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Project Summary

Ultraviolet Disinfection of Water for Small Water Supplies

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Ultraviolet (UV) radiation was considered as an alternative means of disinfecting small drinking water supplies. A major impetus for this study was the U.S. increase in reported waterborne disease outbreaks caused by *Giardia lamblia*, an organism that is highly resistant to conventional chlorination.

Both field and laboratory studies were used to evaluate the effectiveness of UV radiation in reducing the viability of *Escherichia coli*, *Yersinia* sp., and *Giardia* sp. UV sources included commercial UV reactors and an excimer laser.

***G. muris* was used as a surrogate for *G. lamblia* so that reliable excystation and a consistent population of infective organisms could be attained throughout the seasons and through the project study period.**

***G. muris* cysts were significantly more resistant to UV than *E. coli* and more resistant than *Yersinia* sp. The effectiveness of disinfection depended on the amount of UV radiation reaching the organisms and on any hydraulic shortcircuiting. The presence of entrapped air in the commerical UV reactors decreased the efficiency of the reactor.**

Natural or added color in the test waters decreased the effectiveness of UV disinfection on *G. muris*. For the range and type of turbidity examined, the shielding effect against bacterial disinfection noted in other studies was not observed.

Studies on *G. muris* cysts indicated that storage time and temperature affected the viability of the cysts and that the rate of decrease in viability approximately doubled with each 10°C increase in temperature above freezing. Below freezing, however, cyst viability was shortened to hours rather than to days for above-freezing conditions.

Physical stress produced by pressure and alum addition in water treatment processes appeared to damage and even destroy cysts.

This Project Summary was developed by EPA's Water Engineering Research Laboratory, Cincinnati, OH, to announce key findings of the research project that is fully documented in a separate report of the same title (see Project Report ordering information at back).

Introduction

The germicidal effects of sunlight have long been known, but artificial ultraviolet (UV) radiation can obtain much better microbiocidal action at a wavelength of about 260 nm. A common form of artificial UV radiation with a wavelength of 253.7 nm can be produced by low-pressure mercury vapor lamps. The inactivation of microorganisms by UV radiation is based on photochemical reactions in the DNA that result in coding system errors.

Early attempts to use UV radiation for public water supply treatment began in 1910. They were not very successful and were abandoned in favor of chlorination. However, two factors have renewed interest in UV radiation for pub-

lic water supply disinfection: (1) the fact that chlorination can produce byproducts that may be carcinogenic to humans and possibly toxic to aquatic life, and (2) a large increase in giardiasis in the United States, a waterborne disease caused by the chlorine-resistant *Giardia lamblia* cyst. The major impetus for this study was the dramatic increase in waterborne disease, specifically giardiasis, in the United States since the mid-1960's. The present investigation uses both field and laboratory studies to evaluate the effectiveness of UV radiation in reducing the viability of *Escherichia coli*, *Yersinia* sp., and *Giardia* sp. UV sources included commercial UV reactors and an excimer laser.

Germicidal Characteristics of UV Radiation

Ultraviolet (UV) radiation is defined as electromagnetic radiation occupying a small portion of the electromagnetic spectrum lying beyond visible light. The wave lengths of UV radiation range from about 4 to 400 nm, with the narrow band between 200 and 310 nm having the greatest injurious and lethal effects on microorganisms. The maximum microbiocidal action occurs at about 260 nm for practically all microorganisms and is essentially congruent with the UV absorption and photochemical sensitivity of deoxyribonucleic acid (DNA). The inactivation of microorganisms is then essentially based on photochemical reactions in the DNA that result in errors and faults being introduced into the coding systems. Nature has developed various means of molecular-biological error correction for the protection of the vital DNA, and the selectivity of the reactions may be influenced by changes in the organisms in different phases of their life cycle. Thus photo repair systems may resuscitate a seemingly dead organism by either longer wavelength photo irradiation or dark incubation. In addition, spores have been found to be very resistant to radiation.

The germicidal action of UV radiation results from its exposure to or direct contact with the organisms, and it can only be effective if it is absorbed. The lethal effect of UV radiation results from a photochemical reaction initiated by absorption of a photon by the molecular structure rather than by formation of a toxic substance in the medium.

