	3.38 $\pm$ 0.15	4.43 $\pm$ 0.15	9.00	1.16 (1.01–1.58)
B3 (n = 3)	8.63 $\pm$ 0.27	3.18 $\pm$ 0.14	5.45 $\pm$ 0.14	8.67	1.07 (0.68–1.69)
Mean	8.24 $\pm$ 0.31	3.22 $\pm$ 0.08	5.02 $\pm$ 0.19		1.21 $\pm$ 0.07

* PFU = plaque-forming units; n = number of experiments conducted using the specific canister.

† Excludes the outlier canister (C3) because of C3 being statistically different from canister 1 ($F_{2,41} = 3.18$, $P < 0.001$) and canister 2 ($F_{2,41} = 3.38$, $P < 0.001$).

experiments for each canister tested. The bacteriophage influent $\log_{10}$ concentrations (PFU/100 mL) were 7.11 ± 0.41 , 6.15 ± 0.07 , and 7.95 ± 0.74 for the three chlorine canister challenges, respectively (Table 1). The chlorine HaloPure® canisters resulted in an average of 3.41 ± 0.29 ($n = 45$), 2.54 ± 0.38 ($n = 48$), and 8.61 ± 0.63 ($n = 54$) $\log_{10}$ reductions in the three respective units (Table 1). Canister 3, resulting in the $8.61 \log_{10}$ reduction, was found to be statistically different from canister 1 ($F_{2,41} = 3.18$, $P < 0.001$) and canister 2 ($F_{2,41} = 3.38$, $P < 0.001$). The overall average $\log_{10}$ reduction for all three units was 4.86 ± 0.70 ($n = 147$), and the average $\log_{10}$ reduction for canisters 1 and 2 only was 2.98 ± 0.26 ($n = 93$).

For the three bromine canister challenges, the bacteriophage influent $\log_{10}$ concentrations (PFU/100 mL) were 8.28 ± 0.77 , 7.81 ± 0.40 , and 8.63 ± 0.27 , respectively (Table 1). The Bromine HaloPure® canisters resulted in an average of 5.18 ± 0.48 ($n = 30$), 4.43 ± 0.15 ($n = 54$), and 5.45 ± 0.14 ($n = 54$) $\log_{10}$

reductions in the three respective units. The overall average was 5.02 ± 0.19 ($n = 84$) $\log_{10}$ reduction. The bromine system achieved reductions $\geq 99.99\%$ (4 logs) (EPA standard for virus removal for water purifiers) for 100% of the trials.

Figure 2 shows the time series for bacteriophage reductions for the chlorine and bromine canisters, respectively. For all units, there was a slight increase in the reductions of bacteriophage with increased time within the unit. However, this reduction was most dramatic for chlorine canister 3. Overall, the chlorine system met the EPA standard for virus removal for POU systems ($> 99.99\%$ or 4 logs) 44.4% of the time. The respective chlorine unit $\log_{10}$ reduction values were significantly different ($F_{2,15} = 52.14$, $P < 0.001$). The bromine units log reduction values were not significantly different ($F_{2,15} = 3.11$, $P = 0.074$). However, bromine canister 1 showed a statistically significant increase in bacteriophage reduction at the 60-minute time point. It should be noted that phage reduction

A. chlorine

B. bromine

FIGURE 2. Bacteriophage $\log_{10}$ reductions using HaloPure® chlorine canisters (A) and bromine canisters (B), where the error bars are ± 1 SE and the method detection limit is 1 plaque-forming unit/mL. The total average $\log_{10}$ reduction after exposure to the respective halogen is listed next to the symbols in the legend with ± 1 SE and n representing the total number of individual samples for each time point.

when evaluated at less than 5 minutes after the effluent passed through the housing with the beads achieved an average $0.45 \log_{10}$ reduction by the chlorine units and $1.6 \log_{10}$ reduction by the bromine units.

