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Biocidal Efficacy of a Flocculating Emergency Water Purification Tablet

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Chlor-Floc (CF) emergency water purification tablets were tested for bactericidal, virucidal, and cysticidal efficacy in water at temperatures ranging from 5 to 25°C. The minimal required log reduction was achieved for bacteria, *Giardia muris*, and rotavirus, but CF did not achieve the required log reduction of poliovirus at any of the temperatures or times investigated. The biocidal properties of the CF tablet were equivalent to if not greater than those of the Globaline iodine tablet, and the CF tablet was a more rapid cysticide under several potential use conditions. Therefore, it is a suitable substitute for iodine tablets for emergency purification of drinking water. Clarification of turbid waters was effective, but filtration through a cloth is necessary to prevent flocculated sediment from entering the canteen. The CF tablets met military requirements for emergency water purification and are safe and acceptable for use by the military.

American soldiers have been using iodine tablets (31) to purify canteen water under emergency conditions in the field since 1952 (19). The U.S. Army Natick Research, Development and Engineering Center was tasked by the Water Resources Management Action Group in 1987 to provide a water purification tablet which will eliminate the deficiencies experienced with the standard iodine tablet. The deficiencies identified with iodine tablets included slow kill of *Giardia* cysts at low temperatures, medicinal taste and odor, and the fact that undissolved solids, color, and odor in field water are not removed.

A market search for a new emergency water purification tablet or compound was undertaken. The tablet had to be commercially available and nondevelopmental and to satisfy new military requirements. The search resulted in the selection of a tablet called Chlor-Floc (CF) (7a). CF tablets combine sodium dichloroisocyanurate, the active ingredient, with proprietary flocculating agents that clarify the treated water by coagulating particles in the water as the water is being disinfected. The heavy coagulated particles settle out, leaving the water above the sediment clear. When dissolved in water, the active ingredient dissociates to hypochlorous acid and chloride ions depending on the final pH of the water. The tablet also contains a buffering system which buffers the treated waters to a pH that is optimal for disinfection of the water.

The objectives of the testing were to validate and verify the effectiveness of CF tablets for (i) the destruction and removal of microorganisms, selected chemicals, and metals from water; (ii) the clarification of water; and (iii) the stability of CF tablets during storage at different temperatures. However, this paper reports only the biocidal effectiveness of CF.

MATERIALS AND METHODS

General procedures. The procedures and standards used were in accordance with U.S. Environmental Protection Agency (EPA) *Guide Standard and Protocol for Testing Microbiological Water Purifiers* (28) unless otherwise indicated. Standard microbiological analytical procedures were used (2, 3, 32).

Tablets. CF tablets (lot Z/1) were manufactured by the Control Chemical Company, South Africa. Their agent in the United States is Deatrick and Associates Inc., Alexandria, Va. The tablets are registered by the U.S. Environmental Protection Agency for treatment of water at 5 to 25°C (30). The active ingredient is 2.5% sodium dichloroisocyanurate (sodium dichloro-*s*-triazinetrione). One 600-mg tablet provides 1.4% available chlorine (8 ppm of free residual chlorine) and enough flocculating agent for the clarification and disinfection of 1 liter of water at 10 to 25°C. At 5°C, two tablets are required per liter of water (7a). Flocculating agents are proprietary. The CF tablets were used in water as instructed by the manufacturer and in compliance with EPA directives. The total contact time specified was 20 min except for use at 25°C, for which it was only 12 min. The CF tablets were dissolved in water by shaking or stirring (magnetic stirrer) for 1 min. After being swirled for a few seconds, the water was allowed to stand for 4 min; it was then swirled for 10 s and allowed to stand for another 7 min at 25°C or for 15 min at 5 to 15°C. The clarified water was not filtered through a cloth in laboratory studies as recommended for soldiers and campers, except in the cysticidal studies, unless turbidity was measured (turbidity data not shown). CF tablets were used as instructed by the manufacturer at 5, 10, 15, and 25°C for several periods ranging from 5 to 40 min (5–7, 10).

The iodine (Globaline) tablets (31) used in these studies were manufactured by Van Ben Industries, Long Island, N.Y. They were dissolved in water by shaking or swirling for 1 min and allowed to react for 5 to 180 min in parallel studies with CF tablets. The recommended treatment time for Globaline tablets is 35 min, and two tablets per liter of water are required regardless of the temperature of the water. Each iodine tablet provided 8 ± 1 ppm of iodine.

