

## DISINFECTION OF CIRCULATING WATER SYSTEMS BY ULTRAVIOLET LIGHT AND HALOGENATION\*

RICHARD W. GILPIN<sup>1</sup>t, SUSAN B. DILLON<sup>1</sup>, PATRICIA KEYSER<sup>1</sup>, ALICE ANDROKITES<sup>1</sup>,  
MARY BERUBE<sup>1</sup>, NICHOLA CARPENDALE<sup>1</sup>, JANE SKORINA<sup>1</sup>, JAMES HURLEY<sup>1</sup>  
and ADELE M. KAPLAN<sup>2</sup>

<sup>1</sup>Department of Microbiology and Immunology and <sup>2</sup>Department of Community and Preventive  
Medicine, The Medical College of Pennsylvania, Philadelphia, PA 19129, U.S.A.

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**Abstract**—Continuous ultraviolet light (u.v.) and chemical disinfection of circulating water systems were evaluated. Direct comparisons of the biocidal effectiveness of u.v. light vs halogenation were tested with *Legionella* spp and *Pseudomonas aeruginosa* because of their association with the acquisition of overt clinical disease from water-containing appliances. Findings indicated that six species of *Legionella* and *P. aeruginosa* were killed by a moderate level of u.v. radiation. *L. pneumophila* and other bacteria in a circulating water system were effectively killed by a biocidal u.v. light. However, free chlorine levels needed to kill *Legionella*, *Pseudomonas*, and *Flavobacterium* within 1 mm were found to be greater than 4 mg ml<sup>-1</sup>. Data from a long-term field trial with u.v. light treatment of evaporative condenser water showed a significant reduction in numbers of bacteria. Ultraviolet disinfection of hospital hydrotherapy whirlpools confirmed the utility of this mode of disinfection under circumstances where chlorination may not be practical for medical reasons. These findings were confirmed during investigations of halogenated or u.v.-treated public hot tub/whirlpools. The effectiveness of routine chemical disinfection for controlling microbial flora in a cooling tower was also evaluated. The 2 month survey indicated that the numbers of bacteria, including *Legionella*, were not affected by two biocides that were used. The observations made during this investigation support the conclusion that u.v. light disinfection of water-containing systems may be an appropriate alternative or supplement to chemical biocides.

**Key words**—circulating water, chemical biocides, cooling tower, disinfection, evaporative condenser, *Flavobacterium*, *Legionella*, *Pseudomonas*, ultraviolet light, whirlpool

### INTRODUCTION

Control of microbial flora in circulating water systems or plumbing fixtures exposed to the environment has taken on added importance since the discovery of *Legionella*, the bacteria responsible for legionellosis. Outbreaks of legionellosis epidemiologically associated with airborne transmission of *Legionella* in water aerosols exhausted from cooling towers and evaporative condensers as well as water fixtures, shower heads and respiratory devices have been reported by Arnow *et al.* (1982), Centers for Disease Control (CDC 1982, 1983 a, b), and Cordes *et al.* (1980, 1981). *Pseudomonas aeruginosa* has also been reported to cause illness among people using whirlpools according to CDC (1982, 1983a), Farmer *et al.* (1982), Jacobson *et al.* (1976), Kush *et al.* (1980), Sausker *et al.* (1978) and Washburn *et al.* (1976).

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tPresent address: R. Gilpin Limited, 2247 Falkirk Dr, Finksburg, MD 21048, USA.

Water that may contain *Legionella*, *Pseudomonas* or other bacteria is usually disinfected by chemical biocides such as halogens or quaternary ammonium compounds, according to Imperato (1981). Maintaining high levels of these biocides may effectively kill bacteria in water-containing equipment, but Haag and Gieser (1983) reported that chlorinated pool water may cause corneal irritation. According to Kush *et al.* (1980), adequate chlorine levels during the day and superchlorination at night may not be sufficient to remove *Pseudomonas* from whirlpool water if total organic levels are elevated. Findings reported by Jacobson *et al.* (1976) and Salmen *et al.* (1983) of *P. aeruginosa* illness among people using whirlpools with acceptable chlorine levels is cause for concern.

Water disinfection by continuous ultraviolet (u.v.) light is suitable in some situations if the intensity of the radiation is at least 2 mW cm<sup>-2</sup>, according to Buttolph (1955). u.v. Disinfection has been used to disinfect commercial products which cannot be treated by pasteurization or chemicals. One advantage of continuous u.v. disinfection of flowing or recirculating water is that repeated additions of chemicals may not be required. Also, u.v. disinfection does not produce residual toxic compounds, such as

the halogenated organics produced by chlorine or bromine. Therefore, u.v. disinfection may be more cost effective.

The purpose of this investigation was to determine the applicability of continuous u.v. radiation for control of bacteria in recirculating water systems. Commercially available u.v. disinfection systems were connected to recirculating water from an evaporative condenser, hospital hydrotherapy whirlpool, and three public hot tub/whirlpools. In addition, a long-term survey of *Legionella*, total bacterial counts and physico-chemical characterization of pool water in a cooling tower routinely treated with two chemical biocides was performed to further evaluate the effectiveness of current chemical disinfection methods. The implications of these findings are discussed.

## MATERIALS AND METHODS

### Bacterial strains

*L. pneumophila* serogroup 1 (Philadelphia 1 and Pontiac 1 strains); *L. bozemanii*, *L. dumoffii*, *L. gormanii*, *L. micdadei* and *L. longbeachae* serogroups 1 and 2 were obtained from Leta Orrison, Center for Infectious Diseases, Centers for Disease Control. *P. aeruginosa* was isolated from a public whirlpool. A second strain of *P. aeruginosa* was a laboratory stock culture. *Flavobacterium aquatile* was isolated from circulating water from an evaporative condenser.

### Culture conditions

*Legionella* cultures were maintained on the buffered charcoal yeast extract (BCYE) agar medium of Pasculle *et al.* (1980). Nutrient agar (NA) and brain heart infusion (BHI) agar (BBL, Cockesville, MD) were used for culture of other bacteria. Plate count agar (PCA) as specified by the American Public Health Association (APHA, 1975) was used for culture of environmental water samples. Stock cultures were stored at -60°C in broth medium containing 10% glycerol, as reported by Kronick and Gilpin (1980).

