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Enteric Virus and Indicator Bacteria Levels in a Water Treatment System Modified to Reduce Trihalomethane Production

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A drinking-water treatment plant with high concentrations of trihalomethanes in its finished water and large numbers of viruses in its source water was located. This plant was used to study the effect of an alteration in the point of chlorination from the first to last step of water treatment on the biological and chemical qualities of its finished water. This alteration caused some reduction in trihalomethane production, but trihalomethane concentrations still exceeded the prescribed limit of 100 μg/liter. No viruses or bacterial indicators were ever isolated from the finished water of the modified plant. Total virus removal by the treatment steps before chlorination (coagulation, sedimentation, and sand filtration) averaged ca. 90%, whereas removal of bacterial indicators by these processes averaged between 88 and 98%. Recoveries of viruses and bacterial indicators in the source water were generally negatively correlated.

Trihalomethanes (THMs) formed during the chlorine disinfection step of water treatment are considered to be potential carcinogens (16). In 1979, the U.S. Environmental Protection Agency set a maximum contamination level of 100 μg/liter for these compounds in drinking water (8). Possible approaches to reduce the production of THMs are removal of organic precursors with which chlorine reacts, removal of THMs from finished water, and use of alternative disinfectants (20). The last approach would clearly be the best and most direct if a safe, effective, and reasonably inexpensive alternative disinfectant was available. None tested have so far proven to satisfactorily meet these criteria. The most feasible method to reduce the production of THMs at this time may be through removal of organic precursors. This method is especially attractive because some precursors are already being removed by standard treatments in many plants. All that may be required to cause a significant reduction in THM production in such plants is to change the point of chlorination from the initial treatment step to the final treatment step. This method has already been tested in several plants with positive results (6, 22).

Chlorine is often added to source water as the first step of drinking-water treatment (prechlorination) to serve as an oxidant for inorganic material, to increase chlorine contact time, and to help prevent bacterial buildup on the sand filters. Even when chlorine is added at this early time in treatment, some microorganisms detected by standard plate count can be found in finished water. Alteration of the point of chlorination to a late stage of treatment naturally reduces the time and exposure of microorganisms to this disinfectant and increases the probability of their survival in water distributed by the treatment plant.

The microbial quality of drinking water is normally monitored through the use of total coliform tests (1). However, it has been repeatedly demonstrated that bacterial indicators are more effectively destroyed by chlorine than are enteric viruses. Therefore, it is not surprising that drinking waters that satisfy coliform standards and contain a chlorine residual have been found to contain hepatitis A (10) and other enteroviruses (15, 17, 18).

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To determine whether an alteration in the point of chlorination during water treatment permits survival of viral pathogens in finished water, it was necessary to find a treatment plant for study that contained the following: (i) source water with a reasonably high concentration of enteric viruses, (ii) a standard treatment sequence of flocculation and filtration with chlorination as the first treatment step, and (iii) a high concentration of THMs in finished water. Once a water treatment plant that conformed to these criteria was located, a 13-month study was conducted to measure the effects on water quality (chemical and biological) due to changing the point of chlorination from the first to last treatment step. The results of this study are presented here.

MATERIALS AND METHODS

Treatment plant operations and modification. The water treatment facility at which these studies were conducted serves a small town located in southern Michigan. The plant is capable of treating 100,000 gallons (378,500 liters) of water during an 8-h work day. The source water is a small river which contains secondary- or tertiary-treated sewage effluents from several small towns. Treatment before this study consisted of the addition of 7 to 10 mg of chlorine per liter followed immediately by the addition of a chemical flocculant (alum). After the floc was formed by rapid mixing, coaggulated particulates were allowed to sediment by gravity during an average retention time of 4 h. Further removal of flocced particulates was by sand filtration. Finished water was held in tanks with a total capacity of 275,000 gallons (1,040,875 liters), a volume normally sufficient to satisfy user demand for at least 48 h.

