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# *Giardia* and *Cryptosporidium* spp. in Filtered Drinking Water Supplies

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***Giardia* and *Cryptosporidium* levels were determined by using a combined immunofluorescence test for filtered drinking water samples collected from 66 surface water treatment plants in 14 states and 1 Canadian province. *Giardia* cysts were detected in 17% of the 83 filtered water effluents. *Cryptosporidium* oocysts, were observed in 27% of the drinking water samples. Overall, cysts or oocysts were found in 39% of the treated effluent samples. Despite the frequent detection of parasites in drinking water, microscopic observations of the cysts and oocysts suggested that most of the organisms were nonviable. Compliance with the filtration criteria outlined by the Surface Water Treatment Rule of the U.S. Environmental Protection Agency did not ensure that treated water was free of cysts and oocysts. The average plant effluent turbidity for sites which were parasite positive was 0.19 nephelometric turbidity units. Of sites that were positive for *Giardia* or *Cryptosporidium* spp., 78% would have been able to meet the turbidity regulations of the Surface Water Temperature Rule. Evaluation of the data by using a risk assessment model developed for *Giardia* spp. showed that 24% of the utilities examined would not meet a 1/10,000 annual risk of *Giardia* infection. For cold water conditions (0.5°C), 46% of the plants would not achieve the 1/10,000 risk level.**

The Surface Water Treatment Rule (SWTR) was enacted by the U.S. Environmental Protection Agency primarily as a means of controlling outbreaks of *Giardia* spp. and enteric viruses (20). For systems that filter and disinfect, the rule stipulates the following. (i) Systems would be required to meet design and operating criteria specified by the state (or other primary agency) to ensure overall removal and/or inactivation of at least 99.9% of *Giardia* cysts and 99.99% of enteric viruses. (ii) Systems would be required to continuously monitor disinfectant residuals and ensure that at least a 0.2-mg/liter disinfectant residual would enter the system at all times. It is expected that the disinfectant residual would supplement the filtration process by achieving at least a 0.5 $\log_{10}$ inactivation factor. (iii) Finally, systems would be required to ensure that filtered water turbidities be less than or equal to 0.5 nephelometric turbidity unit (NTU) in 95% of the measurements taken every month. Turbidity measurements would be required to be taken every 4 h. In some cases the state could impose less-stringent turbidity restrictions where filtered water turbidities could be less than or equal to 1 NTU in 95% of the measurements, but in no cases could the filtered water turbidities exceed 5 NTU.

Exactly how implementation of filter performance criteria will protect finished water from waterborne transmission of parasites is unclear. For example, filter plants are required to meet a 0.5-NTU limit, but research has shown that sudden changes in filter turbidity are more important than the level itself. Logsdon et al. (15) showed that *Giardia* cysts could pass through treatment filters with relatively small changes (0.2 to 0.3 NTU) in turbidity levels. Logsdon et al. reported that, even when filters operated properly, *Giardia* levels ranged between 3 and 10 cysts per liter in filter effluents of plants with source water of poor quality.

Another problem is related to the judgment of what is adequate performance. Improved detection procedures, for example, may change the research conclusions on which the filter performance concept is based. LeChevallier et al. (14) showed that the combined immunofluorescence antibody procedure recovered substantially more *Giardia* cysts than the reference technique upon which most of the data for the SWTR were based. In addition, until questions regarding cyst viability can be adequately addressed, overall plant performance can only be indirectly assessed.

Recently, *Cryptosporidium* spp. have been recognized as waterborne pathogens (3, 6–8, 19). There are no filter performance criteria for *Cryptosporidium* oocysts and little data to make decisions regarding optimum water treatment practices for the organism. Rose (17) detected *Cryptosporidium* oocysts in 2 of 10 filtered water supplies in the western United States. Ongerth, in a roundtable discussion (2), reported finding *Cryptosporidium* oocysts in half of the two dozen filter effluent samples examined from a newly designed slow sand filtration plant.

