Backcountry Water Treatment to Prevent Giardiasis

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Abstract: This study was conducted to provide current information on the effectiveness of water treatment chemicals and filters for control of *Giardia* cysts in areas where treated water is not available. Four filters and seven chemical treatments were evaluated for both clear and turbid water at 10°C. Three contact disinfection devices were also tested for cyst inactivation. Filters were tested with 1-liter volumes of water seeded with 3×10^4 cysts of *G. lamblia* produced in gerbils inoculated with *in vitro* cultured trophozoites; the entire volume of filtrate was examined for cyst passage. Chemical treatments were evaluated at concentrations specified by the manufacturer and for contact times that might be expected of hikers (30 minutes) and campers (eight hours, i.e., overnight). Two of the four filter devices tested were 100 percent effective for *Giardia* cyst removal. Of the other two filters, one was 90 percent effective and the other considerably less effective. Among the seven disinfection treatments, the iodine-based chemicals were all significantly more effective than the chlorine-based chemicals. None of the chemical treatments achieved 99.9 percent cyst inactivation with only 30-minute contact. After an eight-hour contact each of the iodine but none of the chlorine preparations achieved at least 99.9 percent cyst inactivation. None of the contact disinfection devices provided appreciable cyst inactivation. Heating water to at least 70°C for 10 minutes was an acceptable alternative treatment. (*Am J Public Health* 1989; 79:1633–1637.)

Introduction

Giardia lamblia is the most commonly identified human intestinal parasite in the United States. Giardiasis is commonly transmitted between humans, especially among small children. It is also transmitted in water, particularly in the mountainous regions of the U.S. Since 1965, over 80 waterborne outbreaks of giardiasis have occurred in community water systems, affecting more than 20,000 persons.[1] Giardiasis in hikers and campers has also been documented;[2,3] indeed, it is commonly considered a backpackers' illness. *Giardia* cysts in concentrations as high as four per gallon have been detected in untreated surface water in northeastern and western states.[4]

Concern over waterborne transmission of *Giardia* has led to development of a variety of chemical disinfectants and portable filters for individual use in the backcountry. Although some information on such methods has been reported,[2,5,6] there is no comprehensive guide to their reliability in actually removing or inactivating *Giardia* cysts.

We tested four commercially available portable filters and one contact disinfection device for their ability to remove *Giardia* cysts from water. We also evaluated the cysticidal effectiveness of seven chemical disinfectants and three contact disinfection devices.

Methods

Cysts of *G. lamblia* were prepared for use in both the filtration and disinfection tests by propagation in gerbils inoculated with trophozoites from sterile culture. Trophozoites were of two isolates: one from a beaver (Be-4 isolate from Alberta) and one from a human (H-2 CSU isolate from Colorado). Cysts were concentrated from crushed, filtered gerbil feces by flotation on zinc sulfate (sp. gr. 1.18), cleaned, and stored in distilled water at 4°C for up to 10 days before use. Similarly, *G. muris* cysts of an isolate originally obtained from hamsters[7] were purified from feces of infected athymic (nu/nu) mice and stored before use. Cyst concentrations were determined with a Coulter Counter (Model ZBI, Coulter Electronics, Hialeah, FL) and a haemacytometer. Except where noted, cysts were added to water samples in concentrations of about 3×10^4/ml.

Cyst viability was assayed by fluorogenic staining[8] and *in vitro* excystation.[7] In the former method, live cysts are distinguished by two fluorescing dyes. One dye is fluorescein diacetate (FDA), which when absorbed by cysts produces a fluorescent green only in live cysts; the second dye, either propidium iodide (PI) or ethidium bromide (EB), is excluded efficiently by live cysts but absorbed by dead cysts, resulting in red fluorescence.

Filter Testing

The following backpacker-type water filters were purchased from local retailers: First Need Water Purification Device (First Need), General Ecology Inc., Lionville, PA; H_2OK Portable Drinking Water Treatment Unit Model No. 6 (H_2OK), Better Living Laboratories Inc., Memphis, TN; Katadyn Pocket Filter (Katadyn), Katadyn Products Inc., Wallisellen, Switzerland; and Pocket Purifier, Calco Ltd, Rosemont, IL. Also noted in this category is the Water Tech Water Purifier (Water Purifier), Water Technologies Corp., Ann Arbor, MI. Although it is not advertised as a filter and was not specifically tested for *Giardia* cyst removal, we report qualitative observations made during disinfection testing (see below) because its configuration and mode of operation suggest that particle removal may occur. Physical and operating information provided in the filter packaging is summarized in Appendix A. Each device was tested when it was new. Devices that removed all cysts when new were retested after a period of use approximating several months for a regular weekend user.

