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Effectiveness of 1-Bromo-3-Chloro-5,5-Dimethylhydantoin Against *Legionella pneumophila* in a Cooling Tower

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Cooling towers are considered to be man-made amplifiers of *Legionella* spp. Thus, the proper maintenance and choice of biocides is important. The only biocidal measure that has thus far been shown to be effective in field tests is the judicious use of chlorination. Perturbation studies with 1-bromo-3-chloro-5,5-dimethylhydantoin (Bromicide; Great Lakes Chemical Corp., West Lafayette, Ind.) (BCD) were conducted on an industrial cooling tower shown to contain *Legionella pneumophila*. At the concentrations recommended by the manufacturer, neither the density nor the activity of *L. pneumophila* was affected. At concentrations greater than 2.0 ppm (2.0 μg/ml) free of residual, BCD was not effective in reducing *L. pneumophila* to source water concentrations, nor was it effective in reducing the 2-*p*-iodophenyl-3-*p*-nitrophenyl-5-phenyl tetrazolium chloride activity of the bacterium in situ. The data indicate that at concentrations up to 2.0 ppm, BCD is not effective in these tower studies.

Both humans and *Legionella* spp. occupy selected ecological habitats and niches. When these niches overlap, the chance for cross-contamination is increased, but relatively few data are available to indicate the consequences of sharing habitats. The evidence from epidemiological and ecological studies (3, 4, 6; D. W. Tison, Ph.D. thesis, Rensselaer Polytechnic Institute, Troy, N.Y., 1980) indicate that humans and *Legionella* spp. have shared habitats for a considerable length of time. Cooling lakes and cooling towers have been shown to be amplifiers of *Legionella* spp. and have been implicated in the dissemination of the bacteria (2, 8, 9). The significance of *Legionella* spp. in an amplifier is yet to be understood fully, but it is clear that the mere presence of *Legionella* spp. in a habitat implies nothing about either the quality of the habitat or the restrictions to be placed on it. It does, however, indicate that one needs to be aware of the potential for cross-contamination and use of the habitat so as to avoid propagating *Legionella* spp. or causing their dissemination to a susceptible host.

Previous studies have demonstrated that the judicious use of chlorine is effective in decreasing the electron transport activity of *Legionella pneumophila* as well as in removing the organism from cooling towers and air wash systems (7). Because 1-bromo-3-chloro5,5-dimethylhydantoin (BCD) has been suggested as an effective biocide for cooling towers (N. T. Macchairola, C. H. Johnson, and R. S. Hornack, Cooling Tone Inst. TP-2194, 1980) and because BCD has some of the desirable characteristics of chlorine, field tests were conducted to evaluate its effectiveness in reducing both the density and the activity of *L. pneumophila* in a commercial mechanical-draft cooling tower.

MATERIALS AND METHODS

Cooling tower. The cooling tower studied had an induced mechanical draft which received makeup water from wells 200 feet (ca. 60 m) deep in the Tuscaloosa Aquifer. The tower was limited to a four-cycle operation which provided 400-μmho water at a pH of 6.0 to 7.5 for cooling the heat induction equipment. The tower was constructed of zinc-coated steel, and it cooled 1,000 gallons (ca. 3,800 liters) per min from 50 to 31°C at a wet-bulb temperature of 25.7°C. BCD, an oxidizing biocide, and Wrico H-9921 (Wright Chemical Co., Chicago, Ill.), a molybdate-based inhibitor, were injected into the circulating water to control biofouling and corrosion, respectively. Suspended solids were maintained below 200 ppm (200 μg/ml) through the use of side-stream filters.

BCD treatment. Cylindrical sticks of BCD were obtained from Great Lakes Chemical Corp., West Lafayette, Ind. along with the feeder recommended by the manufacturer for a cooling tower of the size to be studied. Halogen residuals were maintained at the concentration recommended by the manufacturer, 0.2 to 0.5 ppm, or were increased as experimental protocol dictated. Free halogen residuals were measured by standard methods (1).