The purpose of this study was to examine 66 surface water filter plants to evaluate how compliance with the SWTR would control the occurrence of *Giardia* and *Cryptosporidium* organisms in drinking water supplies. The study examines the factors related to the occurrence of *Giardia* and *Cryptosporidium* spp. in filtered water and evaluates the data within the context of a risk assessment model.

## MATERIALS AND METHODS

Descriptions of the sampling sites (13) and immunofluorescence methodology (12, 13) have been previously given. In all cases, the treated water was chemically conditioned before filtration. No apparent outbreaks of giardiasis or cryptosporidiosis were observed in any of the systems tested.

Quality assurance tests were performed on sampling units and all materials used in the assay to ensure that they were free of parasite contamination. These negative control samples were processed for detection of *Giardia* and *Cryptosporidium* organisms as previously described (12, 13).

Turbidity and disinfection data were provided by the

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participating utilities. All analyses were performed by state-certified laboratories and were conducted according to accepted procedures (1). Particle counts were performed by analyzing 10-ml water samples with a Hiac/Royco particle counter (model 4111; Silver Spring, Md.). Determinations were performed in triplicate. The counter was set to measure particles in the size ranges of 2 to 4 μm, 5 to 9 μm, 10 to 15 μm, 16 to 19 μm, 20 to 39 μm, and >40 μm. The results of the >5-μm range counts were combined to determine the number of *Giardia*-sized particles. With each use, field checks of the instrument were performed by using a 9.87-μm latex bead standard (Duke Scientific Corp., Palo Alto, Calif.).

## RESULTS AND DISCUSSION

**Distribution of cysts and oocysts in filtered water samples.** A total of 82 finished drinking water samples were examined for *Giardia* and *Cryptosporidium* spp. *Giardia* cysts were detected in 14 samples (17.1%), with a geometric mean (for positive samples) of 4.45 cysts per 100 liters and a range of 0.29 to 64 cysts per 100 liters. *Cryptosporidium* oocysts were observed in 22 samples (26.8%), with a geometric mean (for positive samples) of 1.52 oocysts per 100 liters and a range of 0.13 to 48 oocysts per 100 liters. Overall, *Giardia* or *Cryptosporidium* spp. or both were found in 32 (39%) of the finished water supplies.

Other researchers have detected cysts and oocysts in treated water supplies. Hibler (9), by using the zinc sulfate-Lugol's iodine test, found *Giardia* cysts in 80 of 1,214 unfiltered potable water samples, 148 of 615 direct filter samples, and 12 of 357 conventional treated water supplies. Ongerth et al. (16), by using an immunofluorescence antibody test, reported *Giardia* cysts in seven of nine filtered water samples for a conventional water system which did not practice chemical conditioning. Even with chemical conditioning, pilot plant studies still found cysts in effluent samples after backwash. Ongerth et al. also found cysts in 7 of 13 filtered water samples of another utility which used four, dual-media, pressure filters. Treatment included the addition of a cationic polymer and a 5-min filter-to-waste cycle. In a third utility, Ongerth et al. (16) detected cysts in one of three effluent water samples from a diatomaceous earth filter. In other preliminary studies, Ongerth (18) detected *Cryptosporidium* oocysts in the effluent of a newly designed slow sand filter. Rose (17) has reported detecting *Cryptosporidium* oocysts in 2 of 10 filtered water samples and 2 of 4 unfiltered potable water supplies in the western United States.

The outbreak of cryptosporidiosis in Carrollton, Ga., occurred in a system that practiced full conventional treatment (8). The treatment chain included the addition of alum, lime, and chlorine, rapid mixing, mechanical flocculation, sedimentation, and rapid sand filtration. Although there were a number of deficiencies found in the treatment system, the plant did meet current federal standards for coliform bacteria and turbidity. Similarly, the outbreak in Swindon and Oxfordshire, England, occurred in a system that practiced full conventional, rapid sand filtration (3, 5). The finished water had a turbidity of 0.2 to 0.4 NTU, with a free chlorine residual of 0.4 to 0.5 mg/liter, and contained no coliform bacteria.

