EFFICACY OF BIOCIDES IN CONTROLLING MICROBIAL POPULATIONS, INCLUDING *LEGIONELLA*, IN COOLING SYSTEMS

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ABSTRACT

Ten cooling towers were tested to determine possible correlations between Legionella *numbers and physical, chemical, biological, and operational parameters. Significant correlations were found between total bacteria and* Legionella *levels as measured by direct fluorescent antibody techniques. Five biocides were tested for efficacy in controlling* Legionella *and other microbial populations in four towers. The results showed that most biocides were ineffective at manufacturers' suggested dosages. Only frequent, high doses of organic biocides or low-level continuous treatment with an oxidizing biocide showed promise for long-term control of microorganisms in the towers tested.*

INTRODUCTION

Cooling systems, including open recirculating cooling towers and evaporative condensers, are known to harbor microorganisms both in the bulk water and on surfaces. These microorganisms cause a variety of problems, including loss of heat transfer efficiency and deterioration of system components. These systems have also been implicated in the growth and dissemination of pathogenic microorganisms, particularly those belonging to the genus *Legionella* (Howland and Pope 1983). Early work implied that aerosols from various heat rejection units (cooling towers, evaporative condensers) were responsible for many of the confirmed cases of legionellosis. In one instance it was felt that the role of the aerosol was demonstrated in an outbreak of legionellosis in a hospital in Tennessee (Dondero et al. 1980). The plume from an adjacent tower could have reached the air intakes of the patients' rooms. However, in another case that implicated a cooling tower in an outbreak in Rochester, New York, it was found that the water distribution system of the facility was contaminated with *L. pneumophila* (Nolte et al. 1984). Many hospital outbreaks of legionellosis have been traced back to the water distribution system (see Muraca et al. [1988] for details), and the role of cooling towers in outbreaks has become much less clear. Other cases have further implicated water distribution systems (States et al. 1987; Stout et al. 1985) and their role in contaminating shower heads (Bollin et al. 1985), hot water tanks (Fields et al. 1989), and potable water supplies (Tison and Seidler 1983).

Several investigators have studied the efficacy of biocides in controlling microorganisms, including *Legionella*, in cooling tower environments. Soracco et al. (1983) demonstrated that many commercially available biocides kill *Legionella* and many of them kill the algae capable of supporting *Legionella* growth (Tison et al. 1980) when tested in a laboratory environment. Braun (1982) and Soracco and Pope (1983) demonstrated, however, that some of these same biocides are not as effective in "real world" cooling systems.

Fliermans and Harvey (1984) suggested that bromine-containing biocides might not be as effective as chlorine in controlling *Legionella* in open cooling systems. Later work by McCoy and Wireman (1989) found that bromochlorodimethylhydantoin (BCDMH as 1.0 ppm chlorine) was 99.9% effective against *L. pneumophila* in industrial cooling water. Muraca et al. (1988) state that biocides have been found to be ineffective in "eradicating" *Legionella* from cooling towers and are "only marginally effective in reducing organism numbers." The literature is divided regarding the case for chlorine, especially in potable water systems. In certain instances, continuous chlorination (1.0 to 1.5 mg/L) and shock chlorination ($\geq$ 50 mg/L) were found to be ineffective (Muraca 1988). Studies by Baird et al. (1984) and Massanari et al. (1984) found that hyperchlorination (i.e., greater than 2.0 mg/L) successfully controlled *Legionella*. Although chlorine has been found to be effective in some cases, the above literature suggests that chlorine may be contraindicated in some industrial environments.

Pope et al. (1984) demonstrated that ozone, if properly applied, could effect control of microbial populations, including *Legionella*, in a variety of cooling systems. The degree to which general microbial communities in the cooling system can, or should be, controlled is an open question. Most investigators would contend that maintaining a cooling system in a sterile condition is impractical and uneconomical. Instead, the target is to keep the microorganisms under control and to limit their effects on operation of the system. The situation with respect to

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Legionella control is little different. Some investigators (Fliermans and Nygren 1987) have used fluorescent antibody methods to measure *Legionella* populations in such devices and have proposed a practical "working level" of 10,000 *Legionella pneumophila* per milliliter, above which serious consideration should be given to cleaning and chemically disinfecting the facility. Other investigators (Morris and Shelton 1990) have used culture methods and have suggested that levels of viable (culturable) *Legionella* in the range of 10 to 100 per milliliter might be cause for enhanced treatment.

The objectives of the two-phase study reported here were to

1. determine whether the levels of *Legionella* in cooling systems could be statistically correlated with other cooling system parameters and
2. test the ability of several commercially available biocides to control microorganisms, including *Legionella*, in cooling systems.

TABLE 1
Cooling Systems Sampled in First Quarter

Site Number and Tower Designation	Manufacturer and Model	Type	Location	Volume (gal)
1 - CIIE	BAC CFT 2427 FCR	Open	Outside shelter, roof	7000
2 - CIIW	BAC CFT 2427 FCR	Open	Outside shelter, roof	7000
3 - Cogs	Marley 222-621	Open	Outside, roof	*
4 - CC	BAC VCT 375 CR	Open	Outside, ground	7000
5 - Union	Marley 7128	Open	Outside, roof	*
6 - Wvl	Marley 8202	Open	Outside, ground	*
7 - Lib1	BAC VS1-100-1	Closed	Inside, top floor	1100
8 - Lib2	BAC VS1-100-1	Closed	Inside, top floor	1100
9 - JEC1	BAC VS1-200-3	Closed	Inside, ground floor	1100
10 - JEC2	BAC VS1-200-3	Closed	Inside, ground floor	1100

*Towers were not used after the initial sampling; additional information was not obtained.

MATERIALS AND METHODS

Cooling Systems Studied

The objective of phase I was to determine if significant correlations between *Legionella* levels and other biological and operational parameters existed. In this phase, 10 sites were sampled. These are listed in Table 1, along with system characteristics. As can be seen, the sites included open cooling towers and evaporative condensers, some of which operated as pairs (two identical units operating in the same facility). All operated on the same make-up water (city of Troy, New York) and all had been on similar chemical treatments (using the same biocides and corrosion inhibitors) for more than 10 years. A wide range of system parameters (e.g., volume, temperatures) were represented.

Make-up volumes were recorded from meters on the individual units. Event recorders were installed on all towers used in phase II in which biocides were tested. These were to be used with blowdown rates to determine blowdown volumes over time. Unfortunately, these rarely gave accurate readings due to faulty operation of the towers (e.g., dirt and leaves can clog blowdown valves).

It should be emphasized that all other routine chemical treatments (corrosion/scale inhibitors) continued in the towers during all phases of the study. Biocides that were in use by the host facility during the study were replaced only for the duration of the testing. There were approximately three cycles of concentration (i.e., the number of times the solids in a particular volume of water are concentrated [Drew 1979]) in systems tested.

Microbiological Analyses

Water samples were obtained from the towers, and temperature, pH, and total dissolved solids (i.e., by conductivity) were determined on site using portable probes. Aliquots of each sample were processed on site using serial decimal dilution to an endpoint to determine the numbers of viable bacteria. Acid-producing (APB; facultative anaerobes and anaerobes that produce organic acids) and sulfate-reducing bacteria (SRB) were enumerated in this fashion using commercially available, prepared fluid media (proprietary media formulations; see Table 3). The remainder of the sample was returned to the laboratory within 30 minutes of collection. Dilutions of the samples were performed in autoclaved and filter-sterilized deionized water, and these were plated onto standard plate count agar to obtain the total viable count. Plating was also done to obtain total viable *Pseudomonas* spp. counts.

Legionella analysis was done on both unconcentrated and concentrated samples. Concentration was achieved by filtering aliquots (up to 100 mL) of the samples onto polycarbonate membrane filters with a pore size of 0.2 μm. Aliquots of these concentrates were treated with acid and/or heat according to the procedures outlined by Dennis (1988). These samples were plated onto selective and nonselective *Legionella* agar plates (i.e., buffered charcoal yeast extract (BCYE) with and without antibiotics [APHA 1989]). Media from several sources were used and compared.

Aliquots of each sample were incubated with INT dye (2-[4-iodophenyl]-3-[4-nitrophenyl]-5-phenyltetrazolium chloride) and respiratory substrates for one hour at room temperature in an attempt to determine the numbers of viable cells in the total microbial and presumptive *Legionella* populations. Details of these procedures have been described by Fliermans et al. (1981). Total microscopic counts were done by spotting concentrated, unconcentrated, and diluted aliquots of the samples incubated with INT onto toxoplasmosis slides and then air drying, heat fixing, and staining them with fluorescein isothiocyanate (FITC). The microbial cells were enumerated using an epifluorescence microscope at 1000× magnification. The number of INT-positive cells was determined by counting the percentage of cells having internal formazan deposits characteristic of cells having active respiratory activity (sufficient to reduce the INT to formazan in the cell) at the time of sampling. Separate aliquots of each sample were prepared as above and stained using FITC-labeled polyvalent antisera (i.e., direct fluorescent antibody method or DFA) to *Legionella*. Positive and negative controls were included with each set of samples. These slides were viewed (using the epifluorescence microscope) to enumerate the numbers of DFA-positive cells with morphological characteristics consistent with designation as *Legionella*. The numbers of DFA-positive cells having a positive INT reaction were also determined.