During this study, routine chlorination was moved to a location between sedimentation and sand filtration. This was done to provide less organic matter with which the chlorine could react but still prevent microbial buildup within the sand filters. When sampling was to be performed, chlorine addition was changed to a location after sand filtration early Monday morning. No microbial sampling of finished water was conducted until Tuesday morning after a full 8 h of plant operation followed by a 16-h holding period. Although the chlorine demand was reduced after the plant was modified, maintenance at a residual chlorine level above 0.5 mg/liter within the distribution system required the addition of 5 to 7

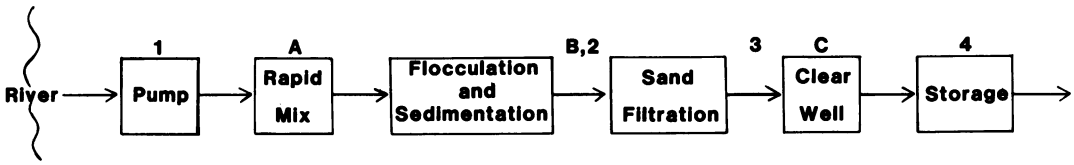

FIG. 1. Treatment plant diagram. Numbers 1 through 4 are sampling sites used throughout the study (1, source water; 2, postsedimentation; 3, post-sand filtration; 4, finished water). Letters A through C are chlorination sites (A, prechlorination; B, routine chlorination during the study period; C, chlorination during microbiological sampling times during the study).

mg/liter regardless of whether the addition was made pre- or post-sand filtration. A schematic diagram of the treatment plant with sampling and chlorination sites is shown in Fig. 1.

Determination of THM levels. Formation of THMs due to chlorination during water treatment was monitored both before and after plant modification. Water samples were collected and stored according to approved Environmental Protection Agency procedures (9). Before plant modification, finished water was tested for THMs on five separate occasions over a 9-month period. For this, finished water held in storage for at least 48 h was collected, immediately treated with sodium thiosulfate to prevent further chlorine activity, and stored at 4°C until analyzed. After plant modification, three samples were collected and analyzed for THMs each time viral concentrates were obtained, i.e., 3 of every 4 weeks throughout the 13-month study period. One sample was obtained from source water before treatment. A second sample was collected from the storage tanks on Monday morning before switching the point of chlorination from prefiltration to postfiltration. This water, which had been treated with chlorine for at least 48 h before sampling, primarily received only the coagulation-sedimentation portion of treatment before chlorination. The third sample was collected from finished water on the following morning; this sample was water primarily treated with chlorine after sand filtration but held in storage for at least 16 h after chlorine addition. The amount of chlorine added at the two different locations was the same and was between 5 and 7 mg/liter throughout the 13-month study period. During this period finished water samples were collected in duplicate. One sample collected on each day was treated immediately with sodium thiosulfate, whereas the other was held for 1 week at 4°C before sodium thiosulfate addition to simulate storage and distribution conditions. All samples were held at 4°C after sodium thiosulfate addition until analyzed.

The samples were analyzed at the U.S. Environmental Protection Agency Laboratory in Cincinnati, Ohio, by the purge and trap method (4). A Tracor 560 gas chromatograph outfitted with a Hall electrolytic conductivity detector 700A LSC-2 sample concentrator, a Tekmar model ALS automatic laboratory sampler, and a Hewlett Packard integrator were used to analyze the samples. The precolumn was a 3% SP1000 on 60/80 mesh Chromsorb W, and the 8-ft (243.84-cm) analytical column was a 1% SP1000 on 60/80 mesh Carbopack B. During the analysis, internal standards were included.

Turbidity measurements. The turbidity was measured with a Hach 2100A turbidimeter. The instrument was calibrated with known standards of different concentrations, and duplicate samples from each water sampling point were measured. The average value was reported in nephelometric turbidity units.

Virus sampling. Viruses were concentrated from both source and treated waters by the filter adsorption-elution technique. Initial samples were collected only from source water. This sampling was done to identify a proper site to conduct the study. During the 13-month period in which the plant was modified, sampling was usually performed 3 out of every 4 weeks. On each occasion, water was collected from four sites. The waters collected included source, postsedimentation, post-sand filtration, and finished. Attempts were made to collect samples of 380, 380, 1,900, and 1,900 liters of the four waters, respectively, although filter clogging sometimes prevented this with source and postsedimentation waters. Three types of microporous filters were used as virus adsorbents. These included one electronegative filter (grade C; borosilicate glass microfiber-epoxy resin filter tube; diameter, 2.5 cm; length, 17.8 cm; nominal porosity, 8.0 μm; Balston, Inc., Lexington, Mass.) and two electropositive filters (1MDS [surface-modified fiber glass and cellulose mixtures; diameter, 7.6 cm; length, 25.4 cm; nominal porosity, 0.2 μm]; and TSM [surface-modified cellulose and filter-aid mixtures, diameter, 7.6 cm; length, 25.4 cm; nominal porosity, 0.5 μm]; AMF Corp., Cuno Div., Meriden, Conn.). For two of every three sampling periods, only Balston filters were used, and sampling was performed on Tuesdays, at least 24 h after the site of chlorination had been switched from postsedimentation to post-sand filtration. During the other week, sampling was carried out with all three filter types from Tuesday through Thursday. The same filter type was used at all four sites during any particular day.

