Wilderness and Environmental Medicine, **11,** 12–16 (2000)

ORIGINAL RESEARCH

Microbiological water purification without the use of chemical disinfection

CHARLES P. GERBA, PhD; JAIME E. NARANJO, BS

From the Department of Soil, Water and Environmental Science, University of Arizona, Tucson, AZ.

Objectives.—Point-of-use (POU) water treatment systems are self-contained units that can be used by recreational enthusiasts who normally obtain drinking water from untreated sources (ie, rivers, lakes, etc). Microbiological water purifier units are capable of removing all waterborne pathogens. The purpose of this study was to evaluate a new technology (Structured Matrix) capable of microbiologically purifying the water without the use of chemical disinfectants or an external power requirement.

Methods.—Each of 3 identical portable water filtration units were evaluated for their ability to remove *Klebsiella terrigena,* poliovirus type 1, rotavirus SA-11, and *Cryptosporidium parvum* oocysts. Units were operated according to the manufacturer's instructions to process 378 L of water. Each unit was challenged with test organisms after 0, 94, 190, 227, 284, 340, and 378 L had passed through it. For the 227-L and 284-L challenges, a "worst-case" water quality (4°C, pH 9, and turbidity 30 NTU) was used that contained 1500 mg/L dissolved solids and 10 mg/L humid acid. At 340-L and 378-L challenges, worst-case water quality was adjusted to pH 5.0. Units were tested after stagnation for 48 hours following passage of 190, 340, and 378 L of water.

Results.—The geometric average removal exceeded 99.9999% for bacteria, 99.99% for viruses, and 99.9% for *Cryptosporidium parvum* oocysts.

Conclusion.—These units comply with the criteria guidelines for microbial removal under the United States Environmental Protection Agency's "Guide Standard and Protocol for Testing Microbiological Water Purifiers."

Key words: water purification, bacteria, viruses, parasites, *Cryptosporidum,* water filtration, poliovirus, rotavirus

Introduction

All surface water supplies can be expected to be contaminated at one time or another, no matter how pristine the source. Because of the difficulty of transporting large amounts of water, military personnel, campers, hikers, and rafters find it necessary to obtain drinking water from rivers, lakes, and other potentially contaminated supplies. The international traveler also is often faced with the need for a microbially safe water supply, particularly when visiting developing nations. Furthermore, emergency situations such as earthquakes, hurricanes, and floods may disrupt the delivery of treated water, or its quality may be impaired. It is essential that such water supplies be treated to eliminate the risk of illness from waterborne, disease-causing microorganisms. Several waterborne pathogens are zoonotic (eg, *Cryptosporidium, Giardia, Salmonella,* and *Campylobacter*).

Iodine tablets for purifying water have been in general use by the United States military and general public for more than 40 years[1] and are known to be effective against waterborne enteric bacteria and viruses.[2] With sufficient dose and contact time, they are also effective against *Giardia* cysts. However, iodine tablets may be ineffective against the oocysts of *Cryptosporidium.*[3] To minimize the risks in consuming poor quality water, several recreational equipment manufacturers have developed small, portable devices that will remove cysts, oocysts, and enteric bacteria by size-exclusion filtration. Enteric viruses are too small to be removed by mechanical sieving filtration, and iodine is typically added to the water to ensure their inactivation.

To ensure the performance of point-of-use (POU) wa-

Direct correspondence and reprint requests to Department of Soil, Water and Environmental Science, 429 Shantz Building, University of Arizona, Tucson, AZ 85721 (Dr Gerba).

Table 1. Sampling plan for test units

Test point (% of estimated test capacity)	*Volume (L)*	*Water test type*	*Influent background*	*Microbiological*
Start	0	General	X	X
25%	94			X
50%	190			X
After 48 hours' stagnation				X
60%	227	Worst case		X
75%	284			X
After 48 hours' stagnation		pH 9.0 ± 0.2		X
90%	340	Worst case		X
100%	378			X
After 48 hours' stagnation		pH 5.0 ± 0.2		X

ter treatment devices, the US Environmental Protection Agency (EPA), Office of Pesticides, Antimicrobial Division, developed its "Guide Standard and Protocol for Testing Microbiological Water Purifiers."[4] The protocol requires lifetime testing using protozoa and enteric bacteria and viruses throughout the claimed or anticipated capacity of POU devices. There are certain minimum reductions for the test organisms under a variety of water quality conditions (Tables 1 and 2). Using this protocol as a guide, we evaluated a new technology for microbiologically purifying water that did not require ultraviolet light or the addition of a chemical disinfectant. This technology removes microorganisms both by size-exclusion filtration and adhesion onto the Structured Matrix.