Test waters. Biocidal efficacy tests (5–7, 10, 20) were conducted in EPA no. 2 test water (28) and several natural waters from rivers and lakes. Residual chlorine and iodine produced by the respective tablets were quenched immediately following treatment of the waters and before conducting any of the plate counts, by adding 1 ml of 10% sodium thiosulfate per liter of test water. Neutralization of the halogens was confirmed by

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culture and by amperometric measurements. The clarified CF-treated water (supernatant) was removed with a pipette from just above the floc or carefully poured into a sterile beaker containing sodium thiosulfate.

The EPA no. 2 test water (pH 9.0) contained 1 liter of deionized water, 10 mg of humic acid (Aldrich Chemical Co., Milwaukee, Wis.), 1,500 mg of sea salt (Instant Ocean; Aquarium Systems, Mentor, Ohio), and 62 mg of SAE Fine Test Dust (General Motors Corp., Flint, Mich.) to obtain a turbidity of 30 nephelometer turbidity units (NTU) (28).

Bacterial challenge. (i) Bacteria. The bacteria used were *Klebsiella terrigena* ATCC 33257 (2), *Escherichia coli* ATCC 15597, and *Pseudomonas aeruginosa* QM-B-1517. Cultures were grown on plate count agar (Difco, Detroit, Mich.) at 35°C for 24 hours.

(ii) Inoculum. Cells were washed off the plate count agar, suspended, and diluted in 0.0145 M saline (4). The inoculum was adjusted turbidimetrically in a Ratio/XR turbidimeter (Hach Co., Loveland, Colo.).

(iii) Challenge. One liter of test water was inoculated to achieve 1×10^6 to 10×10^6 cells per ml (1×10^9 to 10×10^9 cells per liter) before addition of water purification tablets (28). The temperature of the water was equilibrated at 5, 15, and 25°C.

Aerobic plate counts. The aerobic plate counts were performed on plate count agar-containing pour plates incubated at 35°C for 48 h. Plates with minute colonies or no CFU were reincubated for another 24 h. Colonies of sporeformers isolated from natural waters were confirmed as sporeformers by performing simple stains of smears from each colony with methylene blue and observing with a phase microscope (15).

Coliform counts. Selective recovery of injured and uninjured coliforms (*Klebsiella* and *Escherichia* spp.) after treatment, whether inoculated into test waters or naturally present in water, was accomplished on an injury repair medium consisting of tryptic soy agar (Difco) pour plates overlaid with violet red bile agar (Difco) after incubation of tryptic soy agar at 35°C for 2 h (22, 26, 32). Plates were reincubated at 35°C for 48 h.

Plate counts of flocculated sediment in river water treated with CF tablets. To determine the survival of bacteria in the flocculated sediment (floc) of water treated with CF tablets, 1 liter of river water equilibrated at 25°C in triplicate flasks was inoculated with 3×10^6 to 10×10^6 *E. coli* cells per ml. The water in each flask was treated simultaneously with one CF tablet as specified by the manufacturer. The treatment was interrupted by quenching the chlorine in the first flask after 20 min, in the second flask after 40 min, and in the third flask after 60 min. Selective coliform (*E. coli*) counts in the clarified water were performed after 20-, 40-, and 60-min treatment times, on an injury repair medium consisting of tryptic soy agar overlaid with violet red bile agar after 2 h at 35°C, to recover injured as well as uninjured *E. coli* cells (22, 26, 32). Then the floc produced in each of the treated waters was resuspended and harvested by filtration of the entire 1 liter of water through 0.4-μm-pore-size filters (Millipore, Bedford, Mass.). A 2-g portion of the recovered floc was resuspended in 18 ml of 0.0145 M saline (4), and coliform (*E. coli*) plate counts were performed on the same repair medium. No attempt was made to verify the identity of *E. coli* colonies because the inoculum was so much greater than the indigenous microflora that dilutions plated eliminated indigenous bacteria. Colonies recovered could have been produced only by the *E. coli* inoculated into the waters.

Evaluation of cloth filter pouch. The cloth material selected for filtering water after treatment with CF is 100% bleached Oxford cotton. It weighs 7 oz/yd (~198 g/0.9 m) and has an air permeability of 56 to 64 26,429 to 30,205 $cm^3/s/929\ cm^2$. The cloth is available from Holliston Co., Kingsport, Tenn., as product no. 202307. The filter pouch is fabricated with a double layer of the cloth with a double-stitched side seam and is 6 in. (ca. 15 cm) long and 2 in. (ca. 5 cm) in diameter. Test waters 1 and 2 were prepared by adding a measured amount of a dehydrated clay blend to 1 liter of deionized water. Test water 3 was prepared by dissolving a CF tablet in 1 liter of deionized water. Test water 4 was prepared by treating 1 liter of clay water with one CF tablet, as specified by the manufacturer. Except for sample 4, particles were kept in suspension by continuous stirring with a magnetic stirrer. In sample 4, the particles were allowed to settle out, by the action of the tablet, and only the clarified water was passed through the filter. The turbidity of the water samples was measured as NTU at room temperature, before and after filtration, with a model 43900 Hach Ratio/XR turbidimeter.