Microcystin toxin reduction. Table 2 shows influent concentrations, percent reduction, time of first flow, and average residuals for the microcystin toxin experiments. Canisters 3 for chlorine and bromine were not used for these trials. The microcystin influent concentrations (ng/L) were $2,174 \pm 38$ and $2,271 \pm 90$ for the chlorine canister challenges (Table 2). The chlorine HaloPure® canisters reduced the toxin to an average of $1,839 \pm 5$ ng/L ($n = 30$) and $1,362 \pm 266$ ng/L ($n = 30$) for canisters 1 and 2, respectively. The microcystin influent concentrations (ng/L) were $2,282 \pm 66$ and $2,155 \pm 125$ for the bromine canister challenges (Table 2). The bromine HaloPure® canisters reduced the toxin to an average of 376 ± 64 ng/L ($n = 30$) and 142 ± 51 ng/L ($n = 30$) for canisters 1 and 2, respectively. Overall, microcystin was reduced by 27.5% and 88.5% to final concentrations of $1,600 \pm 98$ ng/L and 250 ± 50 ng/L for the chlorine and bromine canisters, respectively.

Figure 3 shows the concentration of the toxin in the effluent over time for chlorine and bromine, respectively. The percent reduction values for chlorine units were significantly different ($F_{1,6} = 32.21, P < 0.005$) (Figure 3B). The percent reduction values for bromine units were not significantly different ($F_{1,6} = 4.62, P = 0.075$). Shorter time intervals were tested for all replicates to examine the immediate effect of contact with the oxidant. After five minutes, only small reductions of toxin were observed. No statistical significance could be demonstrated, although there was a slightly downward trend of approximately 10–20% with increasing time within the unit. Microcystin was reduced below the WHO toxin standard (1,000 ng) 0% and 100% of all trials for the chlorine and bromine system, respectively, with an average influent concentration of 2,222 ng/L.

Flow rates and residuals. The system tested was a gravity-fed system. Thus, the flow rate was fastest at the beginning of the experiment because the entire volume of the influent was in the top chamber. As flow rate decreased, there was increased contact time within the beads and within the lower reservoir. Unlike traditional disinfection studies, a concentration-time (concentration $\times$ time, mg/L/minute) factor can not be fully described with contact disinfectants. The overall average minimum time until first flush (time passed after addition of influent to first effluent flow) for the bacteriophage experiments was 7.55 minutes (range = 4–11 minutes, $n = 9$) and 8.55 minutes (range = 6–12 minutes, $n = 9$) for the chlorine and

bromine canisters, respectively. The overall average minimum time until first flush for the microcystin experiments was 3.50 minutes (range = 3–4 minutes, $n = 4$) and 5.25 minutes (range = 4–7 minutes, $n = 4$) for the chlorine and bromine canisters, respectively.

The flow rates of the chlorine and bromine AquaSure™ systems followed power regression with time ($y = cx^b$, where c and b are constants, as shown in Figure 4A). The average flow rate for the chlorine system was 88.71 mL/minute with a range from 44 mL/minute (90-minute time point) to 171 mL/minute (15 minute time point). The average flow rate for the bromine system was 81.68 mL/minute with a similar range from 46 mL/minute (75 minute time point) to 160 mL/minute (15 minute time point). Flow rate (milliliters/minute) versus $\log_{10}$ reduction of bacteriophage also followed power regression (Figure 4B shows bromine data; chlorine data are not shown but followed a similar trend with lower $\log_{10}$ reductions). The lower flow rates (< 60 mL/minute) had $\log_{10}$ reductions > 5 . However, the lower reservoir at that point was holding most of the volume from the upper reservoir. The high flow rates (> 60 mL/minute) were in the beginning time points of the experiment and demonstrated $\log_{10}$ reductions < 5 . There was no significant difference between the $\log_{10}$ reductions seen at the various flow rates for bromine ($F_{5,12} = 2.01, P = 0.149$) or chlorine ($F_{5,12} = 0.265, P = 0.924$).