Each filter was prepared for testing by filtering four liters of tap water to purge loose carbon particles or debris. The cyst removal performance of each filter was determined by filtering one liter of spring water, turbidity of 0.1 NTU, to which formalin-fixed *G. lamblia* cysts had been added. The

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entire filtrate volume was passed through a 25-mm dia., 5-μm pore size, polycarbonate membrane (Nuclepore, Pleasanton, CA), stained with EB (100 μg/ml), and mounted under a cover slip. Cysts were counted at ×250 magnification with the aid of epifluorescence microscopy. A representative portion of each filter was examined to quantify cyst recovery as described previously.[9] The area examined was inversely proportional to the number of cysts found and ranged from 3.5 percent of seeded positive control filters to 25 percent (one quadrant) of filters with cyst densities less than one per field. Total numbers of cysts present were estimated by extrapolation in direct proportion to the area examined. In extensive work on recovery of *Giardia* cysts using the procedures described above, cyst retention on the 5-μm polycarbonate membrane in a single filtration step has routinely averaged 80 to 90 percent (Ongerth JE: unpublished). Accordingly, the ability to identify high levels of cyst removal, which would result in passage of very few or no cysts, is excellent. This ability is unaffected by the factors that contribute to lack of precision in counting large numbers of cysts on filters; such inaccuracies usually occur when only small representative subareas are examined and the total numbers are estimated by extrapolation. A seeded positive control and an unseeded negative control were processed with each batch of filter evaluations. The cyst removal performance evaluation was replicated three times for each filter device, with results expressed as the arithmetic average and corresponding standard deviation.

Contact Disinfection Testing

The Water Purifier is described in packaging information as a contact disinfection device. Likewise, the H_2OK and Pocket Purifier devices are described as providing disinfection as well as removing cysts by filtration. These devices were therefore tested for their effect on cyst viability in addition to filtration efficiency. A single 500-ml sample for each device was seeded with approximately 2.5×10^4 cysts and passed through the device. Filtrate was collected and filtered as described above to recover cysts. The viability of cysts was then assessed by FDA and EB staining as described below.

Disinfectant Testing

The cysticidal effects of seven commercially available and commonly used disinfectant preparations were tested with identical procedures. Four of the products were iodine-based: Polar Pure Water Disinfectant (Polar Pure), Polar Equipment, Saratoga, CA; Coghlan's Emergency Germicidal Drinking Water Tablets (CEGDWT), Coghlan's Ltd, Winnipeg, Canada; Potable Aqua Drinking Water Germicidal Tablets (Potable Aqua), Wisconsin Pharmacal Inc., Jackson, WI; and 2 percent iodine prepared from I_2, reagent grade (Baker, Phillipsburg, NJ). The remaining three products were chlorine-based: Sierra Water Purifier (Sierra), 4 in 1 Water Co., Santa Fe, NM; Halazone, Abbott Laboratories, North Chicago, IL; and commercial liquid bleach (5.25 percent sodium hypochlorite). Disinfectant solutions were characterized by pH and total halogen concentration (Appendix B), the latter being determined colorimetrically using the DPD method.[10]

Two water sources were used, one to reflect clear high-mountain conditions, the other to reflect downstream, more turbid conditions. Water sources were characterized by pH, turbidity, and free chlorine demand (Appendix C). The upstream source was from a small, spring-fed tributary to the Snoqualmie River near North Bend, Washington. Samples were taken from the stream approximately 50 yards downstream from the spring. The downstream source was the discharge from Lake Washington in Seattle, Washington. Samples were taken in midstream at the entrance to Portage Bay, adjacent to the University of Washington campus. Water samples were prepared for testing by adding disinfectant, according to manufacturers' instructions, to one liter of water in stoppered glass bottles (Appendix B).

Cysticidal properties of the chemical treatments were determined as follows.

1) Water was put in 50-ml disposable plastic centrifuge tubes and placed in a 10°C incubator.

2) *G. lamblia* cysts were added to each test sample at time zero.