Virus challenge. The challenge viruses were poliovirus type 1 (Chat strain [ATCC VR-192]) and simian rotavirus (strain SA-11 [ATCC VR-899]). The challenge viruses were added separately to EPA no. 2 test water at a concentration of 10^7 PFU/liter (10, 28). The poliovirus was grown in Vero cells, and cell-free virus stocks were prepared as outlined by Pancorbo et al. (18). The rotavirus was grown in MA-104 cells, with 0.5 μg of trypsin per liter. Cell free virus stocks were prepared as outlined by Pancorbo et al. (18). Water temperatures were 5, 15, and 25°C (10).

Virus infectivity tests. Two monkey kidney cell lines were used for the virus infectivity tests: MA-104 (Whittaker Bioproducts, Baltimore, Md.) and Vero cells (American Type Culture Collection, Rockville, Md.). All cell lines were grown in Eagle's minimal essential medium (MEM) (modified with glutamine) with 10% fetal bovine serum, 100 U of penicillin per ml, 100 μg of streptomycin per ml, and 0.075% sodium bicarbonate.

Viral plaque assays. Virus infectivity titers were determined by plaque assays (18). The Vero cells were washed twice with Eagle's MEM without serum prior to inoculation with poliovirus. The overlay medium for poliovirus consisted of Eagle's MEM with 1.5% agar overlay, antibiotics (as above), 2% fetal bovine serum, and 25 mM $MgCl_2$. After 2 days of incubation at 37°C, a second overlay with neutral red was added to determine plaque numbers. Rotavirus was assayed with MA-104 cells. The cells were washed three times with Eagle's MEM without serum prior to inoculation with rotavirus. The rotavirus samples were pretreated with trypsin (30 μg/ml) for 1 h at 37°C prior to inoculation of MA-104 cell monolayers. The overlay medium for rotavirus consisted of Eagle's MEM with 0.75% soft agar overlay, antibiotics (as above), DEAE-dextran (100 μg/ml; Pharmacia), 7.5 μg of trypsin per ml, and 25 mM $MgCl_2$. After 4 to 5 days at 37°C, the soft agar overlay was gently removed, and the cell sheet was stained with a 0.1% crystal violet solution.

Cyst challenge. The EPA no. 2 test waters and control waters were challenged with 10^7 *Giardia muris* cysts per liter (5–7). Water temperatures were 5, 15, and 25°C in excystation studies and 5 and 10°C in infectivity studies. The strain of *G. muris* used in this study was obtained from F. W. Schaefer, EPA, Cincinnati, Ohio, and was used by Labatiuk et al. (14).

Cyst production. Cyst production is described in references 5 to 7 and 14. Three-week-old mice (C3H strain; Charles River Laboratory, Wilmington, Mass.) were intubated with 15,000 cysts per 0.1 ml. Fresh fecal samples were collected and processed beginning 5 days after intubation. Cysts were isolated from fecal samples by sucrose flotation followed by Percoll flotation (14). They were suspended in distilled water

TABLE 1. Bactericidal, virucidal, and cysticidal efficacies of CF water purification tablets in EPA no. 2 test water

Agent	Time (min)	Temp (°C)	No. of tablets/liter	MRLR	LR (mean)[a]	FRC (ppm) (mean)[a]	Final pH (mean)[a]
Bacteria[b]	5	5, 15, 25	1	6	>6.0	4.3	4.2
Rotavirus[c]	5	5, 15, 25	1	4	>4.0	4.0	4.7
Poliovirus[c]	20	5	1	4	2.5	4.5	5.0
	20	5	2	4	2.1	10.0	5.0
	20	15, 25	1	4	2.5	5.4	5.0
	30	25	1	4	2.7	5.4	5.0
	40	15	1	4	2.7	5.4	5.0
	5–40	5	2	4	2.3	10.0	5.0
G. muris[d]	10–40	5, 15, 25	1	3	>3.0	4.1	4.3

[a] The mean (three repetitions) values at designated temperatures.
[b] Bacteria were *E. coli*, *K. terrigena*, and *P. aeruginosa*.
[c] Reference 10.
[d] Reference 5.

and refrigerated until used on the following day. Cyst suspensions were inspected microscopically and met the following criteria: phase bright, defined cyst wall, peritrophic space, agranular cytoplasm, no more than 4 to 5% phase-dark cysts, and less than 0.1% empty cyst walls. Cyst counts were made with a hemocytometer (14). More than 90% excystation in the stock suspension was required.