***L. pneumophila* measurements.** The presence of *L. pneumophila* in the cooling tower studied was confirmed by guinea pig infectivity studies, fatty acid composition, and cultural isolations as previously described (1, 5, 7, 8, 12, 14). Water samples were collected aseptically from the cooling tower and were immediately treated with a tetrazolium chloride dye to measure the electron transport activity of *L. pneumophila* under the in situ test conditions (10). Water samples were incubated for 1 h, fixed with formaldehyde, and concentrated by continuous-flow centrifugation (8, 9). As previously described, densities of *L. pneumophila* serogroups 1 through 4 were measured by epifluorescence microscopy with serogroup-specific antibodies for the direct fluorescent antibody technique (8, 9), and cellular electron transport activity was measured by transmitted bright-field microscopy (10).

Chlorination. After BCD treatment at the levels recommended by the manufacturer and at elevated concentrations, the tower was chlorinated with calcium hypochlorite. The levels and the time period of chlorination were 72 h at 1.5 ppm of free residual followed by daily doses of 0.8 ppm of free residual for 1 h, as had been established for our other cooling towers and air wash systems (7).

RESULTS

Table 1 summarizes the experiments. Initial makeup water coming from subterranean wells contained low levels of *L. pneumophila* as shown in the makeup water samples. These levels are similar to those in other deep water wells ca. 200

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TABLE 1. Sampling data from cooling tower treated with BCD at levels recommended by the manufacturer for the removal of *L. pneumophila*

Treatment[a]	Date	Temp (°C)	Conductivity (μmho/cm²)	Dissolved O_2 (ppm)	pH	Free halogen residual (ppm)	*L. pneumophila* (cells/liter)				INT-positive cells (%)
							Knoxville	Togus	Blooming-ton	Los Angeles	
None	9/8/81	19.6	134	9.10	6.53	0	3.00×10^6	BD[b]	BD	5.41×10^4	43
	10/10/81	21.2	571	6.40	7.46	0	BD	1.10×10^6	BD	8.21×10^4	42
	11/20/81	20.8	467	9.10	6.95	0	BD	BD	BD	BD	NT[c]
	12/14/81	19.5	95	6.10	6.84	0	2.40×10^6	BD	BD	BD	38
BCD	1/18/82	19.3	236	10.16	7.18	0.3	BD	2.51×10^6	BD	BD	40
	2/23/83	21.5	455	8.26	7.20	0.3	BD	7.45×10^5	BD	BD	42
	3/31/82	20.7	195	8.26	6.70	0.3	2.50×10^5	4.50×10^6	1.10×10^5	1.40×10^5	48
	4/13/82	21.0	427	8.53	6.48	0.45	3.11×10^5	1.10×10^7	1.30×10^5	6.60×10^5	52
	4/19/82	16.4	132	9.50	6.72	0.20	8.71×10^5	6.60×10^7	BD	6.60×10^5	52

[a] Treatment with BCD (0.2 to 0.5 ppm) began 14 January 1982.
[b] BD, Below detectable limits of 9.1×10^3 cells per liter.
[c] NT, Not tested.

feet (60 m) below the water table (C. B. Fliermans, unpublished results) and demonstrate the presence and the low-level activity of *Legionella* spp. in these systems. Once waters containing *L. pneumophila* enter an amplifier such as a cooling tower, alterations of population density and cellular activity occur. The data indicate that the densities and activity of *L. pneumophila* in the cooling tower fluctuated with time and biocidal treatment, whereas such parameters were relatively constant over time in the well makeup water (Table 2). Previous data indicate that the levels of *L. pneumophila* in the groundwater of deep wells are always less than the densities in surface waters and that cooling towers receiving makeup water from underground sources have *L. pneumophila* densities substantially lower than towers receiving surface makeup water from lakes, rivers, or streams (C. B. Fliermans, Abstr. Annu. Meet. Am. Soc. Microbiol. 1982, N14, p. 180; C. B. Fliermans, Crit. Rev. Microbiol., in press).