3) Tubes were vortex-mixed, sampled, and returned to the incubator.

4) At each sampling time, i.e., time 0, 30 minutes and 8 hours, a 10-ml sample was withdrawn; a portion was used for measuring disinfectant concentration, and in the remainder the disinfectant was quenched with 0.1-mM sodium thiosulphate.

5) Cysts in the quenched sample portion were exposed to aqueous solutions of the viability indicators, FDA (25 μg/ml) and EB (100 μg/ml), filtered on to a 13-mm dia. 5-μm pore-size filter membrane, and rinsed with distilled water (10 ml).

6) Filters were mounted on glass slides, sealed under coverslips and examined by epifluorescence microscopy at ×250 magnification (Model 16, Carl Zeiss, Inc., Thornwood, NY) to enumerate proportions of red and green fluorescing cysts indicating dead and live status, respectively.

The viability baseline of the cysts was established by running a control sample of untreated water seeded with cysts through each test, using procedures identical to those for disinfectant-treated samples. Data are presented in terms of percent survival relative to the controls (Figure 2). The effectiveness of each disinfectant for killing cysts in both upstream and downstream water was determined in triplicate, with results expressed as the arithmetic average and corresponding standard deviation.

The Water Tech Water Purifier, a contact disinfectant, was also tested as a chemical disinfectant. The test water was 100 ml of spring-source water seeded with *Giardia* cysts. The treated water was filtered, stained, and examined for cyst viability as described in steps 5 and 6 above. Three replicates were assayed.

Heat Inactivation

Inactivation of *G. lamblia* and *G. muris* cysts by heating was established as follows. Cysts were added to distilled water in 15-ml glass test tubes. The seeded tubes were incubated for 10 minutes at temperatures ranging from 10° to 70°C. Afterwards, cyst suspensions were cooled immediately by swirling in 10°C water for one minute. Cyst viability was determined either by excystation or by staining. If by the latter, FDA and EB were added to the samples, the tubes were vortex-mixed, and a 1-ml aliquot was filtered through a 13-mm dia. 5-μm pore-size filter membrane. Filters were rinsed, mounted, and examined as described above to enumerate the live and dead cysts.

Results

Filter Device Tests

The four filters differed significantly in their ability to remove *Giardia* cysts (Figure 1). The number of cysts recovered from water having passed through the filter devices ranged from zero to greater than 10^4 in individual tests. The performance of individual devices was consistent as indicated by the standard deviations for each of the three replicate test sets (Figure 1). The percentage of cysts removed by the devices, corresponding to 100 minus the percent of cysts recovered from the filtrate, was 100 percent for the FirstNeed and Katadyn filters and approximately 90 percent for the H_2OK filter. The concentration of cysts in the Pocket Purifier effluent was not statistically different from the seed concentration.

The FirstNeed and Katadyn filters were then subjected to a period of moderate use and then retested. The volume of water processed during the simulated use period was not the same for the two filters owing to differences in their operation. The difference in volume had no apparent effect on performance of the two filters. A total of 88 liters of tap water (turbidity of 0.3 NTU) was filtered with the FirstNeed. During the process it was back-flushed, as recommended in package instructions, because the filtration rate decreased after 50, 71, and 75 liters had been filtered. After 88 liters had been processed, the filtration rate was about 25 percent lower than when the filter was new, and it was retested in that condition. The Katadyn filter was subjected to use by filtering one liter of tap water four times a day for five days. At the end of each day, the filter was cleaned according to package instructions by disassembling, brushing the filter element, and allowing it to air-dry overnight before reassembly. After the respective periods of use, these two filters were tested in triplicate for efficiency of cyst removal. Performance of these filters was the same, 100 percent cyst removal, when they were retested.

Cyst Inactivation

Contact Disinfection Devices—The effect of each of the contact disinfection devices on *G. lamblia* cyst viability was limited. The Water Purifier inactivated about 15 percent of the cysts added in 100 ml of upstream (low turbidity) water; the H_2OK filter inactivated about 5 percent of the cyst challenge, and the Pocket Purifier inactivated about 2 percent of the cyst challenge.