It should be noted that indigenous populations of *Legionella* were used since inoculating the cooling towers with *Legionella* was forbidden by the contract. The disadvantage of this approach was realized prior to initiation of the work: If phase I work showed only low levels of *Legionella* in the systems under consideration, documentation of biocide kill of *Legionella* (specifically) would be unlikely.

Surface samples for the enumeration of surface-associated microorganisms (SAM) were obtained by swabbing 4 cm^2 of the interior wetted surface of the cooling tower. This was processed into a slurry in sterile diluting solution and treated as for water samples.

Algal components were enumerated by direct microscopic observation using both brightfield and epifluorescence (to observe red fluorescence of chlorophyll-containing organisms). Very few algae were observed in the samples. Those found were identified as blue-green bacteria (cyanobacteria) using morphological and color characteristics.

Enumeration of protozoans was attempted using several methods. Direct microscopic observation, enrichment in tubes containing samples plus nutrient agar blocks, and enrichment on plates inoculated with bacteria as the food source were all tried with little success. Protozoans in a control sample from a local pond were easily observed by all three methods.

Chemical Analyses

Aliquots of each sample were provided to a water chemistry laboratory. All samples were preserved and analyzed for the chemical parameters given in Table 2, according to methods outlined in APHA (1989).

Methods for measuring biocide residuals were available only for bromochlorodimethylhydantoin (BCDMH). This was measured as free chlorine residual, immediately after sampling, using the *N,N*-diethyl-*p*-phenylenediamine method (DPD). A list of the biocides used and the treatment levels, as ppm product, is given in Table 4.

RESULTS

Phase I Correlation of *Legionella* Populations with Cooling Tower Parameters

The phase I effort attempted to correlate the chemical, physical, and biological parameters for the various towers to the levels of *Legionella* in these towers. Table 2 gives the raw data for all chemical and operational parameters measured throughout the first eight months of the study. Table 3 presents the biological data. It should be noted that 10 towers were sampled. This is six more than were to be studied in phase II (biocide testing). The other towers were included in order to broaden the data base and assist in attempts to find correlations between operational parameters and *Legionella* levels, even though it was known that the additional towers would be shut down in the fall and consequently that only a few samples would be obtained from these towers.

The ranges for most parameters were quite broad and therefore gave a data base that should yield reasonable correlations, if such exist, between *Legionella* levels and other chemical or biological parameters. The exception was pH, which ranged only from 8.0 to 8.9. Many other parameters had reasonably wide ranges but only at quite low absolute values. This was true for nitrate, nitrite, ammonia, and iron. Algae (n up to 45) only had one positive sample and protozoans were not recovered (n = 37). Since there was a reasonable sample size in each case ($n > 27$), low correlations with these parameters indicate that their influence on the *Legionella* populations was minimal. Statistical analyses were performed using two statistical packages. Pearson pairwise correlation coefficients and stepwise regression analysis using *Legionella*-DFA (*Legionella* by DFA method) numbers as the dependent variable all revealed good correlations between total bacterial levels (FITC) and *Legionella*-DFA levels (r = 0.770). This was true for both water and SAM samples. The level of iron in the water correlated positively with total bacterial levels in the water (r = 0.630). Excellent correlations were found between those chemical parameters that are expected to correlate well, e.g., calcium, magnesium, hardness, and alkalinity (r = 0.860 through 0.995). A positive correlation was seen between nitrite levels and *Legionella*-DFA levels (r = 0.510; with FITC, r = 0.720). However, as discussed above, the values for nitrite were all quite low and thus the interrelationship must be considered with caution. Interestingly, there seemed to be little effect of temperature on the *Legionella*-DFA levels (r = −0.1980).

When a comparison of surface and water (or planktonic) samples was made, the correlations among the

TABLE 2
Chemical and Operational Parameters for Cooling Towers during Phase I Tests

Date	Month	Site	Label	Temp C	pH	TDS mg/L	Ortho Phosphate ug/L	Total Phosphate ug/L	Nitrate mg/L	Nitrite mg/L	Ammonia mg/L	Cl mg/L	Ca mg/L
10/16/89	2	1	CIIE	20	8.8	280	360	120	0.65	0.01	0.01	46	74
11/21/89	3	1	CIIE	23	8.6	220	155	1250	0.32	0.02	0.01	22.5	38
12/18/89	4	1	CIIE	18	8.9	270	28	1900	1.36	0.02	0.01	39	53
1/29/90	5	1	CIIE	22	8.4	250	265	3120	1.2	0	0.01	35	59.7
3/12/90	6	1	CIIE	20	8.2	290	215	2100	2.39	0.01	0.2		67
4/6/90	7	1	CIIE	40	8	300	380						
5/11/90	8	1	CIIE	32	8.1	280							
10/16/89	2	2	CIIW	20	8.8	280	360	3370	1.1	0.01	0.01	46	69
11/21/89	3	2	CIIW	23	8.6	220	155	1250	0.32	0.02	0.01	22.5	38
12/18/89	4	2	CIIW	18	8.9	270	28	1900	1.36	0.02	0.01	39	53
1/29/90	5	2	CIIW	22	8.4	250	265	3120	1.2	0	0.01	35	59.7
3/12/90	6	2	CIIW	20	8.2	290	215	2100	2.39	0.01	0.2		67
4/6/90	7	2	CIIW	40	8	300	380						
5/11/90	8	2	CIIW	32	8.1	280							
10/16/89	2	3	Cogs	23	8.7	210	105	120	0.65	0.01	0.01	45	74
10/16/89	2	3	Cogs Sediment										
10/16/89	2	3	Cogs Scale										
9/22/89	1	4	CC	28	8.6	290	180	200	0.32	0.03	0.01		63.2
10/16/89	2	4	CC	26	8.8	390	280	1890	2.2	0.01	0.01	75	126
10/16/89	2	5	Union	17	8.8	400	400	465	2.36	0.02	0.01	77.5	111
9/22/89	1	6	Wvl	27	8.6	260	190	200	1.24	0.01	0.01		87.8
10/16/89	2	6	Wvl	14	8.5	300	320	745	0.38	0.08	0.01	22.5	106
9/22/89	1	7	Lid1	26.5	8.6	110	1	105	0.21	0.06	0.03		34.3
10/16/89	2	7	Lib1	27	8.6	100	8	40	0.23	0.01	0.1	22.5	51
11/21/89	3	7	Lib1	28	8.8	270	21	600	0.2	0.01	0.02	40	52
12/18/89	4	7	Lib1	19	8.4	280	1	20	0.2	0.01	0.01	8	41
9/22/89	1	8	Lib2	26.5	8.5	180	89	200	0.26	0.08	0.02		49.8
10/16/89	2	8	Lib2	23	8.2	240	420	4500	1.13	0.5	0.01	87.5	96.5
11/21/89	3	8	Lib2	16	8.6	220	130	2750	0.9	0.31		37	43.5
12/18/89	4	8	Lib2	17	8.4	320	19	2900	0.9	0.4	2.08	36	21
9/22/89	1	9	JEC1	28	8.4	240	47	200	0.67	0.05	0.01		96.7
10/16/89	2	9	JEC1	27	8.7	400	99	4500	2.02	0.01		77.5	107.5
11/21/89	3	9	JEC1	9	8.8	230	134	2700	0.5	0.08	0.23	42.5	64.5
12/18/89	4	9	JEC1	20	8.9	150	0	14550	0.5	0.08	0.01	16	25
1/29/90	5	9	JEC1	23	8.4	430	48	2430	2.2	0.05	0.02	56	96.7
3/12/90	6	9	JEC1	29	8.3	110	20	300	0.7	0	0.03		20
4/6/90	7	9	JEC1	25	8.5	300							
5/11/90	8	9	JEC1	28	8.4	150							
9/22/89	1	10	JEC2	28	8.4	190	2	27	0.15	0.04	0.01		26.5
10/16/89	2	10	JEC2	27	8.6	110	4	140	0.21	0.01	0.01	22.5	37
11/21/89	3	10	JEC2	23	8.9	260	1	8	0.38	0.02	0.01	15	26.5
12/18/89	4	10	JEC2	22	8.8	110	28	10	0.38	0.03	0.07	13	17
1/29/90	5	10	JEC2	14	8.7	80	5	10	0.5	0.02	0.02	13	21
3/12/90	6	10	JEC2	18	8.3	100	1	20	0.6	0.13	0.02		22
4/6/90	7	10	JEC2	22	8.4	75							