Cysticidal efficacy. Cysticidal efficacy is described in references 5 and 6. One or two tablets of each type were added separately to 1 liter of EPA no. 2 water (worst case) and deionized water controls containing the cysts and mixed as above, as specified by the manufacturers. The number of CF tablets added to water depended on the temperature of the water tested. Two iodine tablets were added per liter of water regardless of temperature. Treatment times ranged from 10 to 180 min, depending on the tablets tested (see Tables 1 and 2). Following CF treatment, the water was passed through a cloth filter into a beaker containing 1 ml of 10% sodium thiosulfate per liter of water to quench the chlorine. Iodine in treated water was quenched in the same manner, but the water was not filtered since there was no flocculation. The cysticidal efficacy of the disinfectant tablets was determined by an excystation method (14). The minimum number of cysts examined was 1,000.

Infectivity assay. The median-infective-dose experiments (11) established that the 50% infective dose was less than 10 cysts per animal (7). To detect a log reduction (LR) of 1 to 3, mice were challenged with 100, 1,000, and 10,000 pretreated cysts each. Cysts in EPA no. 2 test water at 5 and 10°C were pretreated with two CF tablets or two iodine tablets per liter. Three groups of five male CF-1 mice (3 to 4 weeks old) were intubated for each trial. Determination of infectivity was based on examination of fecal samples 6 to 8 days postexposure (14) and by necropsy of negative animals at the highest uninfecting dose on days 10 to 12 (7).

Measurement of FRC and iodine. Free residual chlorine (FRC) produced by CF tablets dissolved in water was determined by amperometric titration (2) (series A-790 titrator; Wallace and Tiernan, Belleville, N.J.). Free iodine was also determined by amperometric titration in a manner similar to measurement of total residual chlorine (2). However, when titrating for iodine, the burette reading in milliliters was multiplied by 3.58 since 1 ml of phenylarsine oxide solution is equivalent to 3.58 ppm of iodine. Iodine residuals were determined by using buffer solution (pH 4) and potassium iodide (KI) solution (Wallace and Tiernan). The accuracy of the amperometer was checked with a standard chlorine solution (1 ml of Clorox added to 1 liter of deionized water to provide a 50-ppm stock chlorine solution) and free chlorine standards (no. 14268; Hach Co., Ames, Iowa). Deterioration of the KI solution was determined by back titration (Wallace and Tiernan).

TDS. Total dissolved solids (TDS) were measured with a model 532 T1 and T2 DS meter (Myron L Co., Carlsbad, Calif.). Standard solutions of known conductivity were purchased from Myron L Co.

RESULTS

Bactericidal, cysticidal, and virucidal efficacies of water purification tablets. The bactericidal, cysticidal, and virucidal efficacies of CF tablets were compared with those of Globaline iodine tablets (5, 10, 18, 21) in EPA no. 2 test water (stress challenge) and are presented in Tables 1 and 2, respectively. Both tables show the treatment times, water temperature, number of tablets per liter, residual chlorine or iodine levels, and final pH. The average values (three repetitions) are shown for both types of tablets. The LRs are the maximum average values achieved in EPA no. 2 water at any of the three temperatures tested, 5, 15, and 25°C. Bacteria included *E. coli*, *K. terrigena*, and *P. aeruginosa*. Cysticidal and virucidal data, chlorine and iodine levels, and treatment times were presented in contract reports (5, 6, 10).

Table 1 shows that one CF tablet per liter achieved the minimum required LR (MRLR) for bacteria and rotavirus after only 5 min of contact time at 5, 15, and 25°C. The virucidal efficacy of one or two CF tablets per liter for poliovirus was less than the 4 log unit (99.99%) reduction required, even after the contact time of CF was increased to 40 min at 5°C and 15°C and the temperature was increased to 25°C for 30 min. Only CF reduced *G. muris* in excystation studies (5) by the required 3 log units at all three temperatures after the prescribed 20-min contact time. Table 2 shows that one iodine tablet per liter also achieved the MRLR for bacteria after 5 min at all three temperatures. Two iodine tablets per liter achieved the MRLR for rotavirus after 20 min at 5°C (10), the minimum time and the only temperature tested with iodine in the virucidal and cysticidal studies. Iodine was no more effective than CF against poliovirus, and two tablets per liter failed to achieve the MRLR even after 60 min at 5°C (10). Against *G. muris*, two iodine tablets per liter at 5°C achieved only a 2.98 LR after 45 min and a 2.99 LR after 60