In this study, despite the frequent detection of *Cryptosporidium* spp. in filtered drinking water, no outbreaks of cryptosporidiosis were apparent. The levels of oocysts in the outbreaks in Georgia and England were much higher than the levels observed in this study. Analyses performed after the peak illness in the Carrollton outbreak showed 46 oocysts per 100 liters (17). Oocyst levels during the active portion of the outbreak were undoubtedly higher. In the England outbreak, *Cryptosporidium* levels in the distribution system ranged between 0.2 and 7,700 oocysts per 100 liters (5).

TABLE 1. Detection of *Giardia* and *Cryptosporidium* organisms in filter effluents

| Filter type | % Positive[a] | Mean raw water densities[b] | |
|---|---|---|---|
| | | *Giardia* cysts | *Cryptosporidium* oocysts |
| Sand | 36 (5/14) | 3.74 | 5.22 |
| Dual media | 25 (3/12) | 2.46 | 1.03 |
| Mixed media | 17 (4/23) | 0.76 | 0.80 |
| GAC | 61 (20/33) | 5.68 | 5.20 |

[a] Percent plant effluent samples positive for *Giardia* or *Cryptosporidium* or both. Numbers in parentheses are number of positive samples/total number of samples.

[b] Values are geometric means for *Giardia* cysts and *Cryptosporidium* oocysts per liter.

**Estimate of viability.** Microscopic examination of oocysts found in the current study showed that only 2 of the 23 *Cryptosporidium* oocysts found in potable water samples contained sporozoites or a densely packed cytoplasm. In contrast, approximately one-third of the 242 *Cryptosporidium* oocysts observed in raw water samples contained sporozoites within the oocyst (13). Without the sporozoites, these oocysts were probably not viable. While these results are preliminary and a larger data base is needed, they do suggest that the current disinfection practices of the plants studied (98% used prechlorination, 25% used postchloramination) were effective for inactivation of *Cryptosporidium* oocysts. These field data are in contrast to the laboratory results of Campbell et al. (4) and Korich et al. (11) which showed that *Cryptosporidium* spp. were unaffected by even high chlorine doses. Korich et al. reported that *Cryptosporidium parvum* exposed to 80 mg of free chlorine per liter for 90 min showed only a 90% decrease in viability. They concluded that disinfection alone would be ineffective for control of *Cryptosporidium* spp. in drinking water. Our results suggest that treatment, in some way, inactivates *Cryptosporidium* spp. Future research should investigate the combination of environmental exposure and disinfection on *Cryptosporidium* viability.

Observation of 46 *Giardia* cysts in drinking water samples showed that 13.3% of the cysts had a viable type morphology. A viable type morphology does not imply that an organism can excyst or infect animals; rather, a cyst that does not have a viable type morphology, i.e., one that has a distorted or shrunken cytoplasm, is probably dead. Five of the six viable type cysts found in tap water samples were from systems that practiced chloramination. Because chloramines react slowly with *Giardia* spp., these organisms may not demonstrate the same level of destruction as cysts exposed to free chlorine (10). There were no apparent outbreaks of giardiasis in the systems studied.

**Treatment of *Giardia* and *Cryptosporidium* spp.** Analysis of treatment plant configurations showed that granular activated carbon (GAC) and rapid sand filters were more likely to have effluent samples positive for cysts or oocysts than dual- or mixed-media filters. More than 60% (20 of 33) of the GAC filter effluents and 36% (5 of 14) of the rapid sand filter effluents were positive for either *Giardia* or *Cryptosporidium*

TABLE 2. Relationship between treatment parameters and detection of *Giardia* and *Cryptosporidium* organisms

| Treatment parameter | Treatment plant effluent[a] | |
|---|---|---|
| | % Positive | % Negative |
| Filter-to-waste process | 12.5 (4/32) | 20.0 (10/50) |
| Surface wash | 65.6 (21/32) | 50.0 (25/50) |
| Good filter condition | 71.9 (23/32) | 76.0 (38/50) |
| Conventional treatment | 75.0 (24/32) | 80.0 (40/50) |
| Coagulant | | |
| Ferric | 15.6 (5/32) | 16.0 (8/50) |
| Alum | 59.4 (19/32) | 72.0 (36/50) |
| Polymer | 25.0 (8/32) | 12.0 (6/50) |