The inactivation of microorganisms resulting from UV exposure is proportional to the intensity (mW/cm^2) multiplied by the time of exposure (sec). The product of the irradiation intensity per area and the time is called the UV dose ($mW\text{-}sec/cm^2$). Note that the only adverse effect of an excessive dose of UV radiation is additional cost.

Sources of UV Radiation

The sources of UV radiation are of two classes—natural and artificial. The sun is the most important natural source of UV light. The oxidizing and germ-killing effects of sunlight contribute considerably to the conservation of our environment by natural photochemical processes in the atmosphere and by natural ultraviolet purification of surface water. UV light can also be generated artificially by a wide variety of arcs and incandescent lamps. One common form of artificial UV radiation can be generated from special low-pressure, mercury-vapor lamps that produce UV radiation as a result of an electron flow between the electrodes through ionized mercury vapor. These artificial UV radiation sources can supply energy in such relatively high doses that in fractions of a second they can accomplish a higher degree of irradiation than the sun can in several hours.

Since the maximum UV sensitivity of microorganisms and the UV emission of the low-pressure mercury vapor lamp are well matched, the nearly monochromatic low-pressure mercury lamp has prevailed as the dominant radiation source in research and practical applications.

Early Experience with UV Disinfection

The first recorded attempt to use UV radiation for public water supply treatment was made in 1910 in France. Subsequently, UV treatment was tried in the United States with limited success, but most systems were abandoned before 1930. The main reasons given for abandoning the UV method of treatment were relatively high operating costs, operating and maintenance problems, and the advent of chlorination, which was found to be more efficient and reliable.

Procedures

The UV equipment consisted of two commercially available UV water disinfection units, a laboratory batch UV unit, and an excimer laser UV unit to provide coherent UV light at several discrete wavelengths.

The early stage of this study used only *G. lamblia* cysts supplied mainly by hospitals and pathology laboratories across the State of Washington. However, the supply of *G. lamblia* cysts was closely related to the outdoor recreational activities in the area and was hence very seasonal. In addition, the cysts proved difficult to excyst consistently, which severely limited the amount of information that could be gathered. Thus we decided to use *G. muris* cysts, which were indicated by current information to be at least as resistant as *G. lamblia* cysts. *G. muris* cysts provided a relatively higher excystability and less fluctuation of the results. Until we develop a reliable method to determine the viability of *G. lamblia*, the use of *G. muris* cysts as a surrogate is warranted. Female Swiss Webster mice were used for cyst propagation.

Strains of fully virulent *Yersinia enterocolitica* were derived from human patients suffering from chronic gastrointestinal disease manifested largely by recurrent and sporadic diarrhea or by acute episodes of ileitis. Three separate media were studied for isolating and enumerating *Y. enterocolitica* because of the lack of an acceptable standardized method. The first two media, MacTween* and mYE, required membrane filtration of the sample, and the third medium, Tergitol-7 was inoculated by surface spreading.

Two different strains of *E. coli* were evaluated in the batch UV studies: a nalidixic-acid-resistant (NAR) strain and a non-NAR strain.

Three different media were used to isolate and enumerate the two different strains of *E. coli*. The first two media, eosin methylene blue (EMB) and m-Endo, are commonly used media for enumerating coliforms from water samples. Both media were substrates for membrane filters. The third medium, Tergitol-7 TTC, was used to differentiate between *E. coli*-NAR and background coliforms. Water samples were surface-spread on Tergitol-7 TTC agar.

During the course of this investigation, efforts were made to obtain a UV disinfection unit with a self-contained dosimeter so that the actual UV dosage could be read directly. These efforts were fruitless, and it was necessary to rely on actinometry to gauge the out-

*Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

put of the UV unit. Since the geometry of the unit, the flow patterns at various Reynolds numbers, the degree and intensity of reflection, the average distance between lamps, the penetration depth, the cleanliness of the liquid and of the UV lamp, the degree of short-circuiting, and the degree of light scattering all have potential effects on the dosages applied, photo actinometry was used to access the UV light intensity of exposure.

Giardia cysts, *Yersinia*, and *E. coli* organisms were spiked into the UV treatment unit influent stream through a manifold and then mixed by a static mixer. The organisms then passed through the UV contactor with various detention times and hence different radiation doses. Color and turbidity were added to determine their influence on cyst and organism survival. The effect of storage time on *G. muris* cysts at various temperatures was also investigated, as well as their settling characteristics in quiescent conditions. Finally, zeta potentials were determined for *G. lamblia* over a wide range of pH values.