Four separate virus concentrator setups, similar to those described by Hill et al. (11), were used to perform the sampling. Each concentrator was mounted on a wooden frame, and each had an in-line pH probe. The finished water concentrator had an additional acid and sodium thiosulfate additive pump. Separate concentrators were used to prevent cross contamination during the sample collection. Also, the pH probe permitted continuous monitoring which facilitated the maintenance of optimum pH for virus adsorption.

Before collection of viruses on the Balston filters, the water pH was adjusted to 3.5 with HCl for maximum virus adsorption (12). Virus collection on the positively charged filter cartridges was done after a pH adjustment of the water to 7.0 to 7.5 with HCl or NaOH as needed (19). After virus collection, the filters were either eluted on site and the eluate was stored on ice for transport to the laboratory in Cincinnati or the filters were packed in ice, shipped by overnight express mail, and eluted in Cincinnati.

Elution of viruses from filters. All filters were eluted by a single passage of 1,100 ml of 1% beef extract (Lab-Lemco Powder, lot no. 07012689; Oxoid Ltd., London, England) at pH 9.5. Eluates from the Balston filters were concentrated directly by organic flocculation (13). However, eluates from positively charged filters required an additional 1 g of beef extract powder per 100 ml of eluate to floc efficiently during the concentration procedure. This procedure consisted of dropping the pH to 3.5 with 2 M HCl, stirring for 30 min to promote floc formation, centrifuging at 3,000 × *g* for 10 min to pellet the floc, and immediately resuspending the pellet in

0.15 M Na_2HPO_4. These concentrates were then frozen at −70°C until assayed. The finished samples were processed in a separate room reserved for this purpose to minimize contamination.

Viral assay. Viral concentrates from the source, postsedimentation, and post-sand filtration water samples were assayed by the plaque technique on BGM (African green monkey kidney) cells in 75-cm^2 flasks. The cells were grown in Eagle minimal essential medium supplemented with 10% fetal calf serum. For the assay, the medium was removed and 1 ml of the concentrate was added to each flask. After a 2-h adsorption period at 36°C with periodic rocking, 20 ml of agar overlay medium (Eagle minimal essential medium with 2% fetal calf serum, 25 mM $MgCl_2$, 0.0017% neutral red, and 1% Oxoid purified agar) was added. After 72 h at 36°C, plaques were picked and transferred to tube cultures of BGM cells for confirmation by production of cytopathogenic effect. Nonconfirming plaques were passaged one additional time before being scored as negative. Approximately 10% of the confirming viral plaques were identified, using the Lim Benyesh-Melnick pools.

Because many fewer viruses were expected in the finished water concentrates, these were assayed for viruses by cytopathogenic effect production under liquid overlay to increase recovery efficiency. Four cell lines, all grown in the presence of 50 μg of 5-iododeoxyuridine per ml for 72 h before use (5), were used in this assay. These cell lines were BGM, HEL-299 (human embryonic lung), L-132 (human embryonic lung), and RD (human rhabdomyosarcoma). Before inoculation, all flasks were rinsed twice with Hanks balanced salt solution to remove excess 5-iododeoxyuridine. The concentrates were then equally divided such that two 75-cm^2 flasks of each cell type could be inoculated. After adsorption at 36°C for 2 h, 20 ml of Eagle minimal essential medium with 2% fetal calf serum was added, and the flasks were incubated at 36°C. Flasks were observed daily for cytopathogenic effect over a 14-day period. A second passage was made of the material from all flasks to confirm whether the sample was positive or negative for viruses.