TABLE 2 (continued)
Chemical and Operational Parameters for Cooling Towers during Phase I Tests

Date	Month	Site	Label	Mg mg/L	Fe mg/L	Total Alkalinity mg/L	Hardness mg/L	TSS mg/L	Residue mg/L	Volatile Solids mg/L	Water Meter gal	Blow-down hr
10/16/89	2	1	CIIE	11.5	0.61	127	202.2	8	370	14	7749100	
11/21/89	3	1	CIIE	6	0.61	75	119.6	4	390	190	8038800	
12/18/89	4	1	CIIE	9.6	0.68	.112	171.9	13	800	360	8174200	
1/29/90	5	1	CIIE	10	0.95	106	190.3	4	220	140	8412100	22.75
3/12/90	6	1	CIIE		0.81	118		2	600	280	8673000	39.4
4/6/90	7	1	CIIE			96			310	70	8830900	52.6
5/11/90	8	1	CIIE									77.2
10/16/89	2	2	CIIW	11.5	0.61	126	219.7	24	430	11	7749100	
11/21/89	3	2	CIIW	6	0.61	75	119.6	4	390	190	8038800	
12/18/89	4	2	CIIW	9.6	0.68	112	171.9	13	800	360	8174200	
1/29/90	5	2	CIIW	10	0.95	106	190.3	4	220	140	8412100	22.75
3/12/90	6	2	CIIW		0.81	118		2	600	280	8673000	39.4
4/6/90	7	2	CIIW			96			310	70	8830900	52.6
5/11/90	8	2	CIIW									77.2
10/16/89	2	3	Cogs	11.5	0.05	141	232.1	10	460	17		
10/16/89	2	3	Cogs Sediment									
10/16/89	2	3	Cogs Scale									
9/22/89	1	4	CC	17.2	0.27	192	228.6	15	1044		4160270	
10/16/89	2	4	CC	18	0.05	218	388.7	8	690	25	556960	
10/16/89	2	5	Union	17	0.41	218	347.2	54		24		
9/22/89	1	6	Wvl	16.4	0.17	246	286.8	16	508		7184540	
10/16/89	2	6	Wvl	22.5	0.04	218	357.3	14	570	14		
9/22/89	1	7	Lid1	6.8	0.04	84	113.6	4	92		3396690	
10/16/89	2	7	Lib1	6	0.03	64	152.1	18	160	4	4304350	
11/21/89	3	7	Lib1	8.5	0.06	98	164.8	3	580	320	4451420	
12/18/89	4	7	Lib1	8.4	0.96	42	137	6	1360	360	4473460	
9/22/89	1	8	Lib2	8.6	0.08	168	159.8	2	504		5162280	
10/16/89	2	8	Lib2	15	1.17	196	302.7	16	600	10		
11/21/89	3	8	Lib2	7.5	0.47	102	139.5	2	500	330	7188367	
12/18/89	4	8	Lib2	3.6	0.07	98	67.3	8	520	240	7188100	
9/22/89	1	9	JEC1	14.6	0.07	172	301.6	8	592		53785	
10/16/89	2	9	JEC1	17.5	0.05	198	340.5	4	160	6	3419060	
11/21/89	3	9	JEC1	10	0.08	134	197.2	0	500	140	3465445	
12/18/89	4	9	JEC1	4.8	0.08	66	82.2	3	360	240	3472870	
1/29/90	5	9	JEC1	15.8	0.1	95	306.5	9	360	240	3532159	10.75
3/12/90	6	9	JEC1		0.04	6		2	220	120	3639281	72.8
4/6/90	7	9	JEC1			84			370	110	3965149	86.7
5/11/90	8	9	JEC1								3789690	110.2
9/22/89	1	10	JEC2	5	0.04	60	86.8	2.5	188		627264	
10/16/89	2	10	JEC2	5.5	0.01	60	115	560	10	10	5291110	
11/21/89	3	10	JEC2	4	0.03	44	82.6	0	340	190	5454568	
12/18/89	4	10	JEC2	3.6	0.07	42	57.3	1	560	480	5471055	
1/29/90	5	10	JEC2	3.4	0.11	40	66.4	2	60	60	5505768	1.7
3/12/90	6	10	JEC2		0.03	42		4	260	20	5560476	3.6
4/6/90	7	10	JEC2			58			150	30	5604106	7.1

TABLE 3

Biological Parameters for Cooling Towers during Phase I Tests

Date	Month	Site	Label	APB per ml	SRB per ml	Plate Count per ml	Pseudocel per ml	FITC per ml	LDB/FA per ml	INT per ml	Algae per ml	Protozoa per ml
				WATER SAMPLES								
10/16/89	2	1	CIIE	1.0E+02	1.0E+01	1.1E+04	1.8E+03	1.9E+06	1.3E+05		0	0
11/21/89	3	1	CIIE	1.0E+02	1.0E+01	4.8E+03	4.3E+02	3.2E+04	3.0E+04		0	0
12/18/89	4	1	CIIE	1.0E+03	1.0E+02	9.3E+03	2.0E+00		1.5E+02		0	0
1/29/90	5	1	CIIE	1.0E+02	1.0E+01	5.0E+03	0.0E+00		2.5E+03		0	0
3/12/90	6	1	CIIE	1.0E+01	0.0E+00	1.1E+05	2.0E+00		1.0E+02		0	0
4/6/90	7	1	CIIE	1.0E+02	0.0E+00	1.3E+05	0.0E+00		2.5E+02		0	0
5/11/90	8	1	CIIE	1.0E+02	0.0E+00	6.3E+04	1.0E+01				0	0
10/16/89	2	2	CIIW	1.0E+02	0.0E+00	2.1E+04	2.4E+01	2.4E+06	4.8E+04		0	0
11/21/89	3	2	CIIW	1.0E+02	1.0E+01	4.8E+03	4.3E+02	3.2E+04	3.0E+04		0	0
12/18/89	4	2	CIIW	1.0E+03	1.0E+02	9.3E+03	2.0E+00		1.5E+02		0	0
1/29/90	5	2	CIIW	1.0E+02	1.0E+01	5.0E+03	0.0E+00		2.5E+03		0	0
3/12/90	6	2	CIIW	1.0E+01	0.0E+00	1.1E+05	2.0E+00		1.0E+02		0	0
4/6/90	7	2	CIIW	1.0E+02	0.0E+00	1.3E+05	0.0E+00		2.5E+02		0	0
5/11/90	8	2	CIIW	1.0E+02	0.0E+00	6.3E+04	1.0E+01				0	0
10/16/89	2	3	Cogs	1.0E+02	1.0E+01	1.0E+04	7.0E+00	1.6E+06	4.0E+04		0	0
10/16/89	2	3	Cogs Sediment								0	0
10/16/89	2	3	Cogs Scale								0	0
9/22/89	1	4	CC	1.0E+02	1.0E+02	4.2E+03	4.2E+03	4.2E+03	4.2E+03	4.20E+03	0	0
10/16/89	2	4	CC	1.0E+02	0.0E+00	1.0E+04	6.0E+02	9.6E+05	2.0E+04		0	0
10/16/89	2	5	Union	1.0E+02	0.0E+00	9.2E+03	5.4E+02	1.3E+06	1.6E+04		0	0
9/22/89	1	6	Wvl	1.0E+03	1.0E+05	3.3E+02	2.2E+01	3.5E+05	1.0E+03	1.00E+01	0	0
10/16/89	2	6	Wvl	1.0E+02	0.0E+00	5.0E+04	1.0E+02	1.6E+06	8.0E+03		0	0
9/22/89	1	7	Lid1	1.0E+02	1.0E+01	1.3E+04	1.3E+01	5.0E+04	4.0E+03		0	0
10/16/89	2	7	Lib1	1.0E+01	0.0E+00	1.4E+03	6.3E+01	8.0E+05	4.0E+04		0	0
11/21/89	3	7	Lib1	1.0E+01	0.0E+00	2.4E+03	2.5E+01	2.6E+04	3.6E+03		0	0
12/18/89	4	7	Lib1	0.0E+00	0.0E+00	8.0E+01	0.0E+00		1.0E+02		0	0
9/22/89	1	8	Lib2	1.0E+02	1.0E+02	1.0E+06	1.1E+01	1.4E+06	1.6E+04	1.00E+01	0	0
10/16/89	2	8	Lib2	1.0E+02	0.0E+00	5.3E+04	2.2E+03	9.6E+06	1.2E+05		0	0
11/21/89	3	8	Lib2	1.0E+01	1.0E+01	8.8E+03	7.0E+01	4.6E+04	4.6E+04		0	0
12/18/89	4	8	Lib2	1.0E+02	1.0E+01	1.0E+04	2.0E+01				0	0
9/22/89	1	9	JEC1	1.0E+03	1.0E+03	6.2E+03	1.0E+00	5.9E+05	1.0E+03	1.00E+01	0.1	0
10/16/89	2	9	JEC1	1.0E+01	0.0E+00	1.1E+04	1.0E+00	1.3E+06	2.4E+04		0	0
11/21/89	3	9	JEC1	1.0E+02	1.0E+02	8.3E+02	1.0E+01	2.6E+04	5.9E+04		0	0
12/18/89	4	9	JEC1	1.0E+02	1.0E+01	5.4E+03	0.0E+00		3.0E+02		0	0
1/29/90	5	9	JEC1	0.0E+00	0.0E+00	3.2E+04	0.0E+00		1.0E+02		0	0
3/12/90	6	9	JEC1	1.0E+02	0.0E+00	2.5E+03	0.0E+00		1.3E+03		0	0
4/6/90	7	9	JEC1	1.0E+02	1.0E+02	1.3E+04	0.0E+00		2.2E+04		0	0
5/11/90	8	9	JEC1	1.0E+01	1.0E+01	7.7E+04	0.0E+00				0	0
9/22/89	1	10	JEC2	1.0E+01	1.0E+04	1.2E+03	2.0E+00	9.6E+05	1.0E+03	2.30E+01	0	0
10/16/89	2	10	JEC2	1.0E+03	0.0E+00	1.6E+03	3.0E+03	1.6E+06	2.0E+04		0	0
11/21/89	3	10	JEC2	1.0E+01	1.0E+01	1.2E+04	1.0E+01		4.8E+03		0	0
12/18/89	4	10	JEC2	1.0E+01	1.0E+01	5.6E+03	0.0E+00		5.0E+01		0	0
1/29/90	5	10	JEC2	1.0E+01	0.0E+00	1.5E+04	0.0E+00		0.0E+00		0	0
3/12/90	6	10	JEC2	1.0E+05	1.0E+02	5.7E+05	0.0E+00		5.0E+01		0	0
4/6/90	7	10	JEC2	1.0E+03	1.0E+03	2.0E+03	0.0E+00		1.0E+02		0	0