Results and Discussion

With relatively long exposure times of about 20 min, a 99-percent kill of cysts was obtained by the Pen Ray Batch Reactor (Figure 1). Figure 2 compares data on *Giardia* cyst survival versus UV dosage from another study (Rice, E. W. and J. C. Hoff. 1982. Inactivation of *Giardia lamblia* Cysts by Ultraviolet Irradiation. Appl. Environ. Microbiol. 42:546-547) with data obtained in this study using a commercially available reactor. Note that the relatively low destruction rate of *Giardia* cysts shown in the data from Rice and Hoff in Figure 2 was due to the low UV exposure dose, and that commercial UV disinfection units used in this study can achieve disinfection of the cysts. For all experimental conditions in which color was added to the water (regardless of the source), an increase in absorbance at 254 nm resulted in an increase in the percentage of cyst survival (Figure 3). On the other hand, when turbidity was added, the degree of cyst inactivation was not affected with the retention time held constant (Figure 4).

Though the laser-generated UV radiation has a considerably greater intensity than the mercury-vapor UV lamps, the detention time for the laser pulse is on the order of 10 nano-seconds. Thus equivalent dose ranges can be obtained from both sources. Data from this study suggest that the commerical UV units are much more effective than the excimer laser unit in inactivation of *Giardia* cysts.

Comparison of UV inactivation curves for *Y. enterocolitica, E. coli,* and *G. muris* cysts graphically exhibits the tremendous resistance cysts have to UV inactivation. The fact that both *Yersinia* and *Giardia* cysts are more resistant to UV than *E. coli* has important implications where the total coliform procedure is used to monitor disinfection efficiency and indicate microbiological water quality. Outbreaks of giardiasis in water supply systems that reported satisfactory total coliform concentrations are indicative of the problems associated with using coliforms as indicator organisms for adequate UV disinfection.

A study was conducted on the effects of storage time on *G. muris* cysts at various temperatures. The 1°C, 5°C, 10°C, and 20°C experiments showed differences in die-off rates. No excystable cysts were observed at 1°C, 5°C, 10°C, and 20°C after storage periods of 120, 95, 63, and 26 days, respectively.

The settling characteristics were measured for *G. muris* cysts in distilled water under quiescent conditions. They demonstrated, for example, that after 2 days, approximately 50 percent of the cysts were removed from the water column.

Conclusions

1. The excystation procedure for *Giardia* cyst viability was unreliable for *G. lamblia* cysts and could not be used to provide significant data.
2. To determine cyst viability, *G. muris* cysts were used as a surrogate for *G. lamblia* because of their relatively higher percentage of excystability and more consistent reproducibility under laboratory conditions.
3. Unlike chlorine and ozone, UV radiation has no problems with mixing (mass transfer) in the contactor; it also produces no residual. However, like chlorine and ozone contactors, flow dispersion has a significant impact on UV's biocidal effect.
4. As the UV reactors approached plug flow, greater degrees of disinfection were obtained.
5. Direct measurement of the actual UV dosage in the two commercially available UV contactors was not possible. Instead, it was necessary to rely on actinometry and inactiva-