TABLE 2. Bactericidal, virucidal, and cysticidal efficacies of Globaline iodine water purification tablets in EPA no. 2 test water

Agent	Time (min)	Temp (°C)	No. of tablets/liter	MRLR	LR (mean)[a]	I_2 concn (ppm) (mean)	Final pH (mean)
Bacteria[b]	5	5, 15, 25	1	6	>6.0	5.6	4.8
Rotavirus[c]	20		2	4	4.9	14.3	5.8
	30		1	4	4.7	7.2	6.3
Poliovirus[c]	20		2	4	1.4	12.0	5.1
	30–90		1	4	1.2	8.2	6.4
	30, 45	5	2	4	2.0	12.0	5.1
	60	5	2	4	3.0	12.0	5.1
G. muris[d]	30–90	5	1	3	≤2.5	7.0	6.5
	30	5	2	3	2.3	17.0	7.4
	45	5	2	3	2.98	17.0	7.4
	60	5	2	3	2.99	17.0	7.4
	120	5	2	3	>3.0	17.0	7.4
	180	5	1	3	2.99	7.0	6.5

[a] The average (three repetitions) values at the designated temperature.
[b] Bacteria were *E. coli*, *K. terrigena*, and *P. aeruginosa*.
[c] Reference 10.
[d] Reference 5.

min, which are 10 to 25 minutes longer than prescribed to achieve the required 3 LR (5).

The results of mouse infectivity assays in mice challenged with *G. muris* cysts exposed to CF-treated water at 5 and 10°C were comparable to the results of the excystation assays (7). In the infectivity assays, two CF tablets per liter achieved a >3 LR at both temperatures in the prescribed 20 min. Two iodine tablets per liter also achieved a >3 LR of the cysts in the prescribed 35 min at 5°C (worst case), at least 10 min faster than in the excystation studies (Table 2).

The required total treatment (contact) times are 35 min regardless of water temperature for iodine tablets and 12 min at 25°C and 20 min at 5 to 15°C for CF tablets. In Table 1, one CF tablet per liter at 5°C effectively reduced levels of bacteria, *G. muris*, and rotavirus in less than 20 min (5, 10). The recommended dose for iodine is two tablets per liter of water regardless of temperature, whereas the dose recommended by the manufacturer of CF is two tablets only at 5°C and one tablet at 10°C and higher.

Several investigators have reported increased cysticidal efficacy of chlorine at low pH (<6) rather than at high pH because at low pH, hypochlorous acid, an effective cysticide, predominates (12, 25). At the final pH levels achieved in EPA no. 2 water (initial pH, 9.0), the oxidizing capability of the chlorine solution was near its maximum (17) and chlorine should have been in its most active form (hypochlorous acid) (12, 25). Iodine is an effective cysticide at low pH but a poor virucide (19); it is a more effective virucide at higher pH (pH >7.0). Incorporation of a more alkaline buffer to produce higher pH values which would optimize both the cysticidal and virucidal activities of iodine tablets in treated water may be required.

Bactericidal efficacy of CF tablets in natural waters. Tables 3 and 4 show that CF tablets used as directed effectively reduced or destroyed bacteria in a variety of natural waters. Indigenous bacteria, including coliforms, in several natural waters were reduced to <1 CFU/ml of water, with the exception of harmless spore-forming bacteria found in water, as shown in Table 3. FRC levels depended on the chlorine demand of the water treated and the number of tablets used. When two tablets were used in water below 10°C, FRC ranged from 4.2 ppm in the Concord River, Mass., water to 14.6 ppm in the Lake Cochituate, Mass., water. In Lake Cochituate water at 9°C, one tablet was inadvertently added instead of two tablets. Consequently, the FRC was only 1.8 ppm, which still

TABLE 3. Bactericidal efficacy of CF water purification tablets in natural waters of Massachusetts

Water source	Water temp (°C)	TDS (ppm)	Before treatment			After treatment (20 min)			
			APC/ml[a]	No. of Coliforms/ml	pH	APC/ml	No. of Coliforms/ml	pH	FRC (ppm)[b]
Walden Pond	5	150	300	137	5.5	<1	<1	4.2	
Walden Pond	10	60	1,190	88	5.9	<1	<1	4.1	7.1
Charles River	11	170	3,800	330	5.8	82[c]	<1	4.2	4.0
Concord River	9	150	71	49	5.5	<1	<1	4.1	4.2
Sudbury River	5	130	39	0	5.6	<1	<1	4.2	
Lincoln Creek	9	40	4,200	271	5.9	15[c]	<1	4.1	12.1
Dudley Pond	11	160	101	72	6.4	<1	<1	4.3	6.8
Lake Cochituate	4	200	84	46	6.2	18[c]	<1	4.1	14.6
Lake Cochituate	9	175	93	11	5.2	<1	<1	4.1	1.8
Sherman Bridge, Sudbury River	5	160	250	28	6.0	13[c]	<1	4.1	14.0

[a] APC, aerobic plate count.
[b] One tablet per liter at ≥10°C; two tablets per liter at <10°C. The FRC was quenched before plate counts were performed.
[c] Spore-forming bacteria.