The total chlorine residual was 0.48 ± 0.01 mg/L ($n = 18$), and the total free chlorine residual was 0.24 ± 0.03 mg/L ($n = 18$) for the chlorine HaloPure® systems during the bacteriophage experiments (Table 1); these values did not change significantly during the course of the experiment. The total chlorine residual value at the 60 minute time point was 0.46 ± 0.02 ($n = 9$), and the 90 minute time point showed an average residual value of 0.49 ± 0.2 mg/L ($n = 9$). The free chlorine residual values at the 60 minute time point averaged 0.22 ± 0.05 mg/L ($n = 9$), and the 90 minute time point showed residual values averaging 0.26 ± 0.04 mg/L ($n = 9$). The average total bromine residual was 1.21 ± 0.07 mg/L for the bromine HaloPure® systems during the bacteriophage experiments. The bromine residual values at the 60 minute time point averaged 1.23 ± 0.10 mg/L ($n = 9$), and the 90 minute time point showed residual values averaging 1.20 ± 0.12 mg/L ($n = 9$). Residuals measured as soon as flow began in a separate experiment averaged 0.43 mg/L for total chlorine ($n = 3$), and 0.03 mg/L for free chlorine ($n = 3$). Bromine residuals were not measured for all time points during the experiments. Residuals during the microcystin experiments were below the detection limit because of sodium thiosulfate being added to the bottom reservoir as part of the microcystin study design and were all reported as zeros, thus not listed in Table 2.

DISCUSSION

In the United States, drinking water from surface water sources must comply with the Surface Water Treatment Rule (SWTR).³¹ The surface water treatment facilities must adequately filter and/or disinfect so as to achieve 99.9% reduction of *Giardia* cysts and 99.99% reduction of enteric viruses.³¹ The development of the SWTR also incorporated the Ct concept, where concentration (mg/L) of the disinfectant over time ($C \times T$) was used to aid in reaching the SWTR requirements. Tables for disinfection of viruses were established through controlled studies, recognizing that viruses were generally

TABLE 2

Microcystin challenge and reduction by HaloPure contact disinfectant units*

Canister	Influent concentration (ng/L $\pm$ SE)	Effluent $\log_{10}$ reduction concentration (ng/L $\pm$ SE)	% Reduction	First flow† (n = 2), minutes
Chlorine				
C1 (n = 3)	$2,174 \pm 38$	$1,839 \pm 5$	15	4.18
C2 (n = 3)	$2,271 \pm 90$	$1,362 \pm 90$	40	3.27
Mean	$2,222 \pm 49$	$1,600 \pm 98$	27.5	
Bromine				
B1 (n = 3)	$2,282 \pm 66$	376 ± 64	84	6.21
B2 (n = 3)	$2,155 \pm 125$	142 ± 51	93	4.82
Mean	$2,218 \pm 62$	259 ± 50	88.5	

* Effluent was immediately quenched; thus, residual data were not obtained.

† First flow was only measured in the second and third trials for each canister.

A. chlorine

B. bromine

FIGURE 3. Microcystin toxin reductions using HaloPure® chlorine canisters (A) and bromine canisters (B), where the error bars are ± 1 SE and the method detection limit is < 50 ng/L. The total average concentration after exposure to the respective halogen is listed next to the symbols in the legend with ± 1 SE and n representing the total number of individual samples for each time point.

more resistant than bacteria.³² However, the Ct tables formed were based on the assumptions that disinfection kinetics for buffered demand-free water would reflect the disinfection kinetics for source waters, and that the disinfection kinetics would follow a simple a Chick-Watson relationship for microbial inactivation.³³ Water quality and specific parameters (e.g., pH, turbidity) affect disinfection kinetics.³³ Therefore, these variables should be reported for each microbe and water type. For MS2 coliphage, a residual of 0.5 mg/L of free chlorine was shown to achieve 99.99% removal after 1.2 and 26.5 minutes at a pH of 6 and 10, respectively, at 5°C in buffered demand-free water.³⁴ In this current study, natural waters with a pH of 7.78 were used for the contact disinfection where free chlorine residuals were on average 0.24 mg/L and reductions of 99–99.9% were observed after 30 minutes. This finding appears consistent with published reference values³⁵ and the conditions studied (excluding the results from canister 3,

which showed 7 $\log_{10}$ reductions at 30 minutes). The present and cited studies were conducted at room temperature with the well water ranging between 15°C and 20°C at the time of the challenges.