[a] Data have been tabulated for sites which were positive ($n$ = 32) or negative ($n$ = 50) for *Giardia* or *Cryptosporidium* organisms or both. Also shown are the number of sites which used the particular treatment process (i.e., 4 of the 32 positive sites used a filter-to-waste process while 10 of 50 negative sites used a filter-to-waste process). Filter run time averaged 39 h for parasite-positive sites and 44 h for sites where parasites were not observed.

spp. (Table 1). However, the raw water parasite densities for these filters were generally higher than for parasite-negative treatment plants. GAC filter plants are frequently used when the source water quality is poor. These results suggest that high raw water parasite densities may overcome filtration and enter finished water supplies. This conclusion is supported by the observation that parasite-positive treatment plants had an average 2.14 $\log_{10}$ removal of *Giardia* spp. and an average 2.38 $\log_{10}$ removal of *Cryptosporidium* spp. (parasite-negative plants had a >2.45 $\log_{10}$ removal for *Giardia* spp. and a >2.22 $\log_{10}$ removal for *Cryptosporidium* spp.). The results show that treatment plants can have high removal efficiencies of parasites and still detect organisms in finished drinking water samples. Logsdon et al. (15) reported that even when filters were operated properly, *Giardia* levels ranged between 3 and 10 cysts per liter in filter effluents of plants with source water of poor quality. The fact that filtration is not 100% effective places a significant reliance on disinfection, particularly at locations with high source water counts. In addition, the results discussed are based on microscopic detection and do not reflect cell viability.

It should be emphasized that the utilities examined in this study were well-run and well-maintained facilities. This fact may be related to the reason why no operational parameters could account for the presence of cysts or oocysts in effluent waters (Table 2). For example, 4 of 32 (12.5%) sites that were parasite positive practiced a filter-to-waste process, while 10 of 50 sites (20%) that were parasite negative practiced a filter-to-waste process. Similarly, there was no difference in surface wash procedures, filter run times (and backwashing frequencies), filter condition, plant design (conventional versus other configurations) or choice of coagulant. It should be noted, however, that both direct filtration plants examined contained low levels of cysts or oocysts in treated effluents.

**Relationship between turbidity and parasite removal.** The SWTR prescribes that utilities must maintain effluent turbidities for conventional filters of ≤0.5 NTU in 95% of the monthly samples. We found that the average plant effluent turbidity for sites that were parasite positive was 0.19 NTU. For comparison, the average plant effluent turbidity for sites that were parasite negative was 0.18 NTU. The results show that production of low-turbidity water did not ensure that the plant effluent would be cyst or oocyst free.

The vast majority (78.1%) of sites that were positive for

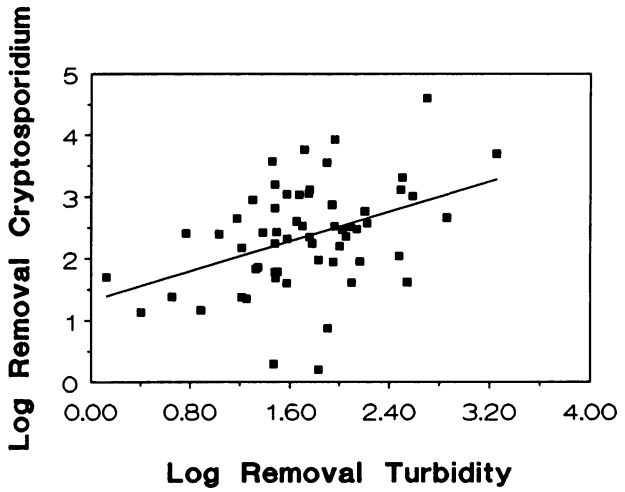


FIG. 1. Relationship between $\log_{10}$ removal of turbidity and $\log_{10}$ removal of *Cryptosporidium* oocysts. Regression line: $y = 0.605(x) + 1.318$; $r = 0.412$, $P < 0.01$.