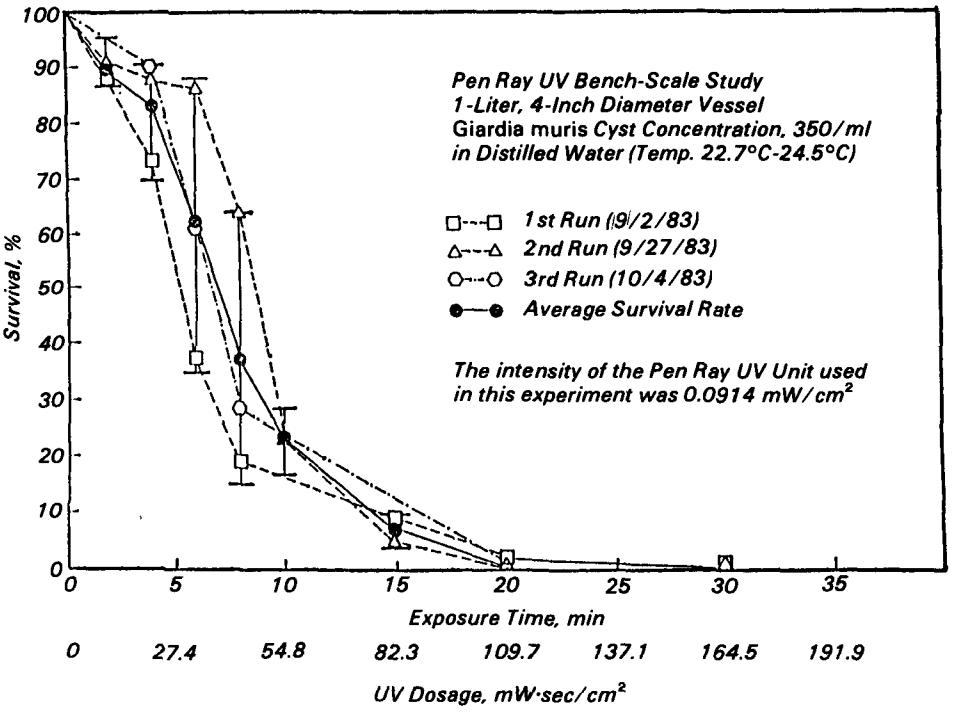

Figure 1. Giardia muris *cyst survival versus UV exposure time with a Pen Ray UV lamp.*