Chemical Disinfectants—The effectiveness of seven disinfecting chemical preparations ranged from only a few percent to greater than 99.9 percent, depending on the chemical and its concentration, the contact time, and the disinfectant demand of the water (Figure 2). None of the disinfectants was more than 90 percent effective after a contact time of 30 minutes. After eight-hour contact, the four iodine-based disinfectants, each caused a greater than 99.9 percent reduction in viable cysts. The chlorine-based disinfectants were clearly less effective than the iodine-based ones at both contact times.

Heating in Water—Experiments conducted with cysts of *G. lamblia* and of *G. muris* indicated that the two species have virtually the same sensitivity to inactivation by heating. Cysts of both species were completely inactivated by heating to 70°C for 10 minutes. Heating to 50°C and 60°C for 10 minutes produced 95 and 98 percent inactivation, respectively (Figure 3).

Discussion

To remove *Giardia* cysts from water, one must use a filter with sufficiently small pores to trap the cysts and sufficiently large capacity to produce a useful volume of treated water before backwashing or replacement is necessary. Although a number of manufacturers advertise that their filters remove *Giardia* cysts, the only previously published account of filter performance was for the Katadyn unit.[6] Our filter evaluation study showed that only the FirstNeed and the Katadyn filters removed cysts with at least 99.9 percent effectiveness. Under the same test conditions, the H_2OK filter was approximately 90 percent effective and the Pocket Purifier was less than 50 percent effective for cyst removal. The analysis of viability for the cysts collected in the effluent of the Water Purifier, H_2OK, and Pocket Purifier indicates that passage through the device did not significantly reduce the percentage of viable cysts.

The current study showed that none of the chemical treatments could inactivate more than 90 percent of cysts with 30 minutes of contact time at 10°C. At both 30 minutes and eight hours of contact time, the iodine-based disinfectants inactivated a higher fraction of cysts than did the chlorine-based products. All methods inactivated a lower percentage of cysts in cloudy or turbid water than in clear water. All disinfectants performed better with eight hours of contact time than with 30 minutes. Only the iodine-based compounds inactivated 99 to 99.9 percent of cysts, within eight hours of contact time for both turbid and clear water. As observed by Jarroll, *et al*,[5] the 2 percent tincture of iodine

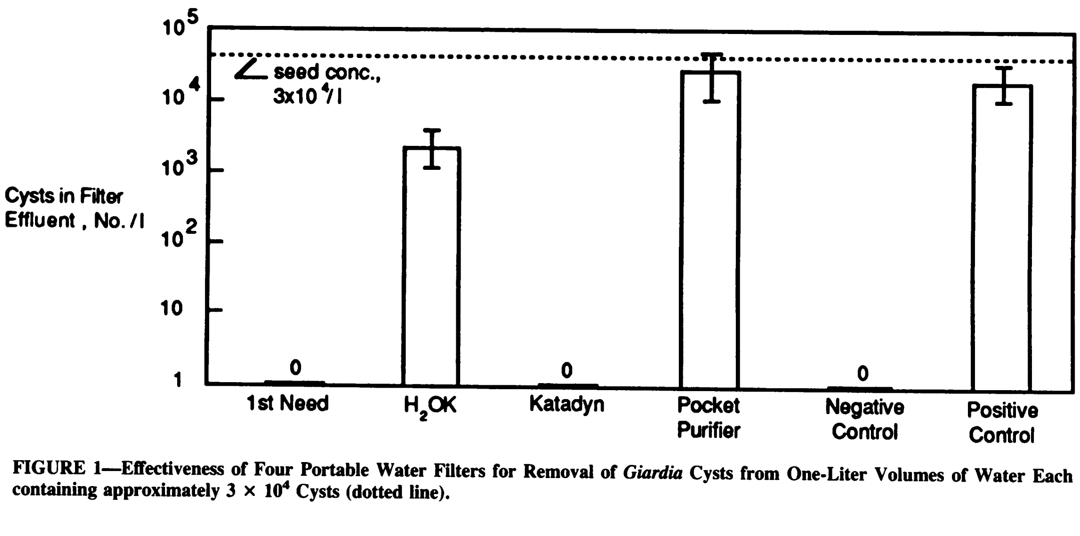

FIGURE 1—Effectiveness of Four Portable Water Filters for Removal of *Giardia* Cysts from One-Liter Volumes of Water Each containing approximately 3 × 10^4 Cysts (dotted line).