*Giardia* or *Cryptosporidium* spp. would have been able to meet the turbidity regulation of the SWTR. Logsdon et al. (15) showed that *Giardia* cysts could pass through treatment filters with relatively small changes (0.2 to 0.3 NTU) in turbidity levels. Conversely, 18% of the sites which were parasite negative did not meet the SWTR guideline of 95% of the samples of <0.5 NTU.

Overall, the removal of turbidity within the treatment process was not a statistically significant ($P > 0.05$) predictor of the removal of *Giardia* spp. However, there was a significant correlation ($P < 0.01$) between removal of turbidity and removal of *Cryptosporidium* spp. (Fig. 1). An even better relationship between turbidity and parasite removal was observed when the data were plotted for an individual site (Fig. 2 and 3). Particle counts performed close to the date of parasite sampling showed a similar pattern (Fig. 4 and 5). The $\log_{10}$ removal of particles in the range of 5 to 15 μm had a correlation coefficient of 0.82 and 0.83 when compared with removals of *Giardia* and *Cryptosporidium* spp., respectively. Additional research is necessary to de-

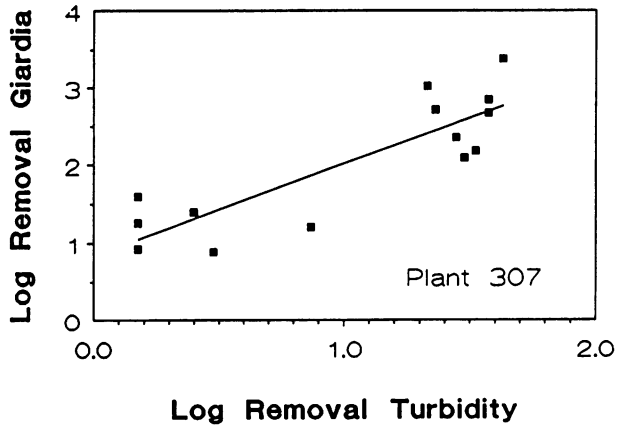


FIG. 2. Relationship between $\log_{10}$ removal of turbidity and $\log_{10}$ removal of *Giardia* cysts at plant 307. Regression line: $y = 0.854(x) + 1.176$; $r = 0.854$, $P < 0.01$.

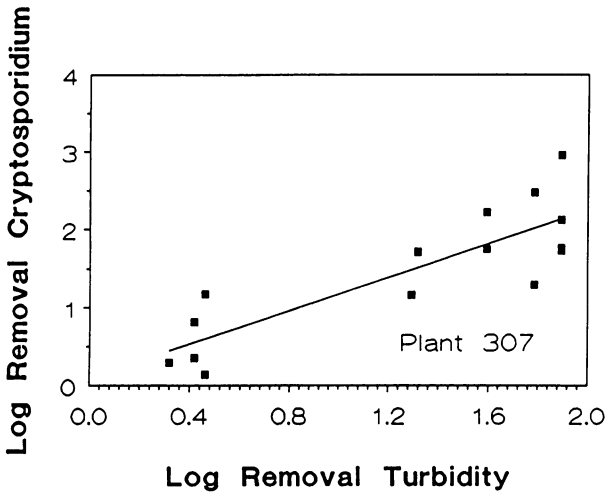


FIG. 3. Relationship between $\log_{10}$ removal of turbidity and $\log_{10}$ removal of *Cryptosporidium* oocysts at plant 307. Regression line: $y = 0.109(x) + 1.071$; $r = 0.847$, $P < 0.01$.