Materials and methods

The experimental design to evaluate the water purification units was based on recommendations by the US EPA's Task Force Report on the "Guide Standard and Protocol for Testing Microbiological Water Purifiers."[4] These are small cylinder units 8 cm long and 7.5 cm in diameter. Water is forced through the unit by a hand pump with the inlet in the water source.

Three units were provided by General Ecology (First Need Systems, Exton, PA) and operated according to the manufacturer's instructions. Water is purified by passage of the water through a block of activated carbon that has been treated to enhance retention of viruses and other microorganisms by association with the surfaces in the Structured Matrix. Larger organisms, particles, or both (0.1 μm) are also retained by size exclusion. The units are furnished with an inlet hose that is placed in the water. Water pumped through the unit exits through another hose and flows into the vessel (eg, canteen, water bottle) from which it is consumed. The unit processes water at a rate of 0.476 L/min, with a total design capacity of 472 L. A lifetime test volume of 378 L was chosen because it is within the manufacturer's recommended capacity for the unit. The unit weighs approximately 425 g and is approximately 12 cm by 12 cm in size.

The units were challenged with the test microorganisms after various points of lifetime operation (Table 1), defined as the volume of water passed through the units. The "general" and "worst-case" tests, as defined in the "'Guide Standard," are shown in Table 2. Before a challenge, test organisms were added to a 100-L tank and mixed for 5 minutes with a submersible pump; a sample was collected for microbial analysis. Ten liters of test water were passed through each unit with the aid of an

Table 2. Test waters used in microbiological challenges

Water type	*Turbidity (NTU)*	*pH*	*Total dissolved solids (mg/L)*	*Total organic carbon (mg/L)*
Average case*	<0.50	7.8	200–300	<1.0
Worst Case	30	9.0 and 5.0†	1500	10

*Well water, University of Arizona campus. Tucson, AZ.

†pH 9.0 for 60% and 75%—estimated life challenges, and pH 5.0 for 90% and 100%—estimated life challenges.

Table 3. Removal of *Klebsiella terrigena*

Test point	Influent concentration per mL (10^7)	Concentration per mL* after treatment				Average % removal
		Unit 1	Unit 2	Unit 3	Geometric average	
Start	1.24	<0.03	<0.03	<0.03	<0.03	>99.9999
25%	1.12	<0.03	<0.03	<0.03	<0.03	>99.9999
50%	1.19	<0.03	<0.03	<0.03	<0.03	>99.9999
After 48 hours' stagnation	NA†	<0.03	<0.03	<0.03	<0.03	NA
60%	0.20	<0.03	<0.03	<0.03	<0.03	>99.9999
75%	0.018	<0.03	<0.03	<0.03	<0.03	>99.9999
After 48 hours' stagnation	NA	<0.03	<0.03	<0.03	<0.03	NA
90%	1.34	2.10×10^1	0.20	<0.03	0.52	>99.9999
100%	2.40	2.00×10^3	<0.03	<0.03	1.30	>99.9999
After 48 hours' stagnation	NA	7.60×10^0	<0.03	<0.03	0.20	NA

*Detection limit was 0.03 colony-forming units/mL.
†NA indicates not applicable.

electric pump before a 100-mL sample was collected. Total water volume and water flow rate were recorded and maintained by in-line water meters and a flow control that limits flow to 2.67 L/min. Between challenges, dechlorinated (by passage through a column of activated carbon) tap water was processed through the units from a 100-L holding tank. Physical and chemical water characteristics are shown in Table 2. Stagnant filtered samples (after 48 hours of nonuse) were collected after 3 challenges (Table 2). Units were challenged with microorganisms suspended in worst-case water quality at 60% and 90% estimated capacities (Table 1). In worst-case challenges, turbidity of test water was increased to 30 nephelometric turbidity units by addition of AC fine dust (General Motors, Flint, MI), 1500 mg/L dissolved sea salts (Sigma Chemical Co, St Louis, MO), and 10 mg/L humic acid (Aldrich, Milwaukee, WI). This mixture was chilled to 4°C before adding test organisms. Additional details are in Abbaszadegan et al.[5]

Physicochemical properties of test water were analyzed according to procedures described in *Standard Methods for the Examination of Water and Wastewater.*[6] Turbidity, total hardness, calcium hardness, magnesium hardness, and pH of water were determined before and after passage through the purification units (Table 2).