TABLE 4. Removal of *E. coli* inoculated into natural waters at 5 and 25°C before and after treatment with CF tablets

Water source	TDS (ppm)	pH	Bacteria	Mean bacterial count/ml[a]	
				Before treatment	After treatment[b]
Lake Cochituate, Mass.	210	5.8	*E. coli*	$(3–5) \times 10^6$	<1
			Indigenous	$30–5 \times 10^6$	<1
Delta River, Alaska	120	7.5	*E. coli*	$(5–10) \times 10^6$	<1
			Indigenous	47–130	<1
Java Creek, Alaska	105	7.2	*E. coli*	$(2–10) \times 10^6$	<1
			Indigenous	21–80	<1
Panama River, Panama	70	7.3	*E. coli*	$(1–10) \times 10^6$	<1
			Indigenous	$(1–9) \times 10^4$	<1
Sudbury River, Mass.	130	6.4	*E. coli*	$(5–6) \times 10^6$	<1
			Indigenous	690–1,400	<1

[a] Mean of three or more trials.
[b] Treatment at 5°C was two tablets for 20 min; treatment at 25°C was one tablet for 12 min.

reduced the counts to <1 CFU/ml. When one tablet was used at 10°C or higher, FRC ranged from 4 ppm in the Charles River, Mass., water to 7.1 ppm in Walden Pond, Mass., water. Although the waters varied in pH, the CF tablets consistently buffered the different waters to pH 4.1 to 4.3. Original water temperatures were maintained within 1°C during the 20-min treatment time by holding water samples in either 5 or 10°C incubators which were set within 1°C of the water temperatures. Table 4 shows the destruction and removal of indigenous microflora as well as *E. coli* inoculated at more than 10^6 cells per ml into five natural waters from different parts of the world at 5 and 25°C. Counts in undiluted samples were <1 CFU/ml after treatment of 1 liter of the water with two tablets for 20 min at 5°C or with one tablet for 12 min at 25°C. The pH varied from 5.8 to 7.5, and TDS ranged from 70 to 210 ppm.

Influence of pH on the bactericidal efficacies of CF and iodine (Globaline) tablets. Table 5 shows the bactericidal efficacy of CF at 25°C after 12 min in deionized water buffered between pH 4 and 10, compared with the efficacy of iodine tablets. Although the water was highly buffered to resist change, the tablets reduced the pH slightly. None of the 2×10^6 to 8×10^6 *E. coli* cells per ml inoculated into the water were recovered after addition of one CF tablet per liter at a final pH ranging from 3.8 to 7.0 (initial pH, 4 to 8). At pH 8.3 (initial pH, 9.0), CF was slightly less effective, allowing 6.0% of the cells to be recovered. CF was not bactericidal at all at a final pH of 9.3. Two iodine tablets per liter were bactericidal at pH 3.8 through 9.0.

Bactericidal end points of FRC from CF tablets. Table 6 shows the bactericidal efficacy of the FRC from CF tablets after 30 min and the pH in a buffered water, Lake Cochituate water, and EPA no. 2 test water (28) inoculated with 2×10^6 to 4×10^6 *K. terrigena* cells per ml. The objective was to determine the bactericidal end point of FRC in canteen water following treatment of the water with a CF tablet. CF tablets were dissolved in each of the waters tested at 25°C. The FRC concentrations were then adjusted by preparing dilutions with the same water and were verified by amperometric titration. After inoculation of the waters at each chlorine concentration, the water was mixed on a magnetic stirrer for 30 min to simulate mixing in a canteen while the soldier is on the move. In all three waters, there was no recovery of *K. terrigena* at ≥0.5 ppm of FRC after 30 min. Below 0.5 ppm of FRC the percent recovery was variable. Therefore, even in a worst-case water such as EPA no. 2 test water, contaminating bacteria, represented by the test organisms, that inadvertently enter the canteen water after treatment with CF tablets will be removed or destroyed within 30 min if the FRC is at least 0.5 ppm at 25°C. In most waters treated with one CF tablet, the FRC can be expected to be four to six times greater.