Once the BCD treatment began, the free halogen residual was maintained between 0.2 and 0.5 ppm. This residual was continuous without interruption during a 1-month study. The data in Table 2 demonstrate that neither the density nor the 2-*p*-iodophenyl-3-*p*-nitrophenyl-5-phenyl tetrazolium chloride (INT) activity of *L. pneumophila* was affected by the concentrations of BCD recommended by the manufacturer. Once a reasonable base line regarding the effectiveness of the BCD had been established, the concentrations were increased to approximately 10 times the levels recommended by the manufacturer. It is not suggested that such high levels be used continuously in the tower, nor are such levels cost effective when compared with established chlorination procedures. The data (Table 2) further indicate that the increased levels of BCD (1.5 to 2.1 ppm of continuous free residual) were not effective in removing *L. pneumophila* from the tower during the 5-day test period, nor were the INT activity levels reduced below background levels.

To determine whether the chlorination procedures remained effective, the established chlorination practices were begun. The results of the procedure (Table 3) indicate that chlorine was effective, as previously described (7).

DISCUSSION

Numerous studies conducted in the laboratory have indicated that a number of biocidal agents are both bacteriostatic and bactericidal against *Legionella* spp. (11, 13, 15; R. J. Soracco, Ph.D. thesis, Rensselaer Polytechnic Institute, Troy, N.Y., 1981). In the laboratory, low concentrations of quaternary ammonium compounds, phenolics, glutaraldehyde, formaldehyde, and hypochlorite were effective against *L. pneumophila* (15). Skaliy et al. (13) demonstrated that biocides containing hypochlorite and 2,2-dibromonitrilopropionamide or a combination of quaternary ammonium salts and isopropanol were bactericidal for *L. pneumophila* suspended in tap water, whereas isothiazolone, thiocarbamates, and chlorophenols were less effective. Grace et al. (11) showed that biocides containing a combination of quaternary ammonium salts and bis(tri-*n*-butyltin) oxide were effective in killing *L. pneumophila* serogroup 1 at 4% of the recommended dosage.

A comprehensive study done by Soracco (Ph.D. thesis) involved the use of 12 commercially available biocides and nine different strains of *L. pneumophila*. The results of the testing indicate that the biocides containing tributyltin oxide and quaternary ammonium compounds were the most effective in controlling *L. pneumophila* in the laboratory. Zedler et al. (16) found that organotin compounds were synergistic with the quarternary salts against both gram-negative and gram-positive bacteria.

Work reported by E. B. Braun (Ph.D. thesis, Rensselaer Polytechnic Institute, Troy, N.Y., 1982) indicated that the use of a biocide containing dithiocarbamates and dithiocarbonate was not effective in controlling the population densities of *Legionella* spp. in a commercial cooling tower during an 18-month study period. Further work on the use of tributyltin in four evaporative heat exchangers established that these biocides at concentrations recommended by the manufacturers were not effective in controlling *Legionella* spp. in selected cooling towers or evaporative condensers.

The results from a series of tests by Braun (Ph.D. thesis) indicated that *Legionella* spp. was not removed from the tower by the use of 23.7% *n*-alkyl (C_{14}, C_{16}, C_{12}, C_{18}) dimethylbenzyl ammonium chlorides and 2.5% bis(tributyltin) oxide at 1 to 3 times the levels recommended by the manufacturer; moreover, another opportunistic pathogen, *Pseudomonas aeruginosa*, was not inhibited by the biocidal treatment either.