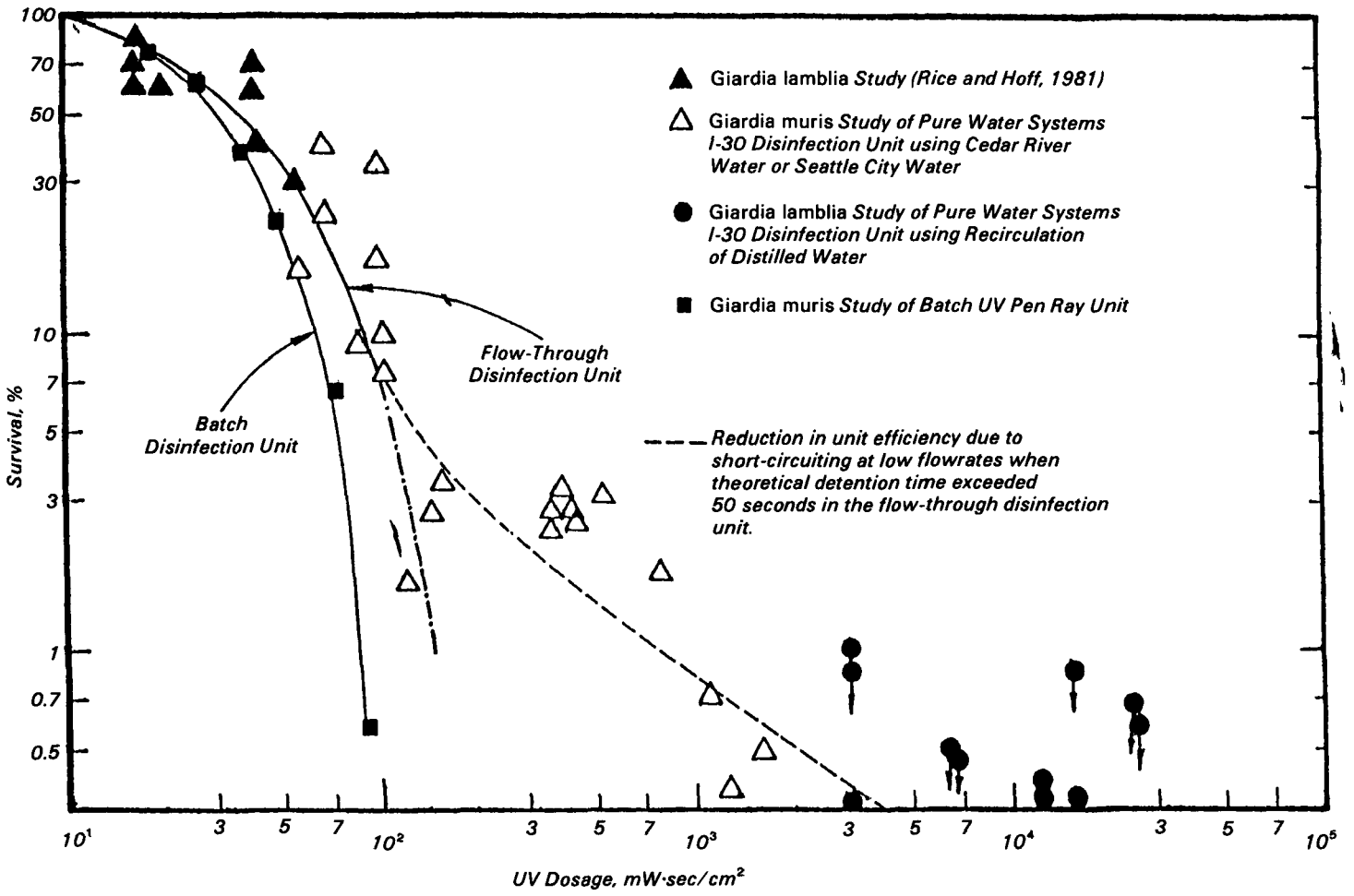

Figure 2. *Survival of* Giardia *sp. cysts versus UV dosage.*

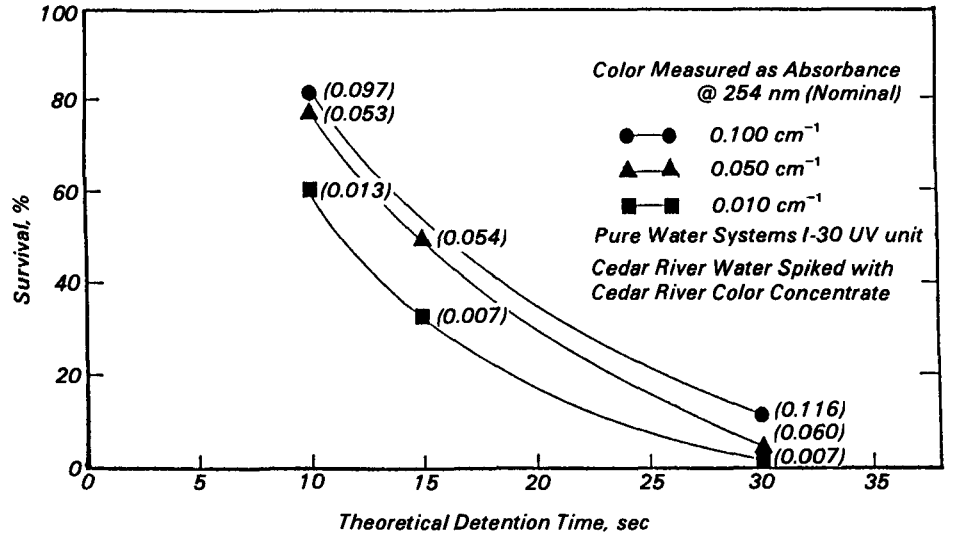

Figure 3. *Effect of Cedar River color on* Giardia muris *cyst survival under UV light at various detention times for Pure Water Systems unit.*

tion of *B. subtilis* spores to indicate the dosage.

6. The low destruction rate for *Giardia* cysts reported by previous investigators was primarily due to low UV exposure dose relative to the actual dose required.
7. *Giardia* cyst inactivation is a function of UV energy absorption and thus depends on the amount of UV light that reaches the cyst and the time of exposure. Where commercial UV unit design permits short-circuiting, the time for 100 percent inactivation can be expected to be protracted.
8. The commercial UV disinfection units tested can achieve disinfection of *Giardia* sp. The design of the units is important in the detention time required for exposure to UV light. For the Pure Water Systems