### Disinfection experiments

**Ultraviolet light.** *Legionella* or *Pseudomonas* were cultured for 3 days at 35°C in a moist air atmosphere supplemented with 2.5% CO<sub>2</sub>. Plates were flooded with 10 ml of 3 mM potassium phosphate buffer, pH 7.0, and bacteria were scraped off the agar with a bent glass rod. Ten ml culture suspensions adjusted to  $1 \times 10^7$  colony forming units (CFU ml<sup>-1</sup>) were placed in the bottom half of 100 mm dia petri dishes and exposed to u.v. radiation from a 15W, u.v. lamp (Model G15T8, General Electric Co., Cleveland, OH) at a distance of 103 cm. This produced a dose of 34 μW cm<sup>-2</sup>. Irradiance was measured at 254 nm by radiometry (Sterimeter, Model SM-600, Westinghouse Electric Corp., Bloomfield, NJ). The suspensions were mechanically rocked during irradiation to ensure even dose distribution. Samples were removed at zero time and 10–20 s intervals and kept in the dark at 4°C to prevent photoreactivation effects as described by Carson and Peterson (1975). Samples were plated within 1 h to determine viability (CFU ml<sup>-1</sup>). Percent survival at various u.v. exposure times was calculated by the following formula; CFU remaining divided by initial CFU times 100. Control cultures, not exposed to u.v. but processed similarly, retained their initial viability.

**Chlorination.** Cultures at a density of  $6 \times 10^6$  CFU ml<sup>-1</sup> were suspended in sterile, chlorine demand-free distilled water, pH 7.4, containing free chlorine levels ranging from 0 to 12 mg l<sup>-1</sup>. After incubation at 25°C for the indicated times, samples were removed, serially diluted and plated uniformly on PCA or BCYE. Chlorine levels were measured before and after each incubation. Calcium hypochlorite

(30–33% available chlorine, J. T. Baker Chemical Co., Phillipsburg, NJ) was used for these experiments. Free chlorine was assayed by the DPD method, according to the American Public Health Association (APHA, 1975).

### Circulating water systems

**Laboratory experiments.** A 6 l. circulating water system with a stainless steel-enclosed u.v. treatment apparatus (Model J-3, Ultra-Hyd, Roslyn, PA) was used. The measured u.v. output of the biocidal lamp (Model B36T6L, Westinghouse) was 7 ergs mm<sup>-2</sup> s<sup>-1</sup>, 100 cm<sup>-1</sup> at 254 nm. A pump circulated water at a flow rate of 85 l h<sup>-1</sup> from a 4 l. water reservoir. Water samples were obtained by syringe from surgical tubing between the pump outlet and the inlet to the u.v. apparatus. A laboratory stock culture of *P. aeruginosa* suspended in 9 ml of 3 mM potassium phosphate buffer, pH 7.0, to  $3 \times 10^8$  bacteria ml<sup>-1</sup> was added to the system and allowed to equilibrate for 10 min with the u.v. apparatus off. After a zero time sample was obtained, the u.v. light was turned on and samples were obtained at the indicated time intervals. The u.v. apparatus and pump were then turned off to determine the amount of bacterial growth that would occur when the u.v. apparatus was not used. After standing overnight, the circulating pump was turned on and samples were obtained at 5 and 60 min. The u.v. apparatus was then turned on and samples were taken at hourly intervals thereafter. This experiment was repeated with *P. aeruginosa* isolated from hot tub water at a public facility that used u.v. disinfection. A similar system was used for laboratory tests with *L. pneumophila*. All samples were serially diluted, plated on PCA or BCYE and incubated at 35°C for 24 or 72 h for *Legionella*.

**Evaporative condenser u.v. treatment.** A u.v. apparatus (Model J-3, Ultra-Hyd) was connected in parallel to the return side of the recirculating water line from a commercial evaporative condenser with a 38 l min<sup>-1</sup> flow reduction valve connected between the return water line and the inlet to the u.v. apparatus. The flow rate of the recirculating water was approx. 280 l min<sup>-1</sup>, but only 13% of the total flow passed through the u.v. apparatus. For 10 weeks, water samples were collected five times per week at a location before the inlet to the inactive u.v. apparatus. The u.v. system was then left on continuously for 6 weeks and samples were collected as before. CFU, water temperature and pH were measured at each sampling time.

**Hospital whirlpool u.v. treatment.** A Model J-3 u.v. apparatus was connected with 3.2 cm dia plastic tubing to a pump with a flow rate of 45 l min<sup>-1</sup>. The water inlet to the pump and the outlet from the u.v. apparatus were positioned below the operating water level of a 340 l, stainless steel hydrotherapy whirlpool. Before each patient, the whirlpool was drained, cleaned with Povadyne (Chaston Medical and Surgical Products, E. Farmingdale, NY), rinsed, and filled with fresh tap water at a temperature of 36°C. Water samples were obtained by immersion of sterile, 300 ml water sample collection bottles before and after each patient. Water samples were filtered (0.45 μm HAWP, Millipore Corp., Bedford, MA), the filters were placed on PCA and incubated. When u.v. treatment was used, the lamp was left on continuously.

**Public hot tub u.v. treatment.** Field experiments were conducted at a public hot tub facility that used a u.v. disinfection system (Model J-32, Ultra-Hyd) with a measured output of 14 ergs mm<sup>-2</sup> s<sup>-1</sup>, 100 cm<sup>-1</sup> at 254 nm. The three hot tubs were each connected to a diatomaceous earth filter system.

The hot tub aerator and auxiliary circulation systems were turned on only during periods of customer use. Tub water was changed twice each week. The tubs were disinfected with 4% hydrogen peroxide and the filters were backwashed and disinfected similarly. The tubs were then filled with fresh water and halogenated once with bromine at 2 mg l<sup>-1</sup>.

Initially, all three hot tubs were not u.v.-treated during the night. Duplicate or triplicate water samples were obtained for 2 weeks each morning when the circulation and u.v. were turned on and each night before they were turned off. All water samples were collected by immersion of sterile, screw cap bottles. Water samples were kept on ice, transported to the laboratory, serially diluted in 3 mM phosphate buffer and uniformly inoculated on PCA. Only plates with colony counts between 30-300 were counted. Also, water samples were obtained from inside the diatomaceous earth filter units and filter drains. Water samples were also obtained before and after the aeration systems were turned on. The diatomaceous earth and baffles were removed from one filter unit to evaluate the absence of a filter on bacterial counts.