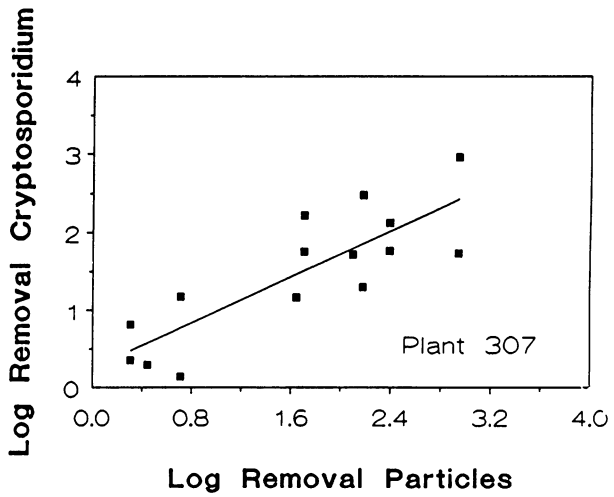


FIG. 5. Relationship between $\log_{10}$ reduction of particle counts of >5 μm in size and $\log_{10}$ removal of *Cryptosporidium* oocysts. Regression line: $y = 0.252(x) + 0.739$; $r = 0.83$, $P < 0.01$.

termine the best predictor of cyst and oocyst removal. With such research, an appropriate surrogate (e.g., turbidity, particle counts, etc.) could be used to reliably predict treatment plant performance.

**Treatment efficiency.** To estimate the annual risk of *Giardia* infection from water consumption examined in this study, the removal and inactivation of *Giardia* cysts on the basis of the credits given in the SWTR were determined. Removal of cysts by treatment was calculated as the logarithmic difference between the raw water (13) and plant effluent *Giardia* counts. Raw water *Giardia* levels were doubled to account for the 50% recovery efficiency, while tap water samples were multiplied by 1.43 to adjust for 70% tap water recovery efficiency. When no cysts were detected in treated effluents, the limit of detection method was used. Values were also adjusted to account for the 12.8% viable type cysts observed in raw water (13). To determine cyst inactivation, data including disinfectant residual, contact time, pH, and water temperature were used to estimate *Giardia* inactivation from published tables of disinfectant concentration × time (C × T) (21). Theoretical contact times were adjusted by using multipliers of 0.6 and 0.1 to account for short-circuiting of treatment basins for pre- and postdisinfection, respectively. The calculated treatment level (i.e., the sum of removal and disinfection treatment levels) was compared with the recommended level of treatment needed to achieve a 1/10,000 annual risk of *Giardia* infection (18).

For presentation of the data in Fig. 6, the actual treatment level was subtracted from recommended level. A value of zero implied that the treated water met the recommended goal of 1/10,000 annual risk of *Giardia* infection (20). Values greater than zero meant that the plant was providing better treatment, while values less than zero signified that the plant was not meeting the 1/10,000 risk assessment level. The results show that 24% of the utilities would not meet the 1/10,000 risk assessment level (Fig. 6). Twelve percent of the sites were more than 1 $\log_{10}$ below the recommended treatment goal.

A worst-case scenario, with disinfection at 0.5°C (assuming that filtration efficiency remained the same), estimated that 46% of the plants would not achieve the 1/10,000 risk level (Fig. 7). During cold weather conditions, almost one-

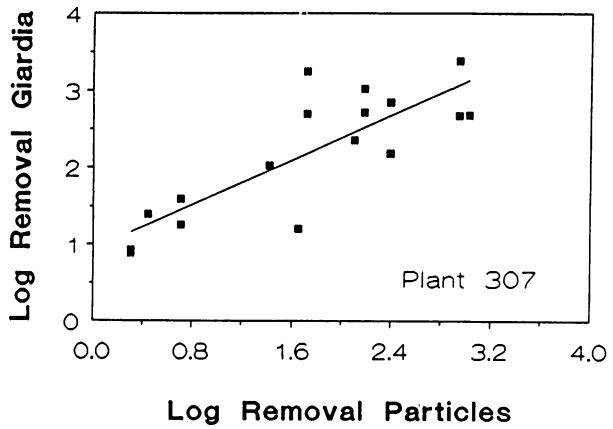


FIG. 4. Relationship between $\log_{10}$ reduction of particle counts of >5 μm in size and $\log_{10}$ removal of *Giardia* cysts. Regression line: $y = 0.939(x) + 0.726$; $r = 0.822$, $P < 0.01$.