TABLE 3 (continued)
Biological Parameters for Cooling Towers during Phase I Tests

Date	Month	Site	Label	SURFACE SAMPLES								
				APB per cm2	SRB per cm2	Plate Count per cm2	Pseudocel per cm2	FITC per cm2	LBD/FA per cm2	INT per cm2	Algae per cm2	Protozoa per cm2
10/16/89	2	1	CIIE	1.0E+05	1.0E+04	1.8E+04	2.5E+01	1.9E+07	2.2E+05		0	0
11/21/89	3	1	CIIE								0	0
12/18/89	4	1	CIIE	1.0E+03	1.0E+02	4.4E+04	0.0E+00		1.5E+02		0	0
1/29/90	5	1	CIIE	1.0E+03	1.0E+03		0.0E+00		5.5E+02		0	0
3/12/90	6	1	CIIE								0	0
4/6/90	7	1	CIIE	1.0E+06	1.0E+02	7.3E+05	0.0E+00		3.0E+02		0	0
5/11/90	8	1	CIIE								0	0
10/16/89	2	2	CIIW	1.0E+04	1.0E+03	1.4E+04	1.0E+01	1.3E+07	4.0E+04		0	0
11/21/89	3	2	CIIW								0	0
12/18/89	4	2	CIIW	1.0E+04	1.0E+04	1.4E+04	0.0E+00		5.0E+01		0	0
1/29/90	5	2	CIIW	1.0E+03	1.0E+03	1.5E+04	0.0E+00		2.6E+03		0	0
3/12/90	6	2	CIIW								0	0
4/6/90	7	2	CIIW	1.0E+03	1.0E+03	9.3E+04	0.0E+00		3.5E+02		0	0
5/11/90	8	2	CIIW								0	0
10/16/89	2	3	Cogs	1.0E+04	0.0E+00	7.3E+03	1.0E+01	2.7E+06	3.3E+04		0	0
10/16/89	2	3	Cogs Sedim	1.0E+05	1.0E+05	1.0E+05		1.6E+07	2.4E+06		0	0
10/16/89	2	3	Cogs Scale	1.0E+05	1.0E+05	2.0E+04	1.0E+01	6.7E+06	1.5E+05		0	0
9/22/89	1	4	CC	0.0E+00		1.2E+05	1.0E+00	4.2E+06	1.3E+05		0	0
10/16/89	2	4	CC	1.0E+03	0.0E+00	5.2E+03	1.0E+01	9.6E+05	2.0E+04		0	0
10/16/89	2	5	Union								0	0
9/22/89	1	6	Wvl			3.3E+03	0.0E+00	2.3E+06	2.7E+04		0	0
10/16/89	2	6	Wvl	1.0E+02	0.0E+00	1.8E+04	1.0E+01	2.1E+06	1.5E+05		0	0
9/22/89	1	7	Lid1	1.0E+02	1.0E+03	2.5E+04	1.0E+00	2.7E+06	1.3E+05		0	0
10/16/89	2	7	Lib1	1.0E+02	1.0E+02	6.7E+04	4.7E+03	3.3E+07	8.7E+05		0	0
11/21/89	3	7	Lib1	0.0E+00	0.0E+00	1.2E+05	1.0E+02	1.4E+05	8.0E+03		0	0
12/18/89	4	7	Lib1	0.0E+00	0.0E+00	7.6E+04	0.0E+00		1.5E+02		0	0
9/22/89	1	8	Lib2	0.0E+00	0.0E+00	7.0E+02	0.0E+00	3.2E+05	7.2E+03		0	0
10/16/89	2	8	Lib2	1.0E+02	1.0E+02	2.4E+04	1.0E+01	6.7E+07	2.7E+06		0	0
11/21/89	3	8	Lib2	1.0E+02	1.0E+02	1.4E+04	1.0E+02	2.0E+05	7.2E+04		0	0
12/18/89	4	8	Lib2	1.0E+02	0.0E+00	2.0E+04	0.0E+00				0	0
9/22/89	1	9	JEC1								0	0
10/16/89	2	9	JEC1	0.0E+00	0.0E+00	8.4E+02	1.0E+01	1.3E+07	6.0E+05		0	0
11/21/89	3	9	JEC1	1.0E+02	1.0E+02	1.7E+03	1.0E+02	6.0E+04	2.4E+04		0	0
12/18/89	4	9	JEC1	1.0E+03	1.0E+02	5.4E+04	0.0E+00		6.0E+02		0	0
1/29/90	5	9	JEC1	1.0E+02	1.0E+02	3.8E+05	0.0E+00		0.0E+00		0	0
3/12/90	6	9	JEC1	1.0E+03	1.0E+02	1.0E+05	0.0E+00		6.3E+03		0	0
4/6/90	7	9	JEC1	1.0E+02	0.0E+00	6.2E+04	0.0E+00		1.2E+04		0	0
5/11/90	8	9	JEC1									
9/22/89	1	10	JEC2			1.1E+04	0.0E+00	1.9E+05	7.2E+03			
10/16/89	2	10	JEC2	1.0E+02	1.0E+02	2.5E+03	3.2E+03	9.3E+11	3.0E+05			
11/21/89	3	10	JEC2	1.0E+02	1.0E+02	1.0E+02	1.0E+02		4.8E+04			
12/18/89	4	10	JEC2	1.0E+04	1.0E+04	1.0E+04	0.0E+00		5.0E+01			
1/29/90	5	10	JEC2	1.0E+04	1.0E+03	1.0E+04	0.0E+00		0.0E+00			
3/12/90	6	10	JEC2	1.0E+03	1.0E+02	1.0E+03	0.0E+00		5.0E+03			
4/6/90	7	10	JEC2	1.0E+05	1.0E+04	1.0E+05	0.0E+00		9.0E+03			

TABLE 4
Biocides Tested in Phase II

Biocide	Concentration of Product (ppm)
Disodium cyanodithioimidocarbonate + potassium N-methyldithiocarbamate	35-454
Polymeric quaternary amine	80
Isothiazolin	150
Glutaraldehyde	150
Bromochlorodimethylhydantoin	0.1-10

various measured populations (FITC total count, plate count, pseudomonads) were generally low ($r < 0.4$). The surface and waterborne populations of *Legionella* by DFA showed a reasonably good correlation ($r = 0.798$). There was no clear trend regarding the size of the surface and planktonic populations. For example, surface-associated and planktonic *Legionella*-DFA values were dominant an equal amount of the time.