*Public hot tub halogen treatment.* Water samples were obtained from a public swimming pool maintained at a chlorine level of 2 mg l<sup>-1</sup> residual. Samples were also obtained from three public hot tubs routinely maintained with bromine at a level of 2 mg l<sup>-1</sup> residual. These hot tubs had cartridge filters. Samples were collected, transported and processed as described above.

#### Cooling tower survey

*Total bacterial counts.* Pool water and interior slats of a 36 m<sup>2</sup> base area, 6 m high cooling tower were sampled at the same time and location at 2-3 day intervals for 2 months. Pool water was collected in sterile, 260 ml bottles. Sterile cotton swabs were used to sample a 30 mm<sup>2</sup> slat area that was continuously exposed to water spray and placed in 9 ml of sterile distilled water. Pool water and swab samples were serially diluted and plated uniformly within 20 min on PCA and other media. Numbers of *Legionella* were determined by concentrating 200 ml samples by filtration (0.45 µm HAWP, Millipore). The filters were thoroughly mixed in 300 µl of distilled water and 5 µl samples of each pool water concentrate were spread in a 6 mm dia well on a Teflon-coated microscope slide (Bioresearch Glass, Inc., Vineland, NJ). Fifty of an estimated 1120 fields observable with an oil immersion objective were counted. The mean number of fluorescent staining bacteria per field was used to calculate the approximate number of fluorescent bacteria ml<sup>-1</sup> of original pool water sample. Numbers of fluorescent *Legionella* were determined with direct fluorescent antibody (DFA) reagents and methods supplied by the Biological Products Division, CDC, according to the procedure of Cherry *et al.* (1978). The limit of sensitivity, 5 *Legionella* ml<sup>-1</sup> of pool water, was determined by testing authentic pool water samples that contained known numbers of *L. pneumophila*.

*Physico-chemical characteristics.* Water samples were collected daily, except weekends, from the recirculating spray water line. Each sample was tested for pH, chromate ion, organic phosphorus and conductivity using standard methods of water analysis, as described by the American Public Health Association (APHA, 1975), with commercially available reagents and test procedures (Calgon Corporation, Pittsburgh, PA). Water pH was determined electrochemically and by colorimetry with bromthymol blue color indicator. Chromate and dichromate were measured by colorimetry with diphenylcarbohydrazide in acidified water samples. Organic phosphorus was assayed by titration of acidified water samples with ammonium molybdate after interference produced by chlorine and soluble iron was removed. Conductivity was determined with a conductivity meter (Model WR 2, Calgon) and measurements were converted to total dissolved solids (mg ml<sup>-1</sup> by multiplying µmhos cm<sup>-1</sup> readings by a factor of 0.75. Pool water temperature was measured with an immersion thermometer. Data describing cooling degree days, related to average air temperature, were obtained from a National Weather Service Station located a few miles from the cooling tower.

*Routine maintenance procedures.* Corrosion and scale were controlled by daily additions of 350–1050 ml of 10% sodium dicromate solution (CL-35, Calgon) and 175-1050 ml of a solution containing 14% sodium l-hydroxy-l-diphosphonate in 1% NaOH (CL-162, Calgon), depending on water sample chemical test results. Water pH was controlled by daily additions of 5.5-9.5 kg of sodium bisulfate (C-120, Calgon), depending on colorimetric pH test results. Two biocides were added on separate days each week; 1.9 l. of 5% dodecylguanidine hydrochloride in 10% isopropanol (HD-1339, Calgon) and 1.4 kg of calcium hypochlorite. Tap water was added ("make up") and recirculating water drainage was increased ("bleed off" or "blowdown") when conductivity measurements exceeded 1500 mg ml<sup>-1</sup> total dissolved solids. If total dissolved solids fell below 900 mg ml<sup>-1</sup> "make up" and "blowdown" were reduced to maintain dissolved solids within a specified range.

#### Statistical analysis

Simple correlations of the means of the logarithm of CFU ml<sup>-1</sup> temperature and pH in the recirculating water from the evaporative condenser before and during continuous u.v. treatment were compared by the Student's t-test. Data collected from the cooling tower survey were analyzed by two-tailed Pearson correlation coefficients. Means and standard deviations for each of the eleven independent physico-chemical and biological variables were compared separately with each other to determine statistically significant relationships.

## RESULTS

#### Disinfection experiments

*Ultraviolet light.* Six *Legionella* species were killed at different rates by a moderate level, 1 µW cm<sup>-2</sup>, of u.v. radiation (Fig. 1). Times required for 50% kill ranged from 5 min for *L. longbeachae* serogroup 2, to 30 min for *L. gormanii*. *L. pneumophila* was at an intermediate time of 17 min. *P. aeruginosa* was killed at a rate similar to *L. micdadei*. Exposure times for 99% kill ranged from 33 min for *L. longbeachae* 2, to 63 min for *L. gormanii*. Times for complete kill (100%) ranged from 45 to 90 min.

*Chlorination.* The levels of free chlorine needed to kill bacteria in chlorine demand-free distilled water, pH 7.4, were established (Table 1). CFU of *Flavobacterium* and *L. pneumophila* were greater than 10 CFU ml<sup>-1</sup> after incubation for 10 min at 25°C in water containing 2 mg l<sup>-1</sup> free chlorine. All bacteria were killed after 30 min of incubation with 2 mg l<sup>-1</sup> chlorine. *P. aeruginosa* and *L. pneumophila* survived 1 min incubation in water containing 5 mg l<sup>-1</sup> free chlorine.