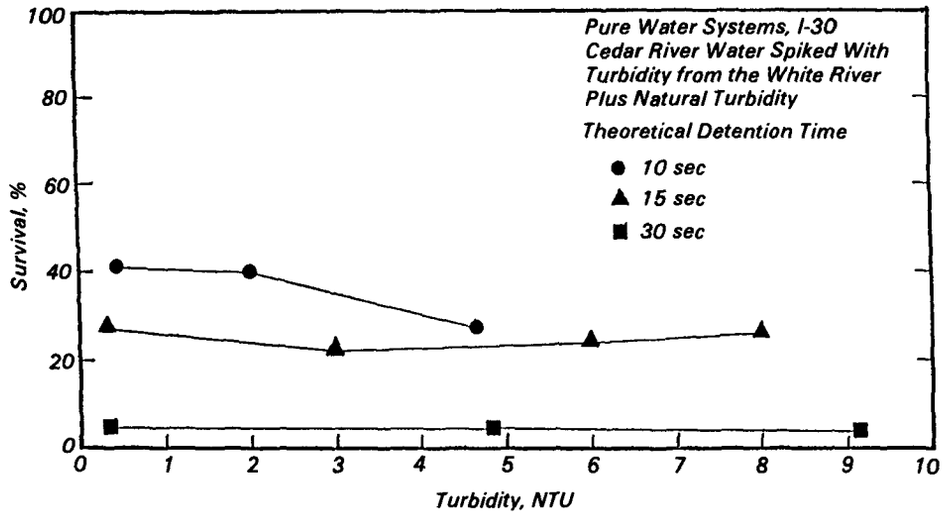

Figure 4. Effect of turbidity from White River on UV disinfection of G. muris *cysts at various theoretical detention times for the Pure Water Systems unit (with trapped gas).*

unit, 1 percent survival was attained at detention times longer than 60 sec. In contrast, detention times longer than about 22 sec were required to reach survival levels of less than 1 percent in the Ultraviolet Technology, Inc., unit.

9. Short-circuiting and collecting of air bubbles in commercial UV disinfection units can seriously impair the disinfection capability of the unit.
10. Commercial UV disinfection units could be modified to make more efficient use of the generated UV light and hence increase the feasibility of UV disinfection.
11. The information presented here indicates that the coherent UV light of the excimer laser is no more efficient in removing *Giardia* cyst viability than is the UV light from mercury-vapor tubes in terms of the energy output.
12. Laser equipment available during the experimental period was unreliable and too difficult to operate to be considered for use in field or commercial disinfection systems.
13. For all experimental conditions, color with an absorbance at 254 nm was found to increase cyst survival. For example, color caused a decrease in UV disinfection effectiveness.
14. The presence of relatively small inorganic or organic particulates (5-μm diameter or less) had no discernible effect on the UV disinfection of cysts.
15. Turbidity in the form of larger suspended particulates (>5 μm) may provide shielding and protection to the organisms.
16. Virulent *Yersinia* were less resistant to UV inactivation than *Giardia*. Hence if *Giardia* are removed by UV, *Yersinia* can be expected to have been destroyed as well.
17. Virulent *Yersinia* that contained a plasmid demonstrated significantly greater resistance to inactivation by UV than its nonplasmid counterpart.
18. UV killed *E. coli* very effectively.
19. The fact that both *Yersinia* and *Giardia* cysts were more resistant to UV than *E. coli* has important implications where the total coliform test is used to monitor microbiological water quality.
20. Size and morphological characteristics of organisms and particles appeared to be very important factors in shielding them from UV radiation.
21. Storage time and temperature affect cyst viability. The decrease in viability approximately doubles with each 10°C increase in temperature above freezing. At freezing temperatures, however, cyst viability is drastically shortened from months and days to hours.
22. Zeta potentials for *G. lamblia* cysts were time dependent, indicating a change in cyst characteristics with storage.
23. The physical stress produced by pressure and alum addition appeared to damage or even destroy cysts.

Recommendations

1. The effect of turbidity on disinfection of *Giardia* sp. needs more study. The particle size and form and its interference with UV disinfection particularly need to be investigated.
2. A more practical method of measuring and recording the actual delivered UV dose in the contactor is desperately needed.
3. Further work is needed to improve the accuracy and precision of the *G. lamblia* excystation procedure.
4. Further studies should be done to determine the effects of storage, temperature, and sedimentation on *Giardia* sp. in the water environment. These factors may significantly influence the operational mode for treatment of surface water supplies.
5. Pressure and coagulant addition may also have drastic influences on the survival of *Giardia* cysts and thus need to be investigated.
6. The design of UV contactors must eliminate or minimize short circuiting and air entrainment, and optimize reflected radiation to improve biocidal effect.

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The complete report, entitled "Ultraviolet Disinfection of Water for Small Water Supplies," (Order No. PB 85-239 960/AS; Cost: $14.50, subject to change) will be available only from:
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