One important conclusion from the study was that each tower is essentially unique. This is even true for the two pairs of towers that were included in this study. It should also be remembered that all of the towers in this study had been using the same water source and chemical treatments for at least the last 10 years. The consequence of this is that "lumping" the data from all the towers together, although useful in trying to find general correlations between *Legionella* and other parameters, can obscure the relationships seen when individual towers are thoroughly examined and tested over fairly long time periods (weeks to months). Examples of this are shown in Figures 1 and 2. As can be seen in Figure 1, the JEC 1 tower, which operates all year, had fairly constant levels of viable (plate count) bacteria throughout the eight months, including the winter. The levels of *Legionella*-DFA in this tower decreased in the colder months and the level of iron was fairly constant at 0.1 ppm. Figure 2 demonstrates that a tower that operates all year, but in which the fans are not operated during cooler months and there are high levels of cyanobacteria (blue-green bacteria) only in the warmer months, had a quite different picture. The levels of plate-count (viable) bacteria were fairly constant throughout the first five months and then increased in the spring, whereas the *Legionella*-DFA lagged behind this increase in total viable cells. This is probably due to the fact that this was the only tower having significant growth of blue-green bacteria. The organisms in these towers grow principally in the form of "mats" on the sides of the tower. Only when the weather gets warm enough for the fans to be operated do the blue-green bacteria grow well. As the authors and colleagues have demonstrated (Tison et al. 1980), cyanobacteria often support the growth of *Legionella*. Therefore, the summer increase in *Legionella* in this tower (the tail end of which was seen in September and October) is probably related to the accumulation of blue-green bacteria and not to the total viable bacteria counts. It should also be noted that the levels of iron in this tower are essentially constant but are almost 10 times the concentration seen in the tower shown in Figure 1. In spite of this, the levels of *Legionella*-DFA are similar in the two towers.

Other than the significant accumulation of cyanobacteria in the CII tower, algae were actually detected only one other time in the JEC tower. It must be remembered that these towers were on chemical biocide treatments and receiving city make-up water (treated with chlorine) throughout this test period.

Attempts to culture viable *Legionella* met with little success, despite the fact that positive controls behaved as predicted. One set of samples was collected according to specifications provided by a laboratory and split, with one portion of the sample sent to the laboratory for analysis and the other being analyzed according to the methods normally employed by the university's team. The same lot number of environmental BCYE agar was used by both laboratories. The university's tests gave no culturable *Legionella*, while the laboratory recovered 30 *Legionella* per mL from the CC tower, 1 isolate from a JEC tower, and 2 *Legionella* isolates each from the CII and MRC towers. The *Legionella* belonged to four different serogroups (1, 4, 6, and CDC #SH2156), and each tower had only one type of *Legionella*. Possible explanations for these results are that the laboratory's personnel are better at recovering *Legionella* than the university's personnel, or the differences arose from the fact that the university's personnel processed samples within 1 hour of collection while the laboratory's personnel processed samples up to 48 hours after sample collection (according to their normal procedures). The low recovery rates for culturable *Legionella* presented a problem in the sense that it was not possible to perform classic "kill curves" for *Legionella* using viable *Legionella* counts. (It should be pointed out, however, that contract guidelines would have made such tests impossible, since towers having numbers of *Legionella* high enough for kill tests would exceed the number at which the towers must be immediately treated.) Accordingly, kill tests done in phase II of this study relied on total viable microbial numbers to determine the potential of biocides to control microorganisms. Use of microscopic counts to determine kill kinetics is possible, as will be seen, only after long-term treatment, with continued measurements of viable bacteria, total microbial cells, and *Legionella*-DFA counts. This is due to the fact that incoming make-up water is very low in *Legionella* and other bacteria. Therefore, if a biocide is capable of controlling microbes in the tower, the level of total cells present (living and dead) will, in the absence of growth in the tower and significant scrubbing of bacteria from the air, *approach* the level of bacterial cells

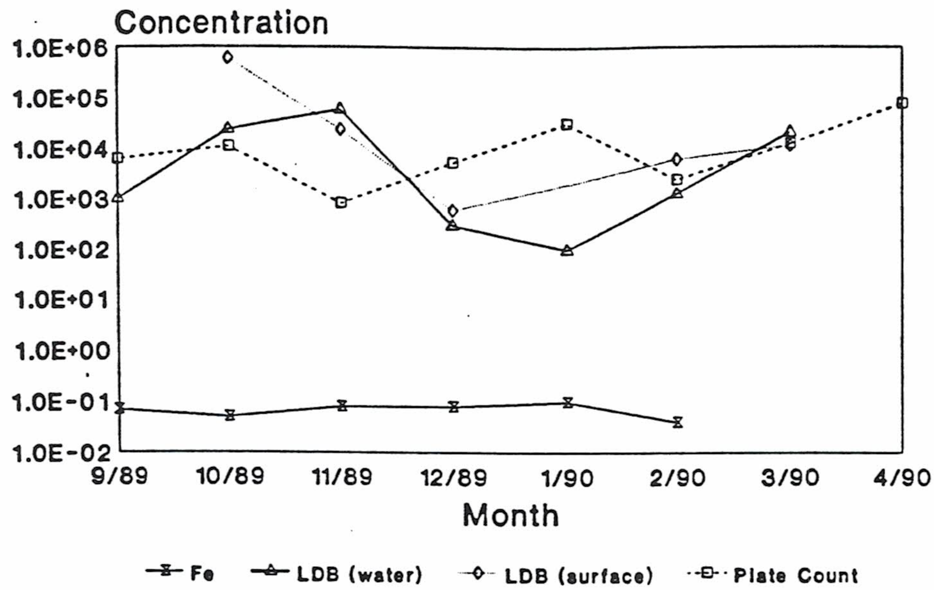

Figure 1 *Example of data for* Legionella-*DFA, plate count (viable) bacteria and iron levels in a cooling tower (JEC1) during phase I of the test program. This tower operates all year; operation during the winter is as a heat pump. A critical point is that the fans on the tower operate year-round. Note the drop in* Legionella-*DFA during the winter months. (Fe = ppm; LDB [Legionnaires' disease bacteria] = mL for water, cm² for surface, mL for plate count.)*

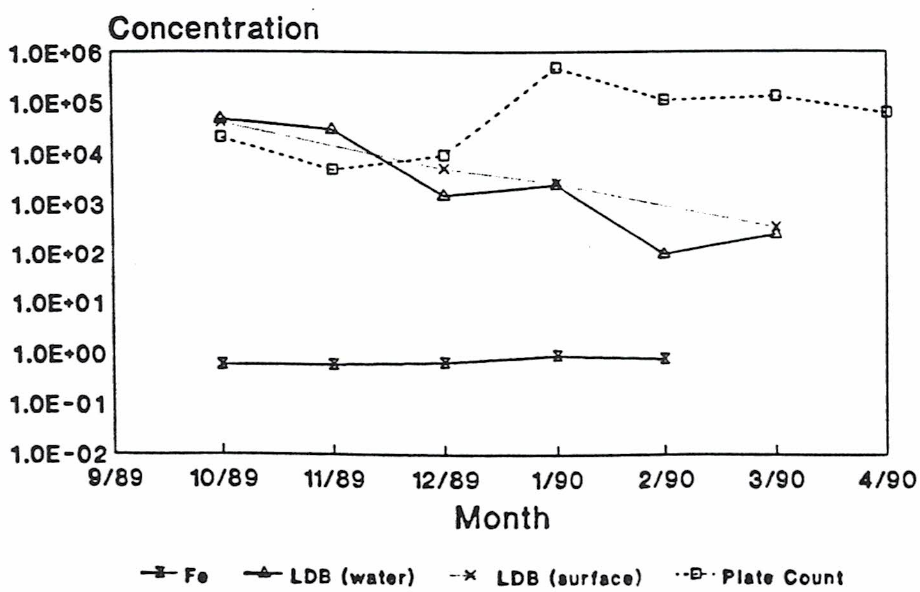

Figure 2 *Example of data as in Figure 1, except that this cooling tower operates all year but without fans during the cool periods of the year (CIIW). This tower supports considerable algal growth in the warm months. Note that the* Legionella-*DFA levels were high during September and October 1989 (late summer and fall) but lagged behind increases in total bacterial levels in the late winter (due to lack of algal regrowth during this period). (Fe = ppm; LDB [Legionnaires' disease bacteria] = mL for water, cm² for surface, and mL for plate count.)*

in the make-up water. Consideration must be given to the cycles of concentration for the tower in question. Failure to at least approach these make-up concentrations of bacteria may indicate that the biocide is not controlling microbial growth in the tower.

Phase II—Tests of Biocide Efficacy

A list of the biocides and dosages used is shown in Table 4. All tests were started by turning off the blowdown, taking a zero time sample, dosing with biocide, and restarting the tower. The blowdown was left off for two hours and restarted after the two-hour sampling. Additional samples were generally taken at 6, 24, 48, and 72 hours after the addition of biocide. In a few cases, samples could not be obtained due to tower malfunction. Make-up water (Troy, New York, city water) was sampled at 0, 24, 48, and 72 hours. The results for isothiazolin will be used to illustrate several points.