TABLE 5. Bactericidal efficacies of CF and Globaline iodine tablets after treatment of water buffered at different pH values at 25°C

Initial pH[a]	Recovery of *E. coli*[b] treated with:			
	1 CF tablet/liter		2 I_2 tablets/liter	
	Final pH	% Recovery/ml	Final pH	% Recovery/ml
4.0	3.8	0	3.8	0
6.0	5.0	0	5.6	0
8.0	7.0	0	7.2	0
9.0	8.3	6		
10.0	9.3	100	9.0	0

[a] pH at which water was initially buffered. pH 4, 0.1 M sodium acetate and acetic acid (Walpole); pH 6 to 8, 1/15 M phosphate buffer (Sorenson); pH 9 to 10, 0.1 M glycine and sodium hydroxide (Sorenson).
[b] *E. coli* added to water ranged from 2×10^6 to 8×10^6 cells per ml. Treatment time was 12 min for both tablets and as recommended for CF at 25°C.

TABLE 6. Bactericidal efficacies of various concentrations of FRC from CF tablets dissolved in three different waters at 25°C

FRC (ppm)	Recovery of *K. terrigena*[a] after 30 min in:					
	Buffered water[b]		Lake water		EPA no. 2 water	
	% Recovery	pH	% Recovery	pH	% Recovery	pH
1.0	0	7.0	0	5.2	0	4.4
0.7	0	7.0	0	6.1	0	4.5
0.5	0	7.0	0	6.2	0	4.3
0.4	0	7.0	12	6.2	1.4	4.6
0.3	72	7.0	68	6.4		
0.0	100	7.0	100	6.4	100	9.0

[a] Cell concentration was 2×10^6 to 4×10^6/ml.
[b] 1/15 M phosphate buffer.

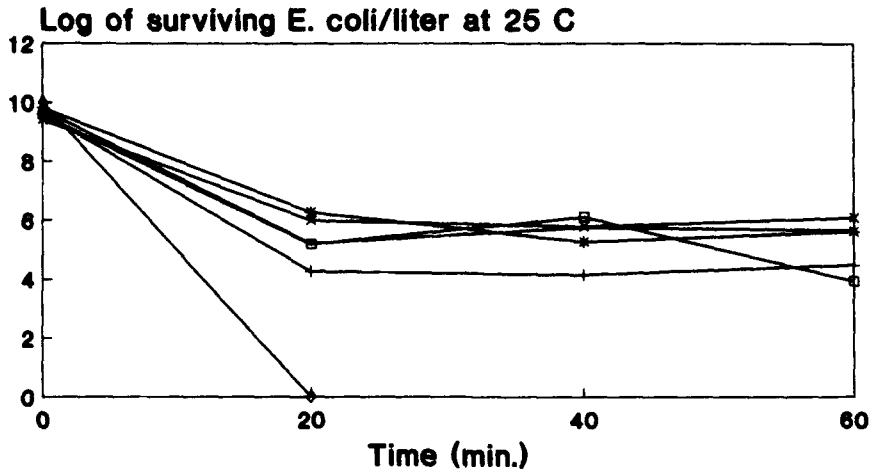

FIG. 1. Survival of *E. coli* in the flocculated sediment produced in five river water samples treated with one CF tablet per liter. Symbols: ■, Panama River (sample 1); +, Panama River (sample 2); *, Sudbury River, Mass.; □, Java Creek, Alaska; ×, Delta River, Alaska; ◇, clarified water.

Survival of microorganisms in flocculated sediment. Figure 1 shows that viable *E. coli* cells may remain in the flocculated sediment (floc) after treatment of some river waters with one CF tablet at 25°C. Before treatment the average pH of the five river waters ranged from 6.4 to 7.5. One CF tablet per liter reduced the average pH of the waters to 4 to 5.9. The FRC levels after treatment with CF ranged from 2 to 3.3 ppm. Although the bacteria were killed or removed from the clarified portion of the treated water after 20 min, they were still viable in the floc after more than 60 min. Surviving *E. coli* cells in the floc ranged from 10^4 to 10^7/liter from an initial population of 10^{10}/liter. Similar results were also obtained with *K. terrigena* in EPA no. 2 test water. The flocculation process in turbid waters (NTU, 150 to 1,400) containing a high level of suspended matter (TDS, 70 to 120 ppm), such as the five river waters shown, may protect bacteria by coating the cells so that chlorine cannot reach them. Aggregation of cells by the flocculants and/or chlorine (25) contained in CF undoubtedly provides some protection against the disinfectant (16). Viable *G. muris* cysts could not be detected in the floc because the floc obstructed the standard microscopic observation of the cysts. However, it appeared that the flocculation process entrapped the cysts so that they were physically removed from the water (5). Enteroviruses were not recovered from the floc (10). It should be noted that the concentration of cells of all test microorganisms inoculated into the waters was much higher than would be expected in most waters encountered and may also play a part in the survival of microorganisms.