#### Circulating water systems

*Laboratory experiments.* The CFU of *P. aeruginosa* suspensions were reduced to below the limit of detection after 45 min (Table 2). After the system was allowed to stand overnight, the *Pseudomonas* count was 2 x 10<sup>3</sup> CFU ml<sup>-1</sup> and colonization of the surfaces of the system was observed visually. After u.v. treatment was resumed, CFU was reduced to 10 CFU ml<sup>-1</sup> within 4 h. *Pseudomonas* isolated from hot tub water produced similar results, but a greater time was required (Table 2). Overnight standing again resulted

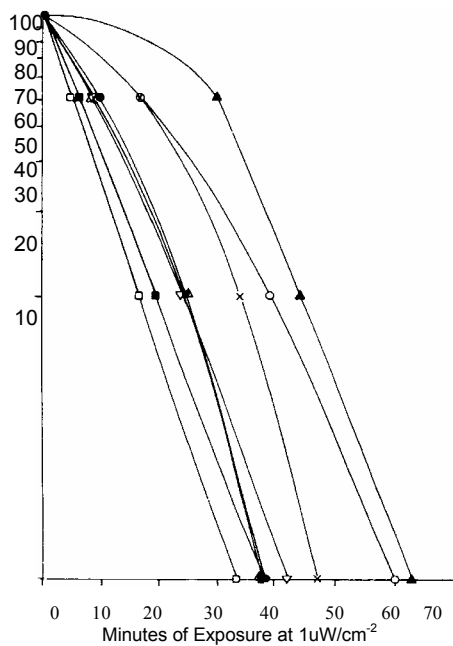


Fig. 1. Ultraviolet sensitivity of *Legionella* spp and *Pseudomonas*. Samples were irradiated as described in the text.

Each point represents the mean from three independent experiments. Time (min) of exposure at u.v. intensity of  $1 \mu\text{W cm}^{-2}$  to produce indicated percent survival of  $1 \times 10^7$  CFU  $\text{ml}^{-1}$  of bacteria. *L. bozemanii* (●), *L. dumoffli* (○), *L. gormanii* (▲), *L. micdadei* (Δ), *L. pneumophila* 1 (x), *L. longbeachae* 1 (■), *L. longbeachae* 2 (□), *P. aeruginosa* (▽).

in high numbers of bacteria which were reduced the next day to  $3 \times 10^1$  CFU  $\text{ml}^{-1}$  after 60 min of u.v. treatment. Therefore, results with both *Pseudomonas* cultures indicated that the u.v. system was effective and clearly suggest a need for continuous u.v. treatment to prevent bacterial colonization of environmental surfaces. Experiments with *L. pneumophila* in a similar, 4 l. recirculating system resulted in a 99% kill within 15 min. No viable *Legionella* could be recovered after 30 min and colonization of surfaces was not observed.

**Evaporative condenser u.v. treatment.** A rigorous field test with an undersized u.v. apparatus connected to an evaporative condenser produced significant reductions in CFU (Table 3). Water temperature and

Table 2. Laboratory survey of *P. aeruginosa* disinfection by u.v. light

Time	Lab strain	Hot tub strain
0	$1 \times 10^5$	$8 \times 10^5$
10	$1 \times 10^3$	$1 \times 10^4$
15	$1 \times 10^2$	$6 \times 10^2$
30	$3 \times 10^1$	$3 \times 10^1$
45	0	$3 \times 10^1$
60	0	$3 \times 10^1$
90	$2 \times 10^1$	$2 \times 10^1$
120	0	$2 \times 10^1$
150	0	ND
180	0	$2 \times 10^1$
210	0	$1 \times 10^1$
240	0	0
Shut Down	—	—
0	$2 \times 10^3$	$4 \times 10^4$
30	ND	$4 \times 10^1$
60	ND	$3 \times 10^1$
180	$1 \times 10^3$	$2 \times 10^2$
240	$1 \times 10^1$	ND

\*A 6 l. circulating system containing either a *P. aeruginosa* lab culture or a *P. aeruginosa* isolated from u.v.-treated hot tub water were treated by a flow-through u.v. apparatus and sampled at the indicated times as described in the text. After a 16 h shut down, sampling of u.v. treated water was resumed.

†Below the limit of detection, less than  $10^1$  CFU  $\text{ml}^{-1}$

‡Not determined.

pH fluctuated over a wide range, but neither statistically correlated with changes in CFU. No viable bacteria could be recovered from water samples obtained from the outlet of the u.v. apparatus. Since only 13% of the return water flowed through the u.v. apparatus on each pass and the condenser had a several thousand liter water volume, many passes of recirculating water would be needed to completely remove all viable bacteria.

**Hospital whirlpool u.v. treatment.** Water samples collected from circulating water before patient use without u.v. treatment had recoverable numbers of bacteria (Table 4). CFU of water samples after 20 min of patient use with the u.v. system off resulted in a  $5 \times 10^5$ -fold increase in CFU. Although bacterial counts varied considerably from patient to patient depending on the presence or absence of open, draining wounds, continuous u.v. disinfection during periods of patient use resulted in bacterial counts below the level of detection, less than  $10 \text{ CFU l}^{-1}$  in 25 of 27 experiments (Table 4).

Table 1. Chlorine killing of bacteria

Culture	Amount ( $\text{mg l}^{-1}$ ) to kill $6 \times 10^6$ CFU $\text{ml}^{-1}$ *		
	1 min	10 min	30 min
<i>F. aquatile</i>	5.0	2.5	2.0
<i>L. bozemanii</i>	ND†	2.0	ND
<i>L. pneumophila</i>	12.0	2.5	0.5
<i>P. aeruginosa</i>	7.0	2.0	1.5

\*Means of three separate experiments in which cultures were suspended in chlorine demand-free distilled water adjusted to pH 7.4 containing 0.5–12  $\text{mg l}^{-1}$  of free chlorine. Samples were incubated at  $25^\circ\text{C}$  for the indicated time. Lowest concentration of free chlorine producing no detectable colonies from 0.1 ml samples are presented.

†ND - not determined.

Table 3. u.v. Treatment of evaporative condenser water\*

	No. of samples	CFU ml <sup>-1</sup>			SD
		Mean	Low	High	
CFU <sup>-1</sup>					
Before u.v.	67	1.1 x 10 <sup>3</sup>	1.6 x 10 <sup>1</sup>	1.0 x 10 <sup>9</sup>	± 1.4 x 10 <sup>1</sup>
During u.v.	60	0.2 x 10 <sup>3</sup> †	0	2.5 x 10 <sup>9</sup>	± 0.5 x 10 <sup>1</sup>
Temperature	128	28°C	18°C	47°C	± 11°C
pH	128	8.3	7.0	9.7	± 0.8

\*Water samples were collected for 10 weeks with the u.v. system off (before) and for 6 weeks with the u.v. system on (during).