The results for the other biocides will be given in an abbreviated form. The data in Figure 3a show the results for isothiazolin treatment in the four test towers. It is obvious that viable bacteria declined over a 24- to 48-hour period and then began to rise to pretreatment levels. Make-up water levels were consistently very low, indicating that most of the viable bacteria in the tower came from air washing and/or growth in the tower. Figure 3b gives the data for total microscopic counts (i.e., total microscopic count = live + dead + culturable + nonculturable bacterial cells). It is clear that these results do not follow those for the viable counts. (The drop in the MRC system was due to a blowdown valve stuck in the open position, which resulted in washout of the microorganisms to *near* make-up water levels for a short period.) The reason for the difference in viable and total counts is explained by the fact that the viable counts represent only that portion of the total counts that was culturable with the methods used at the time of sampling. The total population not only takes into account the contribution of bacteria made by the water but also will include the scrubbing of bacteria from the air passing through the tower. A similar explanation applies to the *Legionella*-DFA results shown in Figure 3c. Note that the MRC tower was again in the washout mode. Figure 3d shows the results for *Pseudomonas* spp. While the data are relatively uniform for most of the towers, a spike in CII at 72 hours may have occurred. Since blooms of *Pseudomonas* would create safety, slime accumulation, and corrosion concerns, it is important to avoid treatments that kill the "normal flora" in the towers while allowing pseudomonads to bloom.

Figure 4 shows the viable count data for the cyanocarbonate/carbamate biocide, which has been used in the towers for the last 10 or more years. It is obvious that it has little effect on the viable counts.

The data in Figure 5 are for a polymeric quaternary amine biocide treatment. It appears generally ineffective in CC and CII towers. The MRC tower was on continuous blowdown due to a stuck valve. After treatment, the viable bacteria in the JEC tower appeared to decline for the duration of the sampling period. This may have been partially due to operational problems with the tower, but the drop in this tower, relative to the other test towers, remains unexplained. In general, however, the data suggest that the polymeric quaternary amine did not work very well at the dose levels employed.

Figure 6 shows the data for glutaraldehyde. The viable bacteria levels fell off in most towers over a 6- to 24-hour period with rapid recovery and return to pretreatment levels in 24 to 72 hours. Viable bacteria in the MRC tower did not return to pretreatment levels due to the faulty control device being in the continuous blowdown mode between 24 and 48 hours.

The data for the bromochlorodimethylhydantoin (BCDMH) test in the CII tower are presented in Figure 7. The biocide residuals are also plotted. It is clear that once the biocide residual rose above about 0.2 ppm free chlorine, the levels of total viable microorganisms rapidly decreased. As the concentration of biocide dropped, the total population rapidly recovered. The MRC tower was also tested, with similar results (data not shown). It should be noted that these towers were chosen for BCDMH testing since they have sumps that allowed buckets containing biocide tablets to be placed in the tower system. It is believed that the good kill over relatively long time periods was due to maintenance of biocide residuals in the tower over these same periods. The fact that levels of viable bacteria rapidly recovered as biocide decreased supports this view.

CONCLUSIONS

The results of the phase I testing to determine correlations between *Legionella*-DFA levels and other parameters can be summarized as follows:

1. Good correlation was found between *Legionella*-DFA levels and total population count, which was performed by the FITC method.
2. Aqueous iron levels correlated well with the total population count as determined by FITC.
3. Nitrite correlated well with the surface *Legionella*-DFA values, but the level of correlation was less with the planktonic *Legionella*-DFA populations.
4. As expected, water quality parameters such as calcium, magnesium, hardness, and alkalinity showed a high degree of correlation.
5. There was little relationship between *Legionella*-DFA levels and temperature.
6. Other parameters tested in the survey gave poor correlations and their effect on the levels of total viable microorganisms and the level of *Legionella*-DFA may be minimal at these sites.

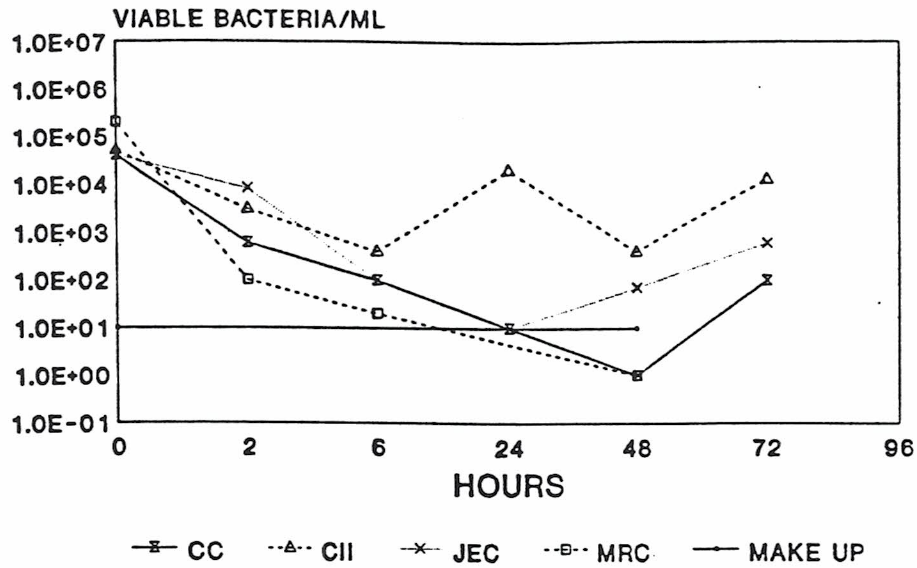

Figure 3a *Effect of isothiazolin treatment on total plate count (viable bacteria) populations in four cooling towers. Biocide was added immediately after collecting the t = 0 samples. Blowdown was left "off" until after the two-hour sampling was completed. For details, please refer to "Materials and Methods" section.*

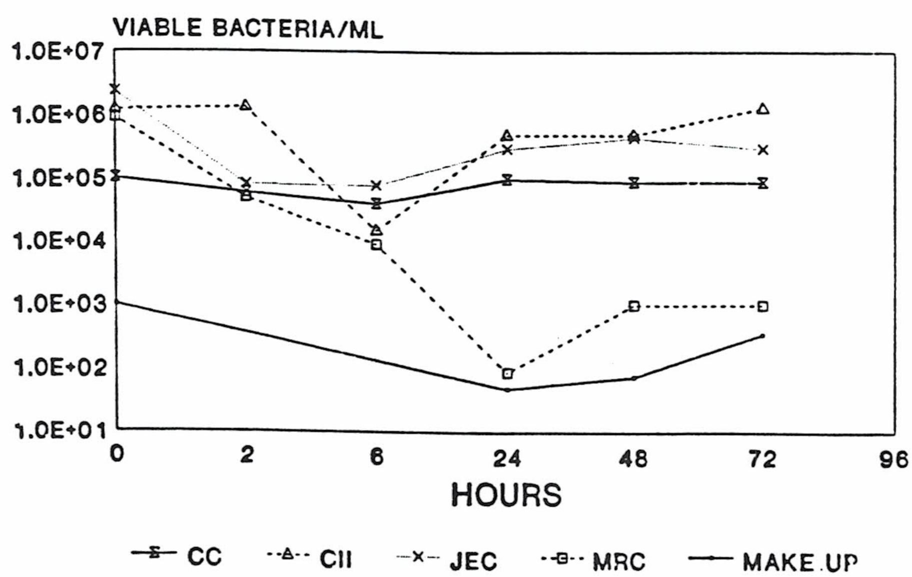

Figure 3b *Effect of isothiazolin treatment on total microscopic count. It should be noted that this procedure measures live + dead + culturable + nonculturable microorganisms. The decrease in the MRC tower is due in part to a malfunctioning blowdown valve. Note that, in general, the levels of total bacteria do not change very much through the study.*