Poliovirus type 1 (strain LSc2ab) ATCC-VR-59 and rotavirus SA-11 (ATCC-VR-899) were obtained from the American Type Culture Collection (Bethesda, MD), grown, and assayed in the MA-104 cell line. The viruses were purified as described in the "Guide Standard."

Initial titers of poliovirus and rotavirus were each determined in the MA-104 cell line by the plaque overlay method described by Smith et al.[7] Before a challenge, each virus was added in equal amounts to achieve a combined titer of approximately 3–4 $\times$ 10^6/L in the challenge water. Assay of viruses in water after treatment was by inoculating each of 3 75-cm^2 plastic tissue culture flasks with 3 mL of undiluted water from each unit. Growth media was decanted from the cell monolayer and test water added. Flasks were incubated at 37°C for 1 hour to allow for virus absorption, and then maintenance media was added.[8] Detection limit for the enteric virus analysis was 0.11 plaque-forming units per milliliter, based on 9 mL of inoculated sample.

Cryptosporidium parvum oocysts were obtained from the feces of infected calves and purified by a discontinuous sucrose gradient procedure.[9] Unit influent (10 mL) and effluent (100 mL) were collected separately. They were centrifuged in an IEC clinical centrifuge (Needham Heights, MA) at 400 $\times$ g for 15 minutes to pellet the oocysts. The supernatant was aspirated to 1 mL above the pellet. After resuspension of the pellet in phosphate base buffer, the oocysts were counted using a Spotlite hemocytometer (Baxter Healthcare Corp, McGraw Park, IL), with a phase microscope (BH Olympus, Japan) at $\times$400 magnification.[10]

Results

The 3 units achieved the required geometric average removal of 6 $\log_{10}$ for *K. terrigena,* 4 $\log_{10}$ for poliovirus type 1 and rotavirus SA-11, and 3 $\log_{10}$ units of *C. parvum* oocysts at all test points (Tables 3 through 5).

Table 4. Removal of *Cryptosporidium* oocysts

Test point	*Influent concentration per mL (10^3)*	*Concentration per mL after treatment*				*Average % removal*
		Unit 1	*Unit 2*	*Unit 3*	*Geometric average*	
Start	1.50	0.92	0.92	0.92	0.92	99.94
25%	1.52	0.92	0.92	0.92	0.92	99.94
50%	3.00	1.90	0.92	<0.92	1.17	99.96
After 48 hours' stagnation	NA†	<0.92	<0.92	<0.92	<0.92	NA
60%	1.45	<0.92	<0.92	<0.92	<0.92	99.94
75%	1.67	<0.92	<0.92	<0.92	<0.92	99.94
After 48 hours' stagnation	NA	<0.92	<0.92	<0.92	<0.92	NA
90%	1.78	1.9	<0.92	<0.92	1.17	99.93
100%	1.74	0.92	<0.92	<0.92	0.92	99.95
After 48 hours' stagnation	NA	<0.92	<0.92	<0.92	<0.92	NA

*Detection limit was 0.92 oocysts per milliliter.
†NA indicates not applicable.

Discussion

Microbiological purification of water for individuals or households has been a major challenge. Such systems must be capable of significantly reducing pathogenic microorganisms in a relatively short period. Filtration in combination with iodine in many situations appears to meet the testing criteria established by the US EPA.[4] This method imparts an undesirable taste to water, however. In addition, iodine effectiveness against enteric viruses is significantly reduced in cold water and at low pH levels.[2]

The device evaluated in this study is capable of removing microorganisms by a combination of microfiltration, broad spectrum adsorption, and electrochemical (net surface charge) separations within Structured Matrix. Currently, other available filtration devices remove microorganisms by size exclusion; their usefulness is limited to protozoan parasites and bacteria. Viruses can be retained on surfaces by a combination of hydrophobic and electrostatic interaction.[11] The units tested in the present study exceeded the required reductions through the lifetime (378 L) testing, even when challenged with water high in organic carbon and at high and low pH. Performance of 1 unit alone was somewhat reduced in the worst-case water challenge. These results demonstrate that this technology is capable of performing under a wide variety of water quality conditions that would be considered extreme challenges to devices that depend on