The obvious discrepancy between laboratory findings and the field data are due in part to the fact that it is very difficult to maintain a residual in cooling tower situation that corresponds to the residual in laboratory testing. L. J. Gawel and R. L. Huddleston (Am. Oil Chem. Natl. Meet., Los Angeles, Calif., 1972) have reported that the initial concentration of

TABLE 2. Sampling data from cooling tower treated with BCD at concentrations higher than those recommended by the manufacturer

Sample location[a]	Date	Temp (°C)	Conductivity (μmho/cm²)	Dissolved O_2 (ppm)	pH	Free halogen residual (ppm)	*L. pneumophila* (cells/liter)				INT-positive cells (%)	Guinea pig isolation
							Knoxville	Togus	Blooming-ton	Los Angeles		
Makeup water	4/26/82	20.6	155	4.45	6.76	0.0	1.91×10^5	1.91×10^5	9.10×10^3	BD	42	–
TNX-CT[b]	4/26/82	21.3	461	8.45	6.85	0.1	8.46×10^5	2.07×10^6	2.46×10^5	3.82×10^5	48	+
TNX-CT	4/26/82	24.1	484	7.75	7.13	0.2	1.64×10^5	3.74×10^6	2.40×10^5	3.82×10^6	NT[c]	NT
TNX-CT	4/26/82	22.6	475	7.94	7.07	0.5	2.24×10^5	2.24×10^5	2.10×10^5	1.64×10^5	53	NT
TNX-CT	4/26/82	23.1	474	7.48	7.12	0.6	2.73×10^4	2.62×10^6	8.19×10^4	3.82×10^5	NT	NT
TNX-CT	4/26/82	24.4	485	7.60	7.14	0.6	5.46×10^4	2.62×10^6	8.19×10^4	2.18×10^5	57	NT
TNX-CT	4/26/82	24.1	486	7.46	7.14	0.9	5.46×10^4	2.95×10^6	5.46×10^4	1.64×10^5	NT	+
TNX-CT	4/26/82	NT	NT	NT	NT	1.4	3.47×10^6	3.47×10^6	5.46×10^4	4.37×10^5	63	+
TNX-CT	4/26/82	24.7	495	7.21	7.06	1.3	3.47×10^4	3.52×10^4	5.46×10^4	4.37×10^5	51	NT
TNX-CT	4/27/82	18.7	380	9.85	7.25	0.75	2.46×10^5	2.51×10^6	8.21×10^4	2.46×10^5	52	NT
TNX-CT	4/28/82	18.9	152	12.0	6.92	0.75	4.69×10^5	1.09×10^6	6.09×10^5	3.00×10^5	48	+
TNX-CT	4/29/82	15.8	297	11.70	7.18	0.9	5.42×10^4	1.12×10^6	5.46×10^4	1.64×10^5	52	NT
TNX-CT	5/3/82	19.1	269	9.27	6.31	1.85	1.30×10^5	1.20×10^6	BD	3.55×10^5	49	NT
TNX-CT	5/5/82	21.9	310	7.43	6.61	1.5	2.18×10^5	1.01×10^6	BD	1.09×10^5	44	+
TNX-CT	5/7/82	20.4	273	9.90	6.25	2.1	1.09×10^5	9.01×10^5	BD	1.64×10^5	49	–
TNX-CT	5/10/82	19.3	369	10.00	6.54	0.7	8.74×10^5	2.07×10^6	BD	1.09×10^5	63	+
TNX-CT	5/14/82	19.8	380	11.48	6.36	0.8	4.91×10^5	2.10×10^6	BD	1.09×10^5	54	+
Makeup water	5/14/82	20.7	155	4.50	6.75	0.0	1.91×10^5	1.91×10^5	BD	9.10×10^3	40	–
TNX-CT	5/17/82	23.4	372	8.30	6.77	0.4	4.10×10^5	3.99×10^6	BD	1.45×10^6	48	NT
TNX-CT	5/26/82	25.5	658	6.34	7.31	0.4	1.91×10^5	5.81×10^6	BD	1.45×10^6	51	+
TNX-CT	6/30/82	25.9	235	8.2	7.03	0.3	1.85×10^5	6.83×10^5	1.37×10^5	BD	50	NT
TNX-CT	7/27/82	26.3	438	9.0	7.32	0.9	5.19×10^5	1.37×10^6	3.22×10^6	1.09×10^5	83	+
TNX-CT	10/8/82	23.2	462	8.3	6.78	0.8	5.46×10^5	1.75×10^6	4.00×10^6	BD	75	NT

[a] BD, Below detectable limits of 9.10×10^3 cells per liter.
[b] TNX-CT, Designation of cooling tower.
[c] NT, Not tested.