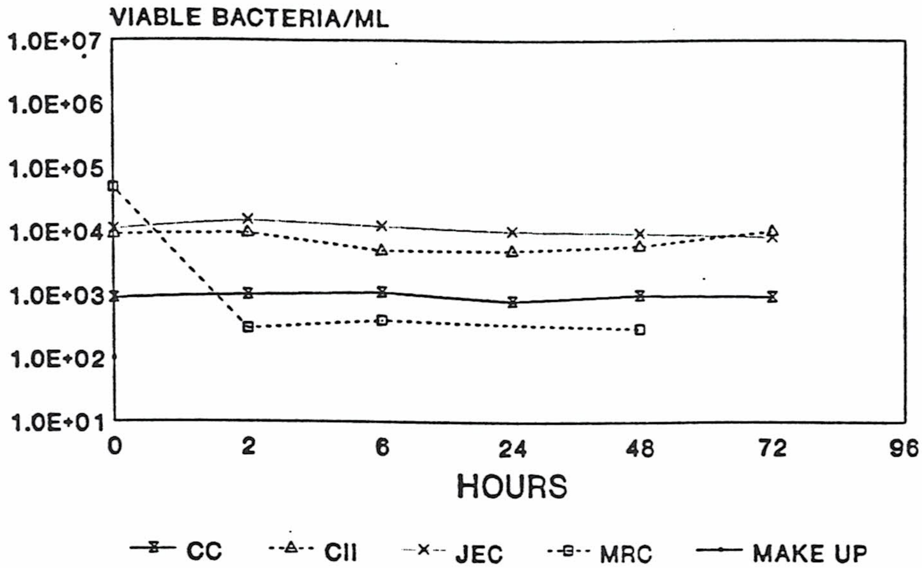

Figure 3c *Effect of isothiazolin treatment on* Legionella-*DFA, that is, the level of cells, whether live or dead, reacting specifically with fluorescent antibodies to* Legionella *sp. See "Materials and Methods" section for details of analytical procedures.*

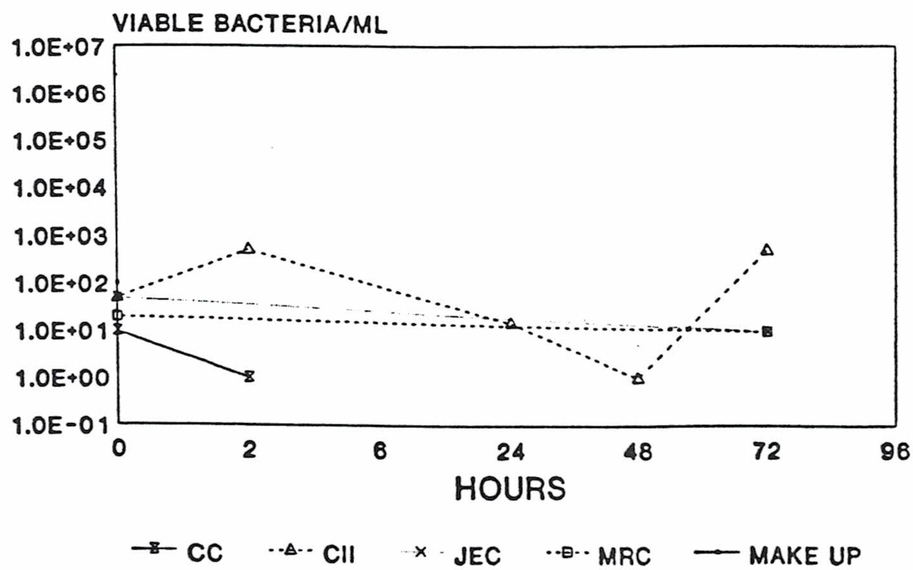

Figure 3d *Effect of isothiazolin treatment on* Pseudomonas *populations. Note that the levels of pseudomonads spiked in one tower at two points after treatment.*

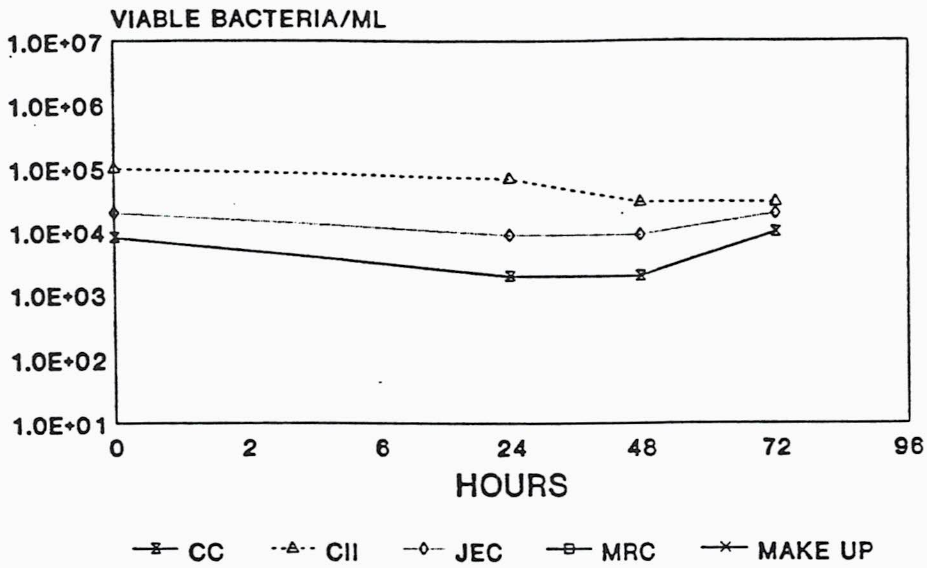

Figure 4 *Effect of cyanocarbonate/carbamate treatment on viable bacterial populations in the test towers. Note that there was little negative effect. The other parameters, as were shown for isothiazolin, showed essentially no change during the test. Biocide was added immediately after collecting the t = 0 samples.*

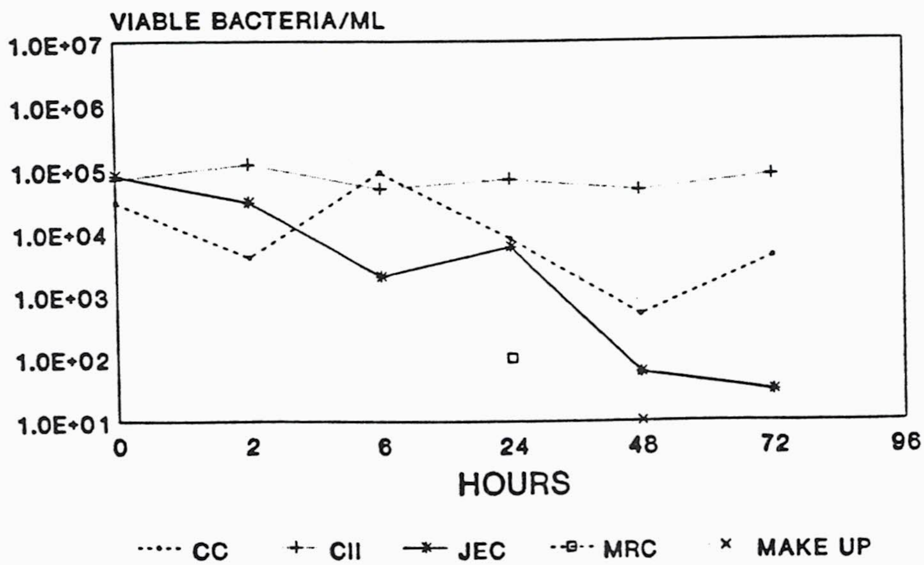

Figure 5 *Effect of polymeric quat treatment on viable bacterial populations in the test towers. This biocide was generally not effective at the dosage used. Note that the MRC tower was on continuous blowdown during most of this test. Biocide was added immediately after collecting the t = 0 samples.*

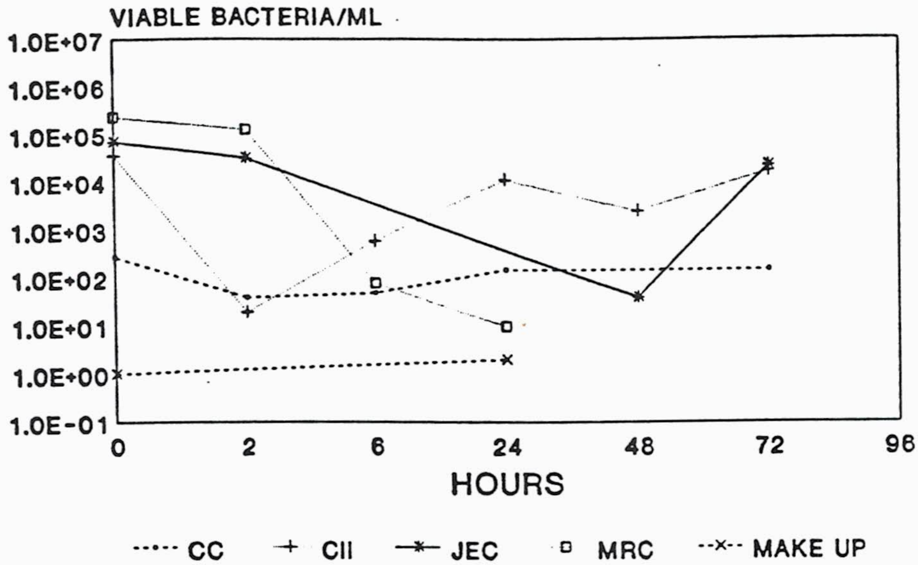

Figure 6 *Effect of glutaraldehyde treatment on viable bacterial populations in the test towers. Biocide was added immediately after collecting the* t = *0 samples.*