The HaloPure® canisters were designed for household use in India on tap water or well water of indeterminate and suspect microbial quality. Adapted from a system that originally used iodine, the canisters also are sold with granulated activated carbon to remove tastes and odors, although our studies were conducted without the carbon element. The bead system has been rated by the manufacturer to treat $\leq 3,000$ liters of water, or last approximately 75% of the year (272 days at 11 liters/day). The system also had built in a pre-filter (approximately 500 μm ; HaloSource, unpublished data) to protect the bead system from clogging because of sediment in the influent water. In most cases, the reservoir is filled with water the night before and water is used the next day,

FIGURE 4. Changes in flow rate in the chlorine and bromine HaloPure® systems during the bacteriophage challenges are shown in **A**, where each error bars represents ± 1 SE. **B**, Relationship between flow rate and $\log_{10}$ reductions of bacteriophage using the bromine canister, where each error bars represents ± 1 SE.

thus providing time for the residual to act further upon any surviving pathogens or regrowth. The design of having the chemical disinfectant delivered as microbes pass through the halogenated media alleviates the problem of the user having to carefully calculate what to add and when, which is one advantage of the contact disinfectant approach (i.e., removing the responsibility from the user). For example, increased user error can be associated with HWT or POU systems that require mixing and holding time. Such additional steps by the user also decreases acceptance and assimilation into everyday water practices.²⁶ Therefore, the HaloPure® system could have a higher rate of sustainability. In addition, the cost of producing water using the N-halamine technology is comparable to municipally treated water (Bridges M, HaloSource, unpublished data).

One aspect of user control to further improve water quality using the contact disinfectant could be in the volume of water added to the top reservoir. As shown in Figure 4, the flow rate decreases over time because of the decreasing pressure and the decrease in water volume in the top reservoir as it passes through the canister. This slower flow rate is advantageous in that pathogens will be exposed to the contact disinfectant longer, thus resulting in higher reductions. The $\log_{10}$ reductions seen in the later time points (Figure 2) may have been caused by this phenomenon. Thus, it should be communicated to the user that better water quality could be achieved by adding smaller volumes of water at a time. However, enough volume is needed for appropriate pressure to enable adequate flow.

When compared with the chlorine canister, the bromine HaloPure® system showed more consistent results in reducing

concentrations of bacteriophage and microcystin toxin. The ability of bromine to effectively disinfect microorganisms at an equivalent or higher rate to chlorine has been documented,^{28,35} although Floyd and others³⁵ reported that the reasoning for such results is still unclear. Presently, to our knowledge, the reasoning for the superior biocidal ability of bromine compared with chlorine has yet to be defined. In addition, despite the fact that cruise vessels, navy ships, and off-shore platforms commonly use this halogen for water treatment,³⁶ bromine has not been widely recognized as a viable disinfectant. A resin brominated by exposure to elemental bromine was first registered in 1976 to treat potable water aboard U.S. Navy ships.³⁶ Although standards for dietary intake tolerance with regards to bromine (1 ppm) have been established,³⁶ requirements in drinking and potable water for bromine and the bromine residuals, bromate (BrO_3^-) and bromide ion (Br^-), have not been fully examined.