Monitoring of viral recovery efficiencies. The efficiency of viral recovery by the adsorption-elution procedure was monitored once each month, using standard strains of enteroviruses. The method used was the addition of ca. 100 PFU of poliovirus type 1, coxsackievirus B3, or echovirus 7 into 380 liters of finished water treated with excess sodium thiosulfate to neutralize the effects of chlorine. This was done at the treatment facility, and the viral concentrator used was the same as that used for the sand-filtered sample. After the concentrator was used, it was chlorinated for 30 min with 10 mg of free chlorine per liter, flushed with finished water for 10 min, and dechlorinated just before use. All three filters but only one virus were tested every fourth week such that during the 13-month study each virus had been concentrated at least three times on each filter. After adsorption, elution, and concentration, the viruses were assayed by plaquing in BGM cells. Recoveries were determined relative to the concentration of virus added to finished water and measured in parallel.

Bacteriological analyses. On each date of viral sampling throughout the 13-month study period, all four sites were sampled to determine bacterial concentrations. The bacteriological analyses performed were total plate count, fecal coliform, total coliform, and fecal streptococcus. Each was measured by standard methods (2).

TABLE 1. THM levels in finished water with chlorination as the first treatment step

Date	THMs (μg/liter)[a]			
	$CHCl_3$	$CHCl_2Br$	$CHClBr_2$	$CHBr_3$
8/80	330	43	9	1
10/80	360	97	33	<1
1/81	120	105	86	10
3/81	205	40	5	<1
4/81	230	16	1	<1

[a] The average total THM was 338 μg/liter.

RESULTS

The location of the study site for this report was based initially on finding significantly higher viral concentrations during the sampling periods of August and October 1980 and January 1981 than those found in other U.S. drinking-water sources sampled by our laboratory. These included numerous sites with high coliform concentrations in Missouri, Pennsylvania, Ohio, Virginia, West Virginia, and Michigan. The virus concentrations found in the study site, using all three filter types, averaged 67, 615, and 108 PFU/380 liters during the three sampling periods, respectively. This site also had THMs in finished water well in excess of the maximum (100 μg/liter) prescribed in the 1979 amendments to the Safe Drinking Water Act (Table 1). Because chlorination was the first step in water treatment at this plant, followed by coagulation, sedimentation, and sand filtration, it appeared to be an ideal site to study the effects of an alteration in the point of chlorine addition on the biological and chemical quality of finished water. With the cooperation of the plant supervisor, this 13-month study was begun in May, 1981.

Effect of plant modification on THM production. Alteration of the site of chlorination from the first step of water treatment to a point either postsedimentation or post-sand filtration (Fig. 1) appeared to cause some reduction in total THM production (cf. Tables 1 and 2). Some differences were also found between samples treated with sodium thiosulfate immediately upon collection and those treated 7 days after collection (compare samples 1 with 2 and 3 with 4 in Table 2). Thus, the reaction of chlorine with THM precursors apparently required a longer holding period than that used in this study (>16 h) to be complete. More extensive investigation revealed that THM production in water treated with chlorine both postsedimentation and post-sand filtration reached 20 to 25% of its 9-day value within 1 h and was ca. 55% of this value within 24 h when held at 4°C (data not shown).

The results in Table 2 also suggest that sand filtration removed some of the THM precursors from water treated only by coagulation-sedimentation (compare samples 1 with 3 and 2 with 4). THMs found in finished water throughout this study were the direct result of chlorine addition during treatment because less than 1 μg of total THMs per liter was consistently found in raw water samples (data not shown).

Although alteration of the point of chlorination appeared to cause some reduction in THM production in the treatment plant being studied, the amount of reduction was much less than the observed average reduction in water turbidity during successive treatment steps (Table 3). For example, 1/10 of the amount of turbidity was found in sand-filtered water relative to that in source water, but chlorination of sand-filtered water caused less than a 50% reduction in THM production relative to that caused by chlorination of source water.

TABLE 2. THM levels in finished water after alteration of the site of chlorination

Date	Total THMs (μg/liter)[a]			
	Sample 1	Sample 2	Sample 3	Sample 4
5/81	229	326	189	291
6/81	326	329	221	280
7/81	354	328	242	243
8/81	381	327	277	297
9/81	292	312	262	324
10/81	285	317	187	281
11/81	281	340	176	303
12/81	197	266	124	232
1/82	165	272	107	215
2/82	110	219	114	218
3/82	173	256	113	174
4/82	160	208	131	188
5/82	262	259	149	222

[a] Numbers presented are the mean values from samples, normally three, collected during the month specified. Sample 1 was collected when chlorine addition was postsedimentation; thiosulfate was added immediately; the average THM value was 246 μg/liter. Sample 2 was the same as sample 1 except that thiosulfate was added after the sample was stored for 7 days at 4°C; the average THM value was 289 μg/liter. Sample 3 was collected 24 h after chlorine addition was changed to post-sand filtration; thiosulfate was added immediately; the average THM value was 176 μg/liter. Sample 4 was the same as sample 3 except thiosulfate was added after the sample was stored for 7 days at 4°C; the average THM value was 251 μg/liter.