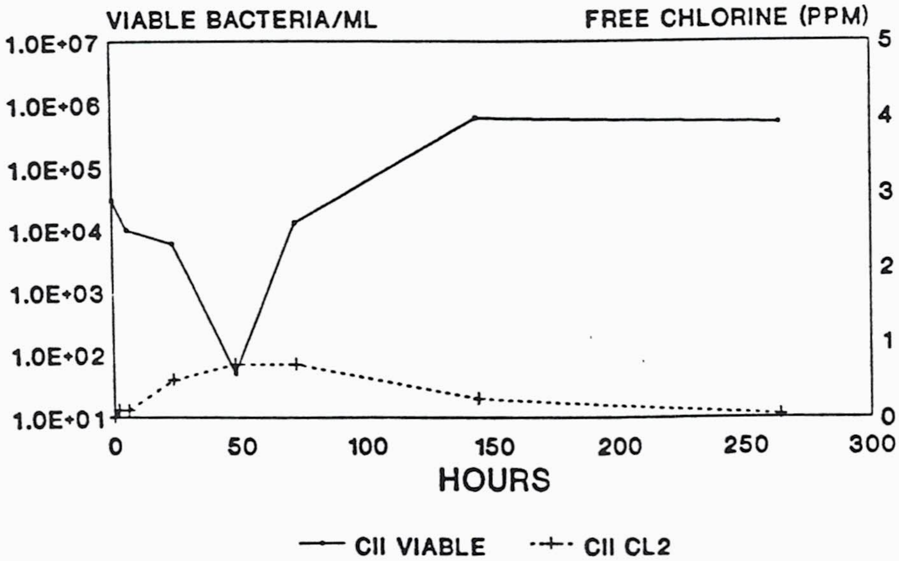

Figure 7 *Effect of bromochlorodimethylhydantoin treatment on viable bacterial populations in the CII test tower. Note that the levels of viable bacteria decreased rapidly as the free residual biocide increased and then rapidly increases as the biocide residual diminished. Biocide was added immediately after collecting the* t = *0 samples.*

The results of the phase II testing to determine the efficacy of commercially available biocides to control microbial populations, including *Legionella*, can be summarized as follows:

1. The data suggest that several commercially available biocides are capable of killing microorganisms in cooling systems. In many cases, however, the kill is very short lived and regrowth results in a tower operating a large percentage of the time with high microbial populations.
2. Use of these biocides at large doses and on a very frequent basis could, undoubtedly, control the microorganisms, but the economics of such treatment may often be unfavorable.

It is possible that continuous treatment of towers with biocides that are effective at low levels is a viable treatment option. This agrees with the findings of one of the authors in a previous study (Pope et al. 1984) in which treatment with ozone was effective as long as the treatment was continuously applied. The economics of the continuous treatment approach are not yet clear and would certainly depend in part on the biocide used and the conditions of the particular tower. In the case of the BCDMH used in the present study, economic analysis is not yet possible, since the minimum long-term dose levels required for control has not been established. If low (< 1.0 ppm) free chlorine residuals with the use of BCDMH are demonstrated to be effective, then there is a good possibility of economical, effective, and reliable continuous treatment. However, the uniqueness of each site must be considered when developing any treatment regime.

REFERENCES

APHA. 1989. *Standard methods for the examination of water and waste water*, 17th ed. Washington, DC: American Public Health Association.

Baird, I.M., W. Potts, J. Smiley, N. Click, S. Schleich, C. Connole, and K. Davison. 1984. Control of endemic nosocomial legionellosis by hyperchlorination of potable water. In *Legionella, Proceedings of the 2nd International Symposium*, p. 333, Thornberry, Balows, Feeley, and Jakubowski, eds. Washington DC: American Society for Microbiology.

Bollin, G.E., J.F. Plouffe, M.F. Para, and B. Hackman. 1985. Aerosols containing *Legionella pneumophila* generated by shower heads and hot-water faucets. *Applied and Environmental Microbiology* 50: 1128-1131.

Braun, E.B. 1982. Ph.D. thesis. Troy, NY: Department of Biology, Rensselaer Polytechnic Institute.

Dennis, P.J.L. 1988. *Isolation of Legionellae from environmental specimens*, T.G. Harrison and A.G. Taylor, eds., chapter 4, pp. 31-44. New York: John Wiley and Sons.

Dondero, T.J., R.C. Rendtoroff, and G.F. Mallison. 1980. An outbreak of Legionnaires' disease associated with a contaminated air-conditioning cooling tower. *New England Journal of Medicine* 302: 365-370.

Drew. 1979. *Principles of industrial water treatment*. Boonton, NJ: Drew Chemical Corporation.

Fields, B.S., G.N. Sanden, J.M. Barbaree, W.E. Morrill, R.M. Wadowsky, E.H. White, and J.C. Feeley. 1989. Intracellular multiplication of *Legionella pneumophila* in amoebae isolated from hospital hot water tanks. *Current Microbiology* 18: 131-137.

Fliermans, C., and R. Harvey. 1984. Effectiveness of 1-bromo-3-chloro-5,5-dimethylhydantoinagainst*Legionella pneumophila* in a cooling tower. *Applied and Environmental Microbiology* 47: 1307-1310.

Fliermans, C., and J. Nygren. 1987. Maintaining cooling systems "free" of *Legionella pneumophila*. *ASHRAE Transactions* 93: 1405-1415.

Fliermans, C.B., R.J. Soracco, and D.H. Pope. 1981. Measure of *Legionella pneumophila* activity in situ. *Current Microbiology* 6: 89-94.

Howland, E.B., and D.H. Pope. 1983. Distribution and seasonality of *Legionella pneumophila* in cooling towers. *Current Microbiology* 9: 319-324.

Massanari, R.M., C. Helms, R. Zeitler, S. Streed, M. Gilchrist, N. Hall, W. Hausler, Jr., W. Johnson, L. Wintermeyer, J.S. Muchs, and W.J. Heirholzer, Jr. 1984. Continuous hyperchlorination of a potable water system for control of nosocomial *Legionella pneumophila* infections. In *Legionella, Proceedings of the 2nd International Symposium*, Thornberry, Ballows, Feeley, and Jakubowski, eds., pp. 334-336. Washington, DC: American Society for Microbiology.

McCoy, W.F., and J.W. Wireman. 1989. Efficacy of bromochlorodimethylhydantoin against *Legionella pneumophila* in industrial cooling water. *Journal of Industrial Microbiology* 4: 403-408.

Muraca, P.W., V.L. Yu, and J.E. Stout. 1988. Environmental aspects of Legionnaires' disease. *Research and Technology*, February: 78-85.

Morris, G.K., and B.G. Shelton. 1990. *Legionella* in environmental samples: Hazard analysis and suggested remedial actions. *PathCon Technical Bulletin* 1(June): 1-4.

Nolte, F.S., et al. 1984. Plasmids as epidemiological markers in nosocomial Legionnaires' disease. *Journal of Infectious Diseases* 149: 251.

Pope, D.H., L.W. Eichler, T.F. Coates, J.F. Kramer, and R.J. Soracco. 1984. The effect of ozone on *Legionella pneumophila* and other bacterial populations in cooling towers. *Current Microbiology* 10: 89-94.

Soracco, R.J., and D.H. Pope. 1983. Bacteriostatic and bactericidal modes of action of bis(tributyltin)oxide on *Legionella pneumophila*. *Applied and Environmental Microbiology* 45: 48-57.

Soracco, R.J., H.K. Gill, C.B. Fliermans, and D.H. Pope.

1983. Susceptibilities of algae and *Legionella pneumophila* to cooling tower biocides. *Applied and Environmental Microbiology* 45: 1254-1260.

States, J.S., L.F. Coley, J.M. Kuchta, B.M. Oleck, M.J. Lipovich, R.S. Wolford, R.M. Wadowsky, R.S. Wolford, A.M. McNamara, J.L. Sykora, G. Keleti, and R.B. Yee. 1987. Survival and multiplication of *Legionella pneumophila* in municipal drinking water systems. *Applied and Environmental Microbiology* 53: 979-986.

Stout, J.E., V.L. Yu, and M.G. Best. 1985. Ecology of *Legionella pneumophila* within water distribution systems. *Applied and Environmental Microbiology* 49: 221-228.

Tison, D.L., D.H. Pope, W.B. Cherry, and C.B. Fliermans. 1980. Growth of *Legionella pneumophila* in association with blue-green algae (cyanobacteria). *Applied and Environmental Microbiology* 39: 456-459.

Tison, D.L., and R.J. Seidler. 1983. *Legionella* incidence and density in potable drinking water supplies. *Applied and Environment Microbiology* 45: 337-339.