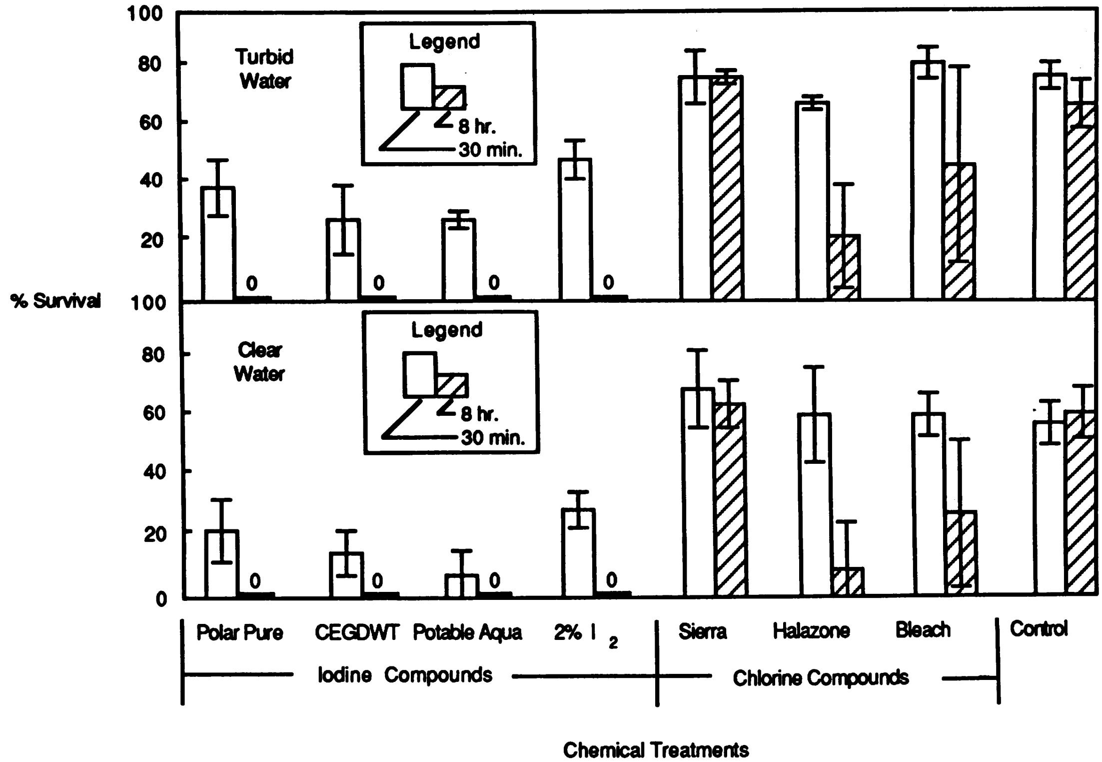

FIGURE 2—Effect of Time and Disinfectant Concentration of Seven Chemical Disinfectants on Survival of *G. lamblia* Cysts in Turbid and in Clear Water.

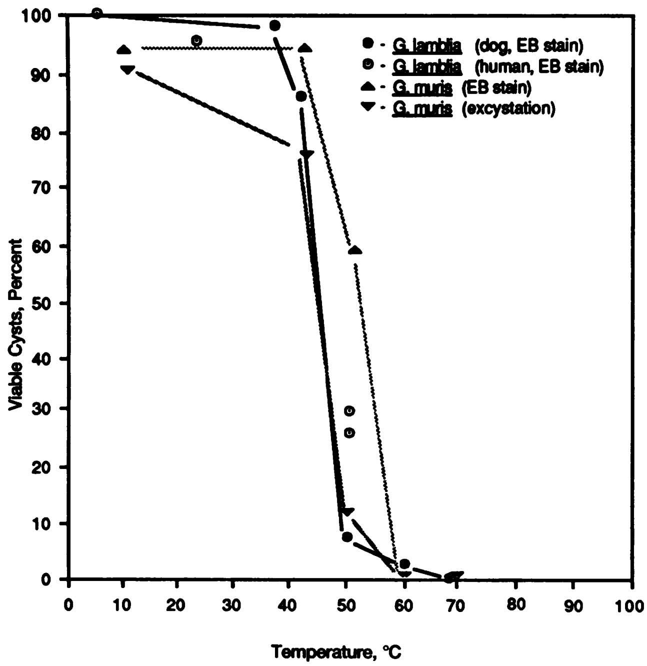

FIGURE 3—Inactivation of *Giardia* Cysts as a Function of Temperature (10-minute exposures) as Indicated by Ethidium Bromide Staining and by *in vitro* Excystation.