The U.S. EPA R.E.D. facts sheet³⁶ reported that this agency does not have enough data on bromate to set a standard; the WHO recommends $< 10 \mu\text{g/L}$.³⁷ However, the WHO recommendation is based on what this organization believes is feasible for treatment systems, rather than a standard of $1 \mu\text{g/L}$.³⁷ The bromide ion is reported to be "exempt from the requirement of tolerance" by the U.S. EPA.³⁶ Despite the apparent advantages of bromine shown in this study and its long-time use aboard vessels, its likely that chlorine will continue to be used in developing countries for POU in household water treatment because of its low cost, widespread availability, and recognized biocidal effectiveness. However, this study shows that more research should focus on the use of bromine-based disinfectants for drinking water disinfection of human pathogens. One key advantage of using bromine versus other halogens, such as iodine and chlorine, is that humans tolerate bromine residuals at higher levels, thereby enabling better protection of treated water during storage (Bridges M, HaloSource, unpublished data).

Publications assessing POU devices (e.g., BSF, CF) for drinking water treatment in communities in developing regions of the world in a laboratory setting¹⁹ and under field conditions,¹⁹⁻²² have focused on the reduction of coliforms and *E. coli* with respects to their ability to decrease rates of diarrheal illness. However, literature measuring the disinfection capabilities of POU devices for the control of bacteriophages or enteric viruses is limited. One study by Elliot and others³⁸ quantified the BSF reductions of echovirus 12 and bacteriophages (MS2 and PRD1) from treated drinking water seeded with low percentages (0–2.5%) of wastewater primary effluent. Mean reductions after 30 days were $2.1 \log_{10}$ and $0.5 \log_{10}$ for echovirus 12 and bacteriophages, respectively.³ Another pilot-scale study using slow sand filtration showed a $1.5 \log_{10}$ to $2.0 \log_{10}$ reduction of MS2 bacteriophage.³⁹ Various POU and HWT studies by Sobsey and others³⁴ and the baseline $\log_{10}$ reductions (field conditions with unskilled persons) for viruses were reported to be $0.5 \log_{10}$ for porous CF, $0.5 \log_{10}$ for BSF, $2 \log_{10}$ for solar disinfection, and $3 \log_{10}$ for chlorination. Bacteriophage $\log_{10}$ reductions with the chlorine HaloPure® canister ($2.98 \pm 0.26 \log_{10}$) were within published virus reduction ranges for other POUs, and the reduction capacity of bromine canisters ($5.02 \pm 0.19 \log_{10}$) surpassed these levels of reduction and showed better and more consistent results. The reasoning for the outlier replicate in the chlorine reduction of the bacteriophage experiment (Figure 3A) cannot be related

to one specific factor but may have been caused by lot-to-lot variation of the canister.

Many developing countries are in tropical regions and have periods of heavy rainfall that transport human wastes and additional nutrients into neighboring sources of drinking water. The combination of excessive pollution, high temperatures, and intense sunlight are ideal conditions for algal blooms in surface waters, thus increasing the potential exposure to algal toxins.^{17,28} The WHO has set a guideline for microcystins in drinking water of $1,000 \text{ ng/L}$ and has stated that drinking water is the major route of human exposure to microcystin toxin. A Canadian study on Shoal Lake, a source of drinking water, reported microcystins at a concentration $> 500 \text{ ng/L}$.²⁸ A collection of 160 surface water samples in a follow-up study showed concentrations ranging from $< 100 \text{ ng/L}$ to $1,000 \text{ ng/L}$.²⁸ Concentrations after conventional treatment ranged from $< 100 \text{ ng/L}$ to $6,000 \text{ ng/L}$.²⁸