Viral and bacterial analyses of waters in modified treatment plant. Viral concentrations were measured at four sites in the treatment plant throughout the 13-month study period. All measurements were made at least 24 h after the point of chlorination was altered from postsedimentation to post-sand filtration. This permitted not only an analysis of the effects of this treatment scheme on the biological quality of finished water but also allowed measurements of the biological quality of the water at each stage of treatment in the absence of a disinfectant. The four sites monitored were source, postsedimentation, post-sand filtration, and finished waters (Fig. 1).

As previously noted, high concentrations of viruses were recovered from samples of source water taken at this site before the 13-month study. During the study, low numbers of viruses were recovered from April through August. However, virus concentrations comparable with those in the prestudy samples were found in samples taken from September through March. Because no obvious differences in viral recoveries were found between the three filters used in this study, the results are combined (Table 4). The coagulation-sedimentation steps of water treatment removed, on the average, ca. 60% of the viruses from source water. Viral recoveries from sand-filtered water averaged ca. 10% of those from the source water. No viruses were ever recovered from finished water during this study (data not shown), even though the assay methods used to detect them in the water concentrates were considerably more rigorous than those used for viral detection in samples from plant sites before chlorination (see above).

A portion of the viral isolates obtained in this study were identified, and all were shown to be enteroviruses. Of the 365 isolates tested, 52% were shown to be echoviruses (variety of types), 23% were polioviruses (64% of these were type 2), 21% were coxsackie B viruses (49% of these were coxsackievirus B5), and 4% were coxsackie A viruses (primarily A16). No apparent differences were detected in the viral isolates obtained after the coagulation or sand-filtration steps from those obtained in source water.

Because enteroviruses were expected to make up most of the isolates of this study, the virus types used to test the efficiency of the concentration procedure were all enteroviruses (poliovirus type 1, coxsackievirus B3, and echovirus 7). Every fourth week during the 13-month study, the recovery of one of these viruses from 380 liters of seeded, dechlorinated finished water was measured after adsorption-elution with each of the three filters used in the study. Average recoveries ranged from 22 to 58%, but no filter was decidedly better or worse (Table 5). The lack of obvious differences among filters was also observed in the recovery of natural viral isolates as noted earlier.

Removal of bacterial indicators by the prechlorination steps of water treatment was generally more effective than that found for viruses. Coagulation-sedimentation removed

TABLE 3. Turbidities of raw and treated water

Date	Turbidity (NTU)[a]			
	Raw water	Post-sedimentation	Post-sand filtration	Finished water
5/81	38.4	4.0	2.6	1.1
6/81	42.1	5.2	1.8	0.6
7/81	68.8	8.4	1.2	0.5
8/81	18.0	3.4	1.3	0.4
9/81	27.8	6.6	3.0	0.4
10/81	52.8	14.2	3.8	1.1
11/81	11.0	5.0	2.5	1.0
12/81	8.3	5.7	2.0	0.9
1/82	15.0	10.6	1.8	2.0
2/82	6.0	10.0	1.0	1.0
3/82	7.0	8.0	3.0	1.0
4/82	20.3	8.7	7.3	0.6
5/82	14.0	7.7	1.0	0.8

[a] NTU, Nephelometric turbidity units. Numbers presented are the averages of two to seven readings taken at each site during the months specified. The average turbidity values were (nephelometric turbidity units): raw water, 25.4; postsedimentation, 7.5; post-sand filtration, 2.5; and finished water, 0.9.