was less effective than the other iodine preparations with 30 minutes of contact time, but it was as effective as the others at eight hours. Comparison of our results with those of Jarroll, *et al*,[5] is complicated by differences between test conditions used. However, our results generally indicate more stringent requirements for effective inactivation of *Giardia* cysts. Differences between cyst populations used in the two studies could account for the observed differences, even though both were *G. lamblia*. Cysts produced in our trophozoite-gerbil system had consistently high intrinsic viability (>80 percent), excysted efficiently when fresh (80 to 90 percent), and have appeared more resistant to halogen disinfectants than reported previously (Ongerth J.E.: unpublished).

The results of heat inactivation in our study correspond to previous reports indicating that heating to between 60° and 70°C kills *Giardia* cysts efficiently. In addition, our data illustrate the correspondence between the fluorogenic staining and *in vitro* excystation procedures for assessing cyst viability. When applied to cysts of the same condition, staining indicates a slightly higher proportion of viable cysts than does excystation. Overall, however, the two procedures provide comparable information.

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APPENDIX A
Walter Filter Characteristics Listed by Manufacturers on Packaging or Instruction Insert

Name	Manufacturer	Filter Type	Operating Mode	Operating Rate	Useful Life	Restrictions/ Limitations
First Need Model #6	General Ecology Lionville, PA	0.4 μm microscreen plus adsorber	hand pump	1 pt/min	up to 800 pints	A
H_2OK	Better Living Labs Memphis, TN	6 μm mesh, 3 in. activated carbon w/Ag	gravity	1 qt/min	2000 gal	A, B
Katadyn Pocket Filter	Katadyn Products Wallisellen, Switzerland	0.2 μm ceramic, Ag-impregnated	hand pump	1 qt/min	many years	A
Pocket Purifier	Calco, Ltd. Rosemont, IL	10 μm (nominal), halogenated resin (38% I), Ag-impregnated carbon	mouth suction	—	—	A
Water[a] Purifier	Water Technologies Ann Arbor, MI	Polystyrene resin bed (46% I_2 as I_5)	gravity	—	100 gal	A, C

A—Does not desalinate; not for saltwater or brackish water.
B—Pretreat with I_2 for bacterially contaminated water.
C—Not for use with muddy water.
[a]Not described as a filter by package information.

APPENDIX B
Characteristics of Disinfectant Preparations

Name	Manufacturer	Active Chemical	Recommended Application	Total Halogen[a] Concentration, (mg/liter)	pH[b]
Polar Pure	Polar Equipment Saratoga, CA	Crystalline iodine, 99.5%	1–7 capfuls per quart depending on temperature	2.4 (1 cap/quart)	6.1
CEGDWT	Coghlan's Ltd. Winnipeg, Canada	Tetraglycine hydroperiodate 16.7% (6.68% titrable iodine)	1 tablet per liter or quart	4.5 (1 tab/quart)	5.6
Potable Aqua	Wisconsin Pharmacal Jackson, WI	Tetraglycine hydroperiodate, 16.7% (6.68% titrable iodine)	1 tablet per liter or quart	5.3 (1 tab/quart)	5.6
2% Iodine	Baker Phillipsburg, NJ	Iodine	0.4 ml per liter	4.5	6.5
Sierra	4 in 1 Water Co. Santa Fe, NM	Calcium hypochlorite & hydrogen peroxide	100 crystals (50 mg) $Ca(OCl)_2$ + 6 drops H_2O_2 per gallon	11.6	6.7
Halazone	Abbott Labs	p-dichloro-sulfamoyl benzoic acid, 2.87%	5 tablets per quart	7.5	6.7
Chlorine Bleach	N. Chicago, IL	sodium hypochlorite, 5.25%	5 ml per gallon	3.9	7.1

a) As prepared according to package instructions.
b) In water treated according to package instructions.

APPENDIX C
Characteristics of Disinfectant Test Water

Source	pH	Turbidity (NTU)	Chlorine Demand[a] (mg/liter)
Spring-fed	6.8	0.09	0.3
Lake Washington	7.1	0.75–0.80	0.7

a) 30 minutes, free chlorine demand[5].