† Comparison of means of logarithms of CFU during u.v. treatment are statistically significant (P = .001) using the Student's t-test

Table 4. u.v. Disinfection of whirlpool water\*

Patients	CFU ml <sup>-1</sup>	
	Mean	Range
Before	1.5 x 10 <sup>1</sup>	5.0 x 10 <sup>0</sup> – 1.2 x 10 <sup>2</sup>
After		
Without u.v.	6.8 x 10 <sup>4</sup>	7.6 x 10 <sup>3</sup> – 9.0 x 10 <sup>5</sup>
With u.v.	0.7 x 10 <sup>0</sup>	0 – 1.0 x 10 <sup>1</sup>

\*Water samples from a freshly filled, 340 l. whirlpool before patient use and after 20 min of patient use with the u.v. system turned on or off.

tMeans of 27 separate experiments.

tStatistically significant reduction (P = 0.001, Student's t-test).

*Public hot tub u.v. treatment.* Comparisons of public hot tub nighttime and morning water samples are presented in Table 5. Generally, numbers of bacteria decreased during the day. The u.v. apparatus on one tub was not turned on for 1 day and the counts increased from 7 x 10<sup>2</sup> to 7 x 10<sup>6</sup> CFU ml<sup>-1</sup> the highest counts obtained during this investigation. Results from the initial surveys indicated that u.v. treatment for only 12 h day<sup>-1</sup> resulted in decreased CFU, but significant numbers of bacteria remained in the water. Later, u.v. treatment was used continuously

and the CFU decreased approx. 100-fold. Samples of water from diatomaceous filters and drains contained high CFU in comparison with CFU in tub water (Table 5). The aeration systems added bacteria to the water when they were turned on. Water samples taken immediately after maximum aeration showed a greater than two log increase in CFU ml<sup>-1</sup> (Table 6). Clearly, aeration systems and diatomaceous earth filters could become a source of bacterial contamination. When the filter system was removed and the hot tub was superbrominated, drained and filled with fresh water, the CFU remained near zero for 72 h with u.v. treatment system on (Table 7). Two other tubs were superbrominated and filled with fresh water, but their filter systems were filled with fresh diatomaceous earth. The subsequent CFU in these two u.v. treated tubs were one log higher than the CFU in the tub without the filter.

*Public hot tub halogen treatment.* Water samples from three brominated public hot tubs and from a chlorinated public swimming pool produced unexpected results. Swimming pool samples contained the greatest CFU and hot tub samples contained

Table 5. Survey of mean of bacterial counts in u.v.-treated hot tubs

Sample site	Viable counts for each hot tub (CFU ml <sup>-1</sup> )		
	Tub 1	Tub 2	Tub 3
Pool water*			
Morning	3 x 10 <sup>3</sup>	1 x 10 <sup>4</sup>	3 x 10 <sup>5</sup>
Evening	6 x 10 <sup>2</sup>	1 x 10 <sup>3</sup>	2 x 10 <sup>3</sup>
Filter material†	2 x 10 <sup>6</sup>	1 x 10 <sup>6</sup>	9 x 10 <sup>4</sup>
Filter drain‡	1 x 10 <sup>7</sup>	3 x 10 <sup>4</sup>	2 x 10 <sup>7</sup>

\*Pool water samples were taken in the morning when u.v. treatment was resumed and in the evening before the circulation and u.v. systems were turned off for 12 h for three weeks. See text for discussion.

†Diatomaceous earth, baffled filter unit material.

‡Filter units were kept pressurized, the drains were opened and allowed to flow for 10 s before samples were collected.

Table 6. Contribution of aeration and auxiliary circulation to bacterial counts

Auxiliary pumps*	Viable counts in pool water (CFU ml <sup>-1</sup> )	
	Experiment 1	Experiment 2
None	6 x 10 <sup>2</sup>	6 x 10 <sup>1</sup>
Aeration	3 x 10	2 x 10 <sup>4</sup>
Circulation	NDt	2 x 10 <sup>3</sup>

\*Hot tub pool water during normal circulation was sampled. The aeration pump was turned on to maximum flow for 30 s before another pool water sample was taken. The auxiliary water circulation pump was then turned on to maximum flow for 30 s before final sampling.

tND - not determined

Table 7. u.v. Treatment of hot tub water after bromination

Treatment	Tub water CFU ml <sup>-1</sup> at indicated times (h)			
	0	12	24	72
Before bromination	6 x 10 <sup>2</sup>	NA*	NA	NA
After bromination <sup>†</sup>	1 x 10 <sup>1</sup>	1 x 10 <sup>1</sup>	1 x 10 <sup>1</sup>	6 x 10 <sup>1</sup>

\*Not applicable.

<sup>†</sup>After the tub was filled with fresh water the two auxiliary pumps were turned on and the water was superbrominated to 10mg ml<sup>-1</sup> for 1 h. The water was then drained and the tub was filled with fresh water. The auxiliary pumps were adjusted to low speed, the u.v. system was turned on and water samples were then collected from the pool.

Table 8. Survey of bacterial counts in halogenated pools

Sample*	Bacterial counts (CFU ml <sup>-1</sup> )
Swimming pool	5 x 10 <sup>3</sup> 1 x 10 <sup>6</sup>
Hot tubs	
water capacity	
1900 l.	7 x 10 <sup>3</sup>
1700 l.	3 x 10 <sup>2</sup>
1200 l.	1 x 10 <sup>1</sup>

\*The public swimming pool was maintained at 2 mg l<sup>-1</sup> total chlorine residual and had a diatomaceous filter system. The hot tubs had 2 mg l<sup>-1</sup> bromine residual with cartridge filter systems.

fewer CFU (Table 8). Therefore, appropriate halogen levels were not found to be completely effective.

Bacteria cultured from u.v. or halogen treated hot tubs included several common bacteria such as *Bacillus* and various gram negative rods. Very few coliform bacteria were isolated and no *Staphylococci* or *Streptococci* were found.

#### Cooling tower survey

**Total bacterial counts.** Previous reports of total bacterial counts by England *et al.* (1982) and Kurtz *et al.* (1982) during their investigations of cooling tower biocide treatments were based on single samples or weekly samples. We obtained 27 samples throughout the summer in order to get more data points. Bacterial counts were greater on slats and less in pool water (Table 9). For purposes of comparison, slat CFU mm<sup>2</sup> data can be converted to approximate CFU ml<sup>-1</sup> volume by multiplying by a factor of 10<sup>4</sup> (1 cm<sup>3</sup> = 10<sup>4</sup> mm<sup>2</sup>). The mean for slat samples then becomes 5.7 x 10<sup>10</sup> CFU ml<sup>-1</sup> or 1.9 x 10<sup>5</sup>-fold greater than pool CFU.