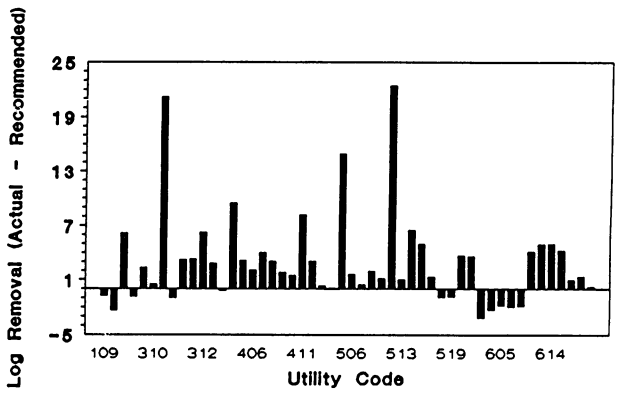


FIG. 6. Analysis of treatment efficiency to meet an estimated 1/10,000 annual risk of *Giardia* infection.

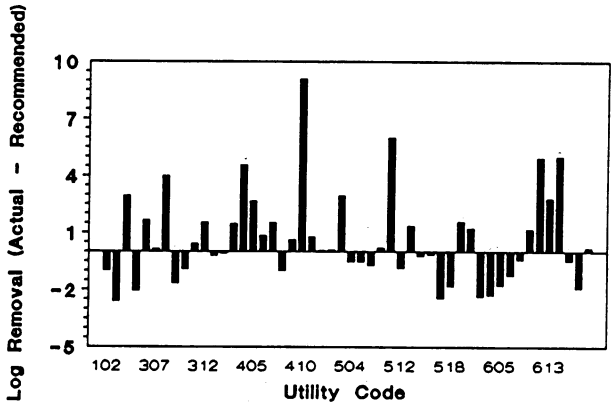


FIG. 7. Analysis of treatment efficiency to meet an estimated 1/10,000 annual risk of *Giardia* infection under cold water conditions.

quarter of the plants would be more than 1 $\log_{10}$ below recommended treatment levels.

It should be noted that these exercises are not intended to imply that the utilities will have outbreaks of giardiasis. Their purpose is to help guide water treatment operators and regulators in applying appropriate treatment technologies.

Most of the utilities examined in this study achieved 2 to 2.5 $\log_{10}$ removal of cysts by clarification and filtration as recommended by the SWTR. It is clear that, in many locations, additional disinfection is needed to treat raw water *Giardia* levels. Overall, the average utility will have to apply 5.0 $\log_{10}$ treatment to achieve an annual risk of *Giardia* infection of <1/10,000. To meet the requirements of the SWTR, many utilities will have to provide a disinfection concentration and contact time within the treatment process to achieve a 3 $\log_{10}$ inactivation. During cold water conditions, substantial increases in disinfectant levels may be required to meet the disinfection concentration and contact time guidelines. This increased requirement for disinfection, however, may pose conflicts with the pending Disinfection By-Product Rule of the U.S. Environmental Protection Agency. The need for increased disinfection may also require engineers to reexamine designs for equipment such as ozonators and disinfectant contact basins.

**Summary.** The current project demonstrates that *Giardia* and *Cryptosporidium* spp. can be frequently isolated from filtered drinking water. Compliance with criteria outlined by the SWTR does not ensure that filtered water will be free of waterborne parasites. Treatment plants with high levels of cysts and oocysts in raw water supplies were more likely to have the organisms detected in finished drinking water. Many treatment plants will have to provide significant levels of disinfection to protect against low levels of waterborne infection. Assays are needed to determine the viability and virulence of pathogenic protozoa found in treated water supplies.

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