TABLE 4. Viral levels in source water, postsedimentation, and post-sand filtration samples

Date	Viral levels (PFU/380 liters)[a]		
	Source	Postsedimentation	Postfiltration
5/81	3 (2–4)	0 (0–1)	0 (0–0)
6/81	14 (1–38)	2 (0–8)	0 (0–0)
7/81	23 (0–64)	1 (1–7)	0 (0–2)
8/81	3 (1–6)	3 (0–7)	0 (0–1)
9/81	90 (2–251)	38 (2–101)	9 (0–23)
10/81	54 (18–77)	26 (8–65)	15 (1–44)
11/81	13 (11–15)	16 (2–29)	2 (1–2)
12/81	64 (16–139)	26 (6–61)	6 (3–9)
1/82	48 (17–138)	23 (1–62)	3 (0–82)
2/82	293 (216–371)	93 (56–129)	22 (2–42)
3/82	71 (12–123)	31 (12–54)	4 (0–11)
4/82	5 (2–11)	3 (0–8)	0 (0–1)
5/82	0 (0–0)	0 (0–0)	0 (0–1)

[a] Values presented are the means of two to seven samples obtained during the months specified at each site. Values in parentheses are the ranges of concentrations obtained. The average viral levels were (PFU/380 liters): source, 52; postsedimentation, 20; and postfiltration, 5.

TABLE 5. Recovery efficiencies with different filters of enteroviruses from seeded finished water

Filter (charge)	Recovery efficiency (%)[a]		
	Poliovirus 1	Coxsackievirus B3	Echovirus 7
Balston (−)	38 (28–48)	58 (29–84)	27 (14–38)
1MDS (+)	36 (32–40)	44 (21–83)	22 (13–28)
TSM (+)	23 (13–33)	35 (5–80)	23 (6–47)

[a] Values represent averages of three or four samplings over the 13-month study period and were determined relative to PFUs recovered from viruses used to seed dechlorinated finished water. Values in parentheses indicate ranges of recoveries.

about 90% of the total coliforms, fecal coliforms, and fecal streptococci, and sand filtration removed an additional 10 to 80% of these indicator bacteria (Table 6). Removal of species measured by total plate count was more similar to that found for viruses. More significantly, no bacterial indicators were recovered from finished water throughout the course of this study.

The concentrations of indicator organisms found in source and treated waters varied widely from one sampling period to the next (see range in Table 6). Furthermore, the concentrations of none of these indicators varied in relation to viral recoveries at the same site on the same day. In fact, indicator concentrations were almost always low to moderate on the days when the largest numbers of viruses were found (Table 7). Similarly, the low numbers of viruses were isolated on the days when total coliforms were highest. Indicator concentrations were usually, but not always, proportional to one another.

DISCUSSION

The availability of safe drinking water is a universal concern. Treatments used to convert contaminated water into potable water must be designed to provide both chemically and biologically safe water. It has been recognized that the use of chemical disinfectants to produce biologically safe drinking water may increase the chemical contamination of that water. For example, the effectiveness of chlorine as a disinfectant is well established, but its use can cause the production of excessively high concentrations of potentially harmful compounds in treated water. Removal of the precursors to these compounds before chlorination is a possible method of limiting their production. However, this in turn raises questions about the biological safety of the treated water. This study answers some of the questions associated with an alteration of the point of chlorination in water treatment and its effect on finished water.

The plant chosen for this study was shown to have high levels of THMs in finished water and relatively high virus concentrations in source water. Movement of the point of chlorination from the first to last step of treatment allowed no detectable survival of viruses or bacterial indicators in finished water and resulted in some measurable reduction of THMs in finished water. Unfortunately, THM levels still consistently exceeded the prescribed limit of 100 μg/liter despite the large amount of suspended matter removed before chlorination, as demonstrated by decreased turbidity. This indicates that the majority of THM precursors in source water did not contribute significantly to turbidity. Apparently, if THM production is to be properly curtailed during water treatment at this plant, either another disinfectant must be used or more extensive measures, such as activated carbon filtration, will be needed to remove THM precursors.