Samples plated on BCYE agar or selective BCYE agar did not result in recovery of *Legionella*, although selective BYCE reduced background counts one log. Lack of *Legionella* isolation by culture was expected since the numbers of *Legionella* determined by DFA were less than the sensitivity obtainable by uniformly plating 0.1 ml water samples on agar because overgrowth by other bacteria precluded isolation of *Legionella* colonies.

The mean fluorescent-labeled *Legionella* from 25 sequential samples of pool water was 5 x 10<sup>3</sup>-fold lower than the total CFU ml<sup>-1</sup> recovered on BCYE agar, PCA or BHI agar. Twenty-eight percent of the pool water samples contained less than 5 x 10<sup>0</sup> *Legionella* ml<sup>-1</sup> the limit of detection by this concentration method. However, some slat samples contained up to 3 x 10<sup>1</sup> DFA-positive bacteria mm<sup>2</sup>, or 3 x 10<sup>5</sup> *Legionella* ml<sup>-1</sup> when converted from mm<sup>2</sup> area to cm<sup>3</sup> volume.

Bacterial species were not routinely identified during this survey of total CFU. Most samples contained gram negative rods, but a few gram positive rods, cocci, yeast, molds and actinomycetes were isolated. BCYE agar plates incubated for 10 days were usually overgrown by other bacteria or fungi. The antibiotic-containing BCYE of Bopp *et al.* (1981) or Edelstein (1981) or acid treatment of water samples according to Bopp *et al.* (1981) did not enhance *Legionella* recovery.

**Physico-chemical characteristics.** Total dissolved solids, chromate, phosphate, and pH were within expected ranges for cooling tower water (Table 9). Relationships between total CFU ml<sup>-1</sup> numbers of DFA-positive *Legionella* and physico-chemical measurements are presented in Fig. 2.

Table 9. Cooling tower physical and chemical parameters

Parameter	No. of samples	Mean	SD	Range of values
Viable bacteria				
Pool water	27	3.0 x 10 <sup>5</sup>	±3.2 x 10 <sup>5</sup>	1.6 x 10 <sup>3</sup> - 1.2 x 10 <sup>6</sup>
Slat surface	27	5.7 x 10 <sup>6</sup>	±7.5 x 10 <sup>6</sup>	1.2 x 10 <sup>4</sup> - 7.5 x 10 <sup>6</sup>
<i>Legionella</i>	25	5.9 x 10 <sup>1</sup>	±6.8 x 10 <sup>1</sup>	5.0 x 10 <sup>0</sup> - 2.2 x 10 <sup>2</sup>
Degree days	62	11.2	±5.7	0 - 19
Pool temp.	22	30.0°C	±1.7°C	27 - 34°C
Diss. solids	41	1.2 g ml <sup>-1</sup>	±240 mg ml <sup>-1</sup>	900 - 1760 mg ml <sup>-1</sup>
Chromate	41	2.4 ug ml <sup>-1</sup>	±0.8 pg ml <sup>-1</sup>	1.0 - 4.5 ug ml <sup>-1</sup>
Phosphate	41	28.1 pg ml <sup>-1</sup>	±8.8 pg ml <sup>-1</sup>	9.0 - 45 pg ml <sup>-1</sup>
pH: Titration	41	7.1	±0.2	6.7 - 7.3
Potentiometric	26	7.0	±0.2	6.6 - 7.6

Pool water plate counts are presented as CFU ml<sup>-1</sup>, slat counts are presented as CFU mm<sup>2</sup> DFA positive *Legionella* are presented as number ml<sup>-1</sup> See text for description of other parameters.

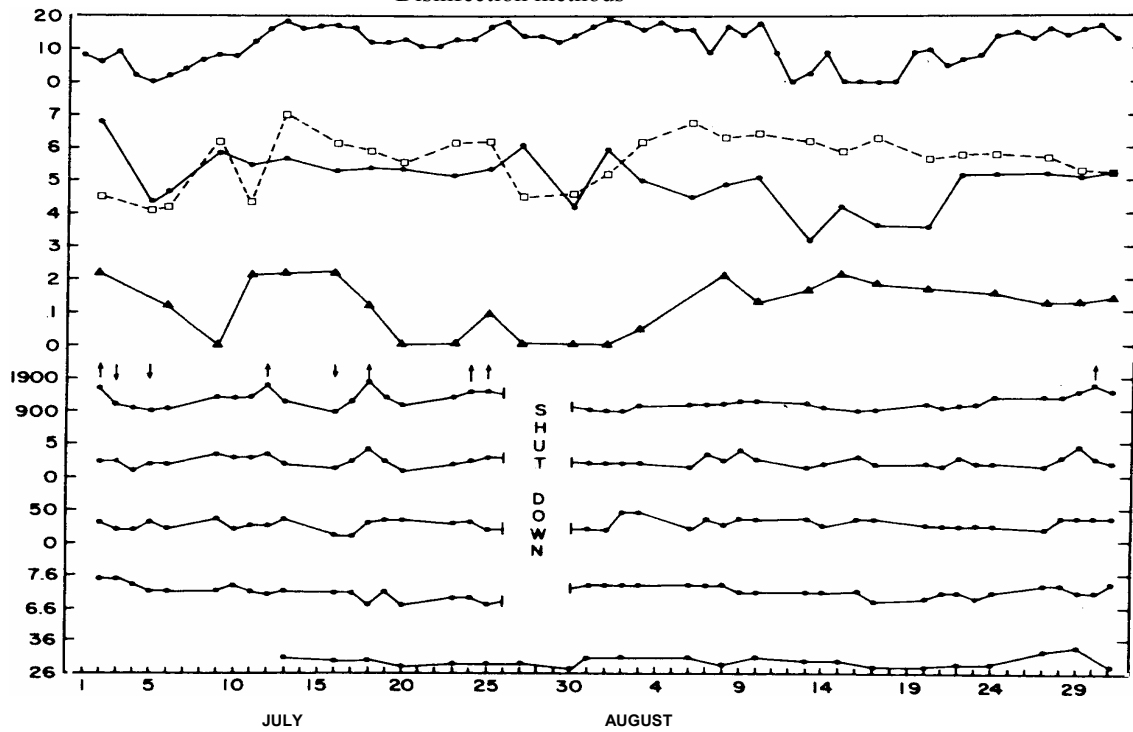


Fig. 2. Biological, chemical and physical parameters of a large cooling tower. Samples were tested on the indicated days during the summer months. Cooling degree days (Degree d) were calculated from a base temperature of 18°C. Viable counts of bacteria from (●) pool water (CFU ml<sup>-1</sup> and (□) slats (CFU mm<sup>-2</sup>); DFA-positive bacteria (▲) in pool water (number ml<sup>-1</sup>) dissolved solids (mg ml<sup>-1</sup>) calculated by multiplying conductivity measurements µmho cm<sup>-2</sup> by a factor of 0.75; chromate and phosphate concentrations are µg/ml. Temperature is °C. Arrows indicate increased (↑) or decreased (↓) blowdown.