Table 5. Removal of poliovirus and rotavirus

Test point	*Influent concentration per mL (10^4)*	*Concentration per mL after Treatment*				*Average % removal*
		Unit 1	*Unit 2*	*Unit 3*	*Geometric average*	
Start	6.00	1.00	<0.11	<0.11	<0.11	>99.99
25%	2.00	<0.11	<0.11	<0.11	<0.11	>99.99
50%	1.90	0.66	<0.11	<0.11	0.20	>99.99
After 48 hours' stagnation	NA†	<0.11	<0.11	<0.11	<0.11	NA
60%	1.95	<0.11	<0.11	<0.11	<0.11	>99.99
75%	2.23	0.33	<0.11	<0.11	0.16	>99.99
After 48 hours' stagnation	NA	<0.11	<0.11	<0.11	<0.11	NA
90%	4.40	0.44	<0.11	<0.11	0.17	>99.99
100%	4.70	3.67	<0.11	<0.11	0.36	>99.99
After 48 hours' stagnation	NA	<0.11	<0.11	<0.11	<0.11	NA

*Detection limit was 0.11 plaque-forming units per milliliter.
†NA indicates not applicable.

filtration technology, adsorption technology, or both. Water high in pH and organically laden is seen as the most difficult challenge to such a technology with regard to viruses.[11] Filtration units for recreational use do not typically remove viruses because of their small size. It is also important that previously retained microorganisms are not released after storage and is the rationale for testing water following a 48-hr stagnation period.

Conclusion

It appears that this technology is capable of meeting the test requirements as a microbiological water purifier as defined by the EPA[4] without the use of chemical disinfection. The small size, lack of external power requirements, and high degree of portability of these units make them suitable for recreational enthusiasts who obtain their drinking supply from rivers, lakes, or other untreated water.

References

1. Schaub SS, Hargett HT, Kamrud KI, Sterling CR, Marshal M. *Evaluation of military effectiveness of chloro-floc water purification tablets for treatment of waterborne microorganism.* Technical Report 9205. Fort Ditrick, Frederick, MD: US Army Biomedical Research and Development Laboratory; 1992.
2. Gottardi W. Iodine and iodine compounds. In: Block SS, ed. *Disinfection, Sterilization, and Preservation.* 4th ed. Malvern, PA: Lea and Febiger; 1991:152–166.
3. Gerba CP, Johnson D, Hasan MN. Efficacy of iodine water purification tablets against *Cryptosporidium* oocysts and *Giardia* cysts. *Wilderness Environ Med.* 1997; 8:96–100.
4. Guide standard and protocol for testing microbiological water purifiers. *Fed Regist.* 1989;54:34067.
5. Abbaszadegan M, Hasan MN, Gerba CP, et al. The disinfection efficacy of a point-of-use water treatment system against bacterial viral and protozoan waterborne pathogens. *Water Res.* 1997;31:574–582.
6. *Standard Methods for the Examination of Water and Wastewater.* 19th ed. Washington, DC: American Public Health Association; 1995.
7. Smith EM, Estes MK, Graham DY, Gerba CP. A plaque assay for the simian rotavirus SA-11. *J Gen Virol.* 1979; 43:513–519.
8. Smith EM, Gerba CP. Laboratory methods for the growth and detection of animal viruses. In: Gerba CP, Goyal SM, eds. *Methods in Environmental Virology.* New York, NY: Marcel Dekker; 1992:15–47.
9. Arrowood MJ, Sterling CR. Isolation of *Cryptosporidium* oocysts and sporozoites using discontinuous sucrose and isopycnic percoll gradients. *J Parasitol.* 1987;73:314–319.
10. United States Environmental Protection Agency. *Guidance Manual for Compliance with the Filtration and Disinfection Requirements for Public Water Systems Using Surface Water Sources.* Washington, DC: Government Printing Office; 1990.
11. Gerba CP. Applied and theoretical aspects of virus adsorption to surfaces. *Adv Appl Microbiol.* 1984;30:133–168.