TABLE 7. Filtration efficiency of cloth filter

Test water	No. of CF tablets/liter	Treatment of particulates[a]	Mean turbidity (NTU) of test waters	
			Before filtration	After filtration
1. Clay	0	Suspended	42	17
2. Clay	0	Suspended	40	13
3. DIW[b] + CF	1	Suspended	56	1
4. Clay + CF	1	Settled	9	1

[a] Particles in test waters 1, 2, and 3 were kept in suspension on a magnetic mixer and during filtration through the cloth (see text). In sample 4, clay particles were settled out by the CF tablet and then the clarified water was filtered.
[b] DIW, deionized water.

Evaluation of cloth filter for clarification efficiency. Table 7 shows that the cloth filter lowered the turbidity of clay suspensions from 40–42 to only 13–17 NTU. The CF particles kept in suspension in deionized water were almost completely removed by the cloth filter, which reduced the turbidity from 56 to only 1 NTU. The CF tablet reduced the turbidity of the clay suspension to 9 NTU, and the cloth filter further reduced the water turbidity to only 1 NTU. The cloth filter also physically removed 96% of the *G. muris* cysts in another study (7).

DISCUSSION

The biocidal efficacy, clarification, and storage stability of CF emergency water purification tablets were verified and compared with those of iodine tablets (20). The biocidal properties of the CF tablet were equivalent to if not greater than those of the Globaline iodine tablet, and the CF tablet was a more rapid cysticide under several potential use conditions (Table 1). In addition, CF effectively clarified turbid water, and its active ingredients remained stable for more than 36 months at 5 and 25°C (20).

As a result of these studies, CF tablets were recommended as a suitable and safe alternative to iodine tablets presently used by U.S. soldiers for emergency purification of field water. The CF tablets received medical clearance by the Office of The Surgeon General of the U.S. Army on 22 July 1992 on the basis of these and other studies (20, 24, 25). CF tablets have been used by the South African Defense Forces for several years (13a).

The CF tablets were registered by the EPA under the Federal Insecticide, Fungicide, and Rodenticide Act on 16 February 1989 and were accepted as a safe and effective disinfectant for recreational and emergency use in water at temperatures ranging from 5 to 25°C.

A health hazard assessment of CF was conducted by the U.S. Army Environmental Hygiene Agency (27) for the Office of The Surgeon General, Department of the Army. No toxicity or other harmful effects were found to be associated with the proper use of CF, provided that the label instructions are

followed (20). Filtration of the treated water to remove the flocculated sediment and to prevent it from entering the canteen is essential. An effective cloth filter was designed and tested for the removal of floc (9, 20). It is provided to the soldier as a component of a kit for emergency water purification (9). Although viable microorganisms were removed from the clarified water, they were found in the sediment of some test waters. However, these test waters were inoculated with millions of bacteria per milliliter. Survival in the floc may not be a problem in natural waters, which usually will have much lower microbial counts. Although CF tablets will remove some chemicals from water, they should not be depended upon for this purpose. They did not produce potentially harmful levels of halogenated organic compounds in water (20, 29).

The failure to achieve the required log reduction of poliovirus in this study (Table 1) may be due to strain differences or experimental procedure and should be investigated further. The effect of the oxidation reduction potential (ORP) of EPA no. 2 water treated with CF tablets on virucidal efficacy should also be determined, because ORP may be more important to disinfection than FRC (17). Schaub et al. (24) reported that CF removed poliovirus type 1 from water at low temperatures even at high pH. Sensitivity of poliovirus to chlorine has also been reported by other investigators (1, 13, 25). The sensitivity of the closely related rotavirus to chlorine was demonstrated in this study by CF tablets.

Soldiers will be provided with an emergency water purification tablet (8) and a kit containing a cloth filter and a plastic water treatment bag (9). On the basis of the results of these studies, soldiers will be instructed to treat all waters with only one CF tablet for 20 min to reduce and minimize the taste of chlorine, thereby producing safe and more acceptable water. This report shows that CF tablets can be used as a safe alternative to iodine tablets when murky, turbid water encountered in the field must be clarified to make it drinkable. Clear, clean-appearing, odor-free water will encourage greater consumption, thus reducing casualties due to dehydration.

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