The two exhaust fans and water circulation were turned off for repairs during a 5 day period, but bacterial counts were not affected. Biocide treatments were not plotted since the same quantities were added each time. Free chlorine levels ranged from 0.5 to 1.0mg l<sup>-1</sup> up to 2 h after addition of calcium hypochlorite. Free or combined chlorine was not detected after this time.

Two-tailed Pearson correlation coefficients between each of the eleven independent variables are presented in Table 10. Positive correlations were found between cooling degree days vs pool CFU ( $P = 0.02$ ) and slat CFU ( $P = 0.05$ ), indicating that CFU increased with air temperature. However, pool and slat CFU did not correlate with pool water temperature. Also chromate ion

correlated with total dissolved solids ( $P = 0.002$ ). It is of interest that a corrosion and scale inhibitor, CL- 162, correlated with slat CFU ( $P = 0.01$ ). When more CL-162 was added to the system, slat CFU increased. The converse was also found to be statistically significant. However, the other corrosion and scale inhibitor, CL-35, and the two biocides did not affect pool water or slat CFU.

DISCUSSION

The purpose of this investigation was to compare biocidal u.v. treatment with chemical biocides. In addition, a cooling tower routinely treated with

Table 10. Statistical correlations among eleven parameters

Parameters	CL-162	C-120	CL-35	Pool temp.	pH	Phos.	Chrom.	Diss. sol.	<i>L. pneumo</i>	Slat CFU	Pool CFU
Deg. days	0.22	-0.03	-0.10	0.32	0.14	-0.14	0.05	0.22	-0.16	0.38*	0.44*
Pool CFU	-0.37	-0.20	0.09	0.24	0.14	-0.24	0.28	0.34	-0.21	-0.21	
Slat CFU	0.51*	0.29	-0.04	0.31	-0.13	0.28	0.03	0.07	0.22		
<i>L. pneumo</i> .	-0.33	0.27	-0.10	0.19	0.23	-0.07	-0.09	0.05			
Diss. sol.	0.23	-0.27	-0.18	0.17	-0.09	0.11	0.61**				
Chromate	0.18	0.21	0.11	0.33	-0.19	0.16					
Phosphate	0.18	0.25	-0.24	-0.02	-0.06						
PH	-0.42*	-0.16	-0.04	0.43							
Pool temp.	-0.10	0.22	-0.02								
CL-35	-0.14	0.31									
C-120	0.15										

\*Significant correlations for two tailed Pearson correlation coefficients at  $P \leq 0.05$ .

\*\*Significant correlations at  $P \leq 0.01$ . See text for description.

chemical biocides was surveyed to determine whether these treatments or other physico-chemical variables affected either total bacterial counts or numbers of *Legionella*.

#### *Disinfection experiments*

**Ultraviolet light.** Data from laboratory experiments indicated that moderate u.v. levels disinfected water contaminated with *Legionella* or *Pseudomonas*. Individual species of *Legionella* had a 10% survival rate that ranged from 17 to 44 min at a dose of  $1 \mu\text{W cm}^{-2}$ . The strains from every species of *Legionella* tested were reduced from  $1 \times 10^8$  CFU to less than  $1 \times 10^1$  CFU after 65 min. An exposure time of 15 min at a dose of  $1 \mu\text{W cm}^{-2}$  was reported by Antopol and Ellner (1979) to produce a 90% kill of *L. pneumophila*. An exposure time of 34 min was found during this investigation. However, Antopol and Ellner cultured their *Legionella* on chocolate agar and exposed it to u.v. light in distilled water. Therefore, our increased 90% kill times may be the result of different experimental conditions. Zelle and Hollaender (1955) reported 90% kill times with a dose of  $1 \mu\text{W cm}^{-2}$  ranging from 14–40 min for *Serratia marcescens* to 92 min for *Pseudomonas aeruginosa*. *P. aeruginosa* tested during this investigation was more sensitive to u.v. radiation, 90% kill in 28 min. Although our irradiated *Pseudomonas* samples were held on ice in the dark after u.v. exposure, different strains may have variable u.v. sensitivities dependent on their ability to repair u.v.-induced damage. However, Carson and Peterson (1975) reported that *Pseudomonas cepacia* exposed to u.v. radiation in the absence of light showed no evidence of dark repair mechanisms.

**Chlorination.** *Pseudomonas* and other cultures used for u.v. treatment experiments were tested with various levels of chlorine to facilitate comparisons with biocidal u.v. treatment. *Flavobacterium*, *L. pneumophila*, *L. bozemanii* and *P. aeruginosa* were killed by a recommended level of  $2.5 \text{ mg l}^{-1}$  chlorine when exposed for at least 10 min at room temperature. However, shorter exposure times or higher water temperatures required greater levels of chlorination.

#### *Circulating water systems*

**Laboratory experiments.** A report by Fredette (1963) on u.v. disinfection of a circulating water system with an enclosed, low-pressure, quartz-shielded u.v. water treatment apparatus indicated that  $10^6$  CFU  $\text{ml}^{-1}$  of *P. aeruginosa* were reduced two logs after three passes. Our laboratory experiments produced similar results,  $4.5 \times 10^5$  CFU  $\text{ml}^{-1}$  of *Legionella* or *Pseudomonas* were reduced by nearly five logs after ten passes.

**Evaporative condenser u.v. treatment.** An undersized u.v. apparatus produced a statistically significant reduction in CFU  $\text{ml}^{-1}$ . This decrease was striking in view of the fact that only 13% of the return water was treated on each pass. Tests with a properly

sized u.v. apparatus are needed to confirm the use of u.v. treatment for evaporative condensers.