It was of interest to note that the coagulation-sedimentation and sand-filtration steps of water treatment caused large reductions in both viral and bacterial concentrations. These reductions are presumably due to removal and not inactivation, a suggestion that has not been directly tested. Howev-

TABLE 7. Comparative recoveries of viruses and bacterial indicators on sampling dates when highest concentrations were found for viruses or total coliforms

Month	Recovery of microorganisms[a]				
	Viruses	Total coliforms	Fecal coliforms	Fecal streptococci	Total plate count
Period of highest viral recoveries					
2/82	371	9,800	1,800	2,100	2,500
2/82	261	5,800	1,700	1,060	1,100
9/81	251	16,000	1,100	10,000	2,000
9/81	142	22,000	1,400	8,500	1,700
12/81	139	1,900	180	240	950
3/82	123	3,400	650	1,700	1,500
Period of highest total coliform recoveries					
7/81	22	123,000	9,000	11,000	15,000
7/81	5	52,000	4,550	8,000	6,200
7/81	0	46,000	900	1,900	13,600
6/81	5	43,000	500	900	12,100
7/81	0	30,000	1,250	8,400	3,000

[a] Recoveries are expressed in PFU/380 liters (virus), CFU/100 ml (total coliforms, fecal coliforms, fecal streptococci), or CFU/ml (total plate count). Data represent single samples taken during the month specified. Average recoveries for the period of highest viral recovery were: virus, 215 PFU/380 liters; total coliforms, 9,800 CFU/100 ml; fecal coliforms, 1,140 CFU/100 ml; fecal streptococci, 3,930 CFU/100 ml; and total plate count, 1,460 CFU/ml. Average recoveries for the period of highest total coliform recoveries were: virus, 7 PFU/380 liters; total coliforms, 58,800 CFU/100 ml; fecal coliforms, 3,220 CFU/100 ml; fecal streptococci, 6,040 CFU/100 ml; and total plate count, 9,980 CFU/ml.

TABLE 6. Average recoveries of indicator organisms from different sites within the treatment plant throughout the study period

Site	Recovery of[a]:			
	Total coliforms	Fecal coliforms	Fecal streptococci	Total plate count
Raw water	12,721 (500–123,000)	790 (70–9,000)	2,138 (47–11,000)	2,779 (70–15,000)
Postsedimentation	1,337 (0–12,000)	72 (0–500)	215 (0–2,200)	913 (20–9,000)
Post-sand filtration	273 (0–3,400)	65 (0–2,350)	58 (0–1,000)	312 (0–3,400)
Finished water	<1	<1	<1	<1

[a] All recoveries are in CFU/100 ml except total plate count which is in CFU/ml. Data is the average of 53 samplings. The range of value is presented in the parentheses.

er, in no case did the average removal exceed 99%. There was clearly no positive correlation between viral and bacterial recoveries (Table 7). In fact, the results suggest a strong negative correlation in most instances. Possibly, certain bacteria in the source water may cause viral inactivation, as has been suggested in studies on virus inactivation in activated sludge (14), lake water (7), and seawater (21). Therefore, high bacterial concentrations may be responsible for low viral recoveries in some instances. This relationship between viral and bacterial concentrations was also observed by Marzouk et al. (15).

There were large variations in bacterial recoveries during the course of this study (Table 6) and even from one sampling period to the next (data not shown). However, viral recoveries were fairly stable over long periods of time with low recoveries from April through August and high recoveries from September through March. This variation was apparently not due to changes in recovery efficiencies because the same methods were used throughout, and seeded viruses were recovered with comparable efficiencies during both periods. Therefore, it appears that viruses were most prevalent in the source water during the fall and winter, possibly due to differences in viral shedding patterns or viral survival. Differences in survival could, in turn, have been due to changes in water temperature or, as discussed above, in concentrations of certain bacteria. Clearly, much more extensive experimentation is needed to predict the cause of this observation.

The report on drinking water by the American Water Works Association Committee recommended that finished water should have a turbidity of ≤ 1.0 nephelometric turbidity unit, a free chlorine residual of 1.0 mg/liter or greater for 30 min, and a pH of <8.0 (3). The finished water analyzed in this study normally met this turbidity requirement and always conformed to the chlorine and pH requirements. In fact, the mean free chlorine residual was 2.3 mg/liter in water leaving the plant (data not shown). This would account for the inability to obtain any viable viruses or bacterial indicators from the finished water. Other investigators have repeatedly found viruses in chlorinated finished waters, but the chlorine residual in these cases was ≤ 0.3 mg/liter (17, 18). However, there is no mention of prechlorination or of the chlorine contact time. These data and the findings of this study tend to support the recommendations of the American Water Works Association Committee for producing virologically safe drinking water. However, further confirmation is needed from field studies conducted at water treatment plants having a known virus challenge and a chlorination practice close to the recommended concentration and contact time.

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