Detection of microcystins can also be dependent on water column depth, as shown in a Turkish study of Lake Sapanca, where toxin was not detected at a depth < 10 meters but was detected at a depth of 20 meters (concentration = $3,650 \text{ ng/L}$).¹⁷ Additionally, 90% of the samples containing microcystin toxin were detected between 15 and 25 meters, where the intake pipe was located for the drinking water supply.¹⁷ The HaloPure® devices would reduce these toxin levels by 27.5% with the chlorine canister and by 87.5% with the bromine canister. The bromine canister would likely reduce the toxin levels to meet the required WHO standard in such waters. Even with high challenge concentrations of $2,500 \text{ ng/L}$, the bromine canister reduced the toxin to an average concentration of 259 ng/L , which is well below the standard. However, the chlorine canister reduced the toxin to an overall average of only $1,600 \text{ ng/L}$. In the study by Acero and others,⁴⁰ the Ct values for chlorine required to reduce microcystins to $1,000 \text{ ng/L}$ were equivalent to those needed to reduce *Giardia* cysts and viruses to the standards of 99% ($2 \log_{10}$) and higher 99.99% ($4 \log_{10}$) at pH 6 and 9, respectively. The bromine canister in our study supports the findings of Acero and others⁴⁰ in regards to the reduction levels needed for viruses, but the chlorine canister did not achieve the standards.

The U.S. EPA guideline for standard and protocol for testing microbiological water purifiers states that a system should be capable of reducing viruses or an acceptable surrogate by at least $4 \log_{10}$ (99.99%).²⁷ This requirement originated for the registration of water disinfectant systems for water to reflect the goals of the SWTR. This goal may or may not be appropriate given the influent concentrations of pathogens challenging these water systems. Regli and others⁴¹ used a risk assessment approach and a safety goal of $1/10,000$ to define viral risk reductions needed in the United States, which was dependent on surface water pollution levels. More information is needed on viral concentrations in the various waters across the globe to fully analyze the risks to public health posed by current water treatment practices and the risk reduction achievable through various POU systems.

Developing countries lacking access to adequate drinking water presents a significant global public health problem, which is aggravated by poor sanitation and hygiene practices, malnutrition, and a high prevalence of chronic diseases such as human immunodeficiency virus/acquired immunodeficiency syndrome. The WHO and the United Nations Children's Fund report that open defecation is still widely practiced by

approximately 1.2 billion persons in many developing areas, which further supports the need for combined action in water and sanitation efforts. In addition, despite the demonstrated ability of available POU devices, such as commercial chemical packets and the BSF to decrease diarrheal illnesses, acceptance and long-term sustainable use in these communities are low.²⁴ The Millennium Development Goal for providing access to clean drinking water to higher percentages of the world's population may only be successful if POU devices could be integrated into everyday practices in these developing countries, particularly in the rural and periurban environments. Contact disinfectants hold promise as one possible approach to help achieve safe drinking water.

Contact disinfectants can be used in real-world situations for reduction of viruses and toxins, two waterborne contaminants of concern. Reductions of 12–88% for microcystin and 99–99.999% for the MS2 virus were documented in seeded well water when chlorine and bromine systems, respectively, were used. We believe that variation in flow rate is a suitable proxy for variation in the contact time in the bead bed, and more significantly, in the bottom reservoir. Such increasing contact time is responsible for the increased inactivation of phage with time. Risk reduction and appropriate use depends on the characteristics of the surface waters and level of contamination. Thus, the appropriate goal for virus inactivation and toxin oxidation needs to be matched to a better understanding of levels in waters that might be treated by POU systems. In the future, a greater exploration of bromine as a disinfectant is warranted because of its superior performance compared with chlorine. However, bromate formation and water chemistry reactions will need to be fully investigated.

Received May 24, 2009. Accepted for publication October 25, 2009.

Financial support: This study was supported by the Department of Homeland Security (DHS) Summer Internship program and HaloSource Inc. (Bothell, WA), and by an appointment to the Department of Homeland Security Scholarship and Fellowship Program administered by the Oak Ridge Institute for Science and Education (ORISE) through an interagency agreement between the U.S. Department of Energy (DOE) and DHS. ORISE is managed by Oak Ridge Associated Universities (ORAU) under DOE contract no. DE-AC05-06OR23100.

Disclosure: All opinions expressed in this paper are those of the authors and do not necessarily reflect the policies and views of DHS, DOE, or ORAU/ORISE.

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