**Hospital whirlpool u.v. treatment.** Current recommendations by the Centers for Disease Control (1981) for chemical disinfection of hot tub and whirlpool water specify maintenance of free chlorine levels, measured hourly, in a range from 1.5 to  $3.0 \text{ mg l}^{-1}$  during operation, followed by superchlorination at the end of the use period. According to Hopkins *et al.* (1981) when these indications are not maintained, bacterial counts may increase and whirlpool users may become infected.

Also, halogen disinfectants may cause irritation among users if pools are located in small, inadequately ventilated rooms. Although this problem may not always apply to outdoor swimming pools, swimming pool chlorination was reported by Mustchin and Pickering (1979) to cause bronchial hyperreactivity known commonly as "coughing water". In light of these considerations, data from our experiments with u.v. treated hospital whirlpool water suggests that bacterial contamination may be controlled without the need for halogenation.

**Public hot tub u.v. treatment.** Investigations of public hot tubs produced novel results. As illustrated by our laboratory experiments, bacteria adhering to surfaces could multiply to high numbers when the circulation and u.v. apparatus were turned off. The CFU found in hot tub water 15 h after no u.v. treatment approximated the numbers reported for u.v. treated pools by De Jonckheere (1982). Therefore, continuous u.v. treatment of circulating hot tub water was necessary. Results obtained from our investigation of intermittently u.v. treated public hot tubs agree with those of De Jonckheere (1982), who found bacterial counts to be slightly greater in pools treated with u.v. instead of halogens. Continuous u.v. treatment, however, produced the opposite result. De Jonckheere did not present the mechanical details of the u.v. systems used on the two pools that were tested. Therefore direct comparisons are not possible.

**Public hot tub halogen treatment.** The public hot tubs maintained at  $2 \text{ mg l}^{-1}$  bromine residual contained appreciable numbers of bacteria. De Jonckheere (1982) reported high bacterial counts in filters used to clear the water. Therefore, we conducted experiments to determine why there were reported differences in CFU between u.v. and chemically treated pools. The CFU in u.v. treated hot tubs seemed to be dependent on several factors: (1) hours of u.v. treatment per day; (2) frequency and duration of aeration; (3) frequency and duration of auxiliary circulating pump use; and (4) type of filtration system.

The auxiliary aeration and auxiliary circulation systems, which may be off for prolonged periods of time, may contribute bacteria to the circulating water when they are turned on because halogen and/or u.v. treatments may not effectively kill bacteria that colonize standing water and surfaces in these areas. It

Is often assumed that halogen treatment will effectively eliminate this problem. However, the dramatic increase in cases of *Pseudomonas* folliculitis reported over the past two years by the Centers for Disease Control (1982, 1983a, b) suggests a need for reevaluation of this assumption. Also, further investigations of diatomaceous earth and cartridge filter systems are needed to determine the best way to prevent establishment of bacterial reservoirs in the filters. For example, we found that u.v. disinfection of public hot tub water was more effective when the diatomaceous earth filter system was removed.

It was of interest that *P. aeruginosa* was often isolated from water of both halogen and u.v. treated hot tubs. This species adapts to warm water conditions and this characteristic is used to differentiate this species from other *Pseudomonas* spp in diagnostic microbiology laboratories, according to Hugh and Gilardi (1980).

#### *Cooling tower survey*

**Total bacterial count.** A large cooling tower was surveyed for an extended period of time to determine whether any of the eleven independent variables statistically correlated with each other. Also, bacterial CFU on environmental surfaces were compared to those in pool water to determine whether bacterial surface colonization should be considered during investigations involving disinfection of cooling towers. To our knowledge, this was the first long-term, frequent survey of a routinely maintained cooling tower. Because calcium hypochlorite and a quaternary biocide did not statistically correlate with reductions in bacterial CFU in pool water or on slats, further controlled disinfection studies of commercial cooling towers may be appropriate. Since the numbers of bacteria, including *Legionella*, on slats were greater than those in pool water, investigations of cooling towers should include sampling of environmental surfaces in addition to pool water.

Fliermans *et al.* (1981), using single or short-term sampling of cooling tower water, found a range of less than 9 up to  $3 \times 10^5$  *Legionella* ml<sup>-1</sup> of pool water. The numbers found during our survey more closely approximate those reported by Fliermans *et al.* (1979) for non-epidemic habitats such as streams. *Legionella* have been isolated from thermally polluted habitats at temperatures ranging from 40 to 60°C, according to Fliermans *et al.* (1981) and Wadowsky *et al.* (1982). A report by Fliermans *et al.* (1981) indicated that pool water temperature was the only factor that correlated with quantity of *Legionella*. Since pool water temperatures during our investigation fluctuated over a narrow range from 28 to 34°C we may have encountered suboptimal growth conditions for *Legionella*. However, we did find a correlation between cooling degree days (average air temperature) and bacterial CFU. Since the slats were constantly exposed to circulating air because bacterial growth at this location

could be directly affected by air temperature.

Bacterial CFU on slats were statistically correlated with the use of CL-162, a scale and corrosion inhibitor. One speculation for this finding is that the chemical affected bacterial slat counts by either promoting growth or by removing growth-inhibiting material from slat surfaces. The lack of statistical correlations between biocide treatment and CFU was not expected. Grace *et al.* (1981), Kurtz *et al.* (1982), Skaliy *et al.* (1980) and Soracco *et al.* (1983) reported that several chemical biocides effectively killed *Legionella* under laboratory conditions. However, Washburn *et al.* (1976) and Winn *et al.* (1982) reported that these biocides may not eliminate *Legionella* from cooling towers. According to Fliermans (1982), most cooling towers contain *Legionella* so the important question is whether *Legionella* are growing to high numbers. The results of the present investigations suggest that monitoring total CFU, possibly *Legionella*, on slats may be a useful method for monitoring effectiveness of cooling tower disinfectants.

In conclusion, data obtained during the present investigations suggest that u.v. disinfection can effectively reduce or eliminate bacteria in circulating water systems and that chemical biocide treatment of cooling towers may not produce statistically significant reductions in CFU. Also, recommended levels of chemical biocides for whirlpools/hot tubs and cooling towers did not consistently reduce bacteria to recommended densities. As with chemical biocides, the overall effectiveness of u.v. treatment depends on the physical and environmental characteristics of each circulating water system.

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