TABLE 3. Sampling data from cooling tower treated with chlorine at concentrations shown to be effective in other towers

Sample location[a]	Date	Temp (°C)	Conductivity (μmho/cm²)	Dissolved O_2 (ppm)	pH	Free halogen residual (ppm)	*L. pneumophila* (cells/liter)				INT-positive cells (%)	Guinea pig isolation
							Knoxville	Togus	Blooming-ton	Los Angeles		
Makeup water	10/8/82	20.6	156	4.48	6.73	0.0	1.81×10^5	2.13×10^5	9.10×10^3	9.10×10^3	41	–
TNX-CT[a]	11/16/82	18.1	427	9.7	7.30	0.8	8.19×10^5	8.19×10^6	3.10×10^6	5.28×10^5	83	+
TNX-CT	11/16/82	15.1	355	9.65	7.36	2.1	8.19×10^6	2.18×10^5	BD[b]	BD	22	–
TNX-CT	11/17/82	13.7	270	10.2	6.73	2.2	5.46×10^4	2.73×10^5	BD	BD	12	–
TNX-CT	11/17/82	18.8	420	8.9	7.00	1.8	BD	1.37×10^5	BD	BD	5	–
TNX-CT	11/18/82	16.1	570	9.3	7.38	1.6	BD	1.37×10^5	BD	BD	5	–
TNX-CT	11/18/82	20.5	542	8.5	7.45	2.0	9.10×10^3	1.09×10^5	BD	BD	5	NT[c]
Makeup water	11/18/82	20.6	156	4.50	6.72	0.0	1.91×10^5	1.89×10^5	BD	BD	40	–

[a] TNX-CT, Designation of cooling tower.
[b] BD, Below detectable limits of 9.10×10^3 cells per liter.
[c] NT, Not tested.

quaternary ammonium salts of 20 ppm in a cooling tower was less than 1 ppm within 3 h of treatment. Thus, it appears that at the present time, the organic biocidal treatment described is not effective in removing *Legionella* spp. from the cooling systems studied.

BCD is a biocidal treatment for cooling water systems. The stated advantage of BCD over other oxidizing biocides, particularly chlorine, is its ease in handling and its effectiveness in the presence of nitrogenous compounds since bromoamines are more effective than chloroamines as microbicidal compounds (Macchairola et al., Cooling Tower Inst.). In addition, the reaction products of bromine are more effective than the reaction products of chlorine over a wide pH range.

Thus, it appears from the literature that BCD would be an effective replacement compound for chlorine and could be effective against *Legionella* spp. But findings from literature studies and laboratory experiments do not always agree with field results (Soracco, Ph.D. thesis), and such is the case with BCD. The data demonstrate that *L. pneumophila* was not readily removed from the cooling tower studied when BCD was used at the concentrations recommended by the manufacturer, nor was the BCD effective at concentrations up to 10 times the recommended levels.

The data do not indicate whether BCD had a bacteriostatic effect on *L. pneumophila*, that is, whether levels were kept low by continuous treatment. It is clear that chlorination as previously described (7) is effective not only in reducing the levels of INT-active cells in the *L. pneumophila* population but also in reducing the levels of *L. pneumophila* to below concentrations in source water.

This study makes the point once again that results in the laboratory are not always comparable to results under in situ conditions. Any product or technique recommended for field use, as in cooling towers, should be tested for effectiveness under expected operating conditions.

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LITERATURE CITED

1. **American Public Health Association.** 1980. Standard methods for the examination of water and waste water, 15th ed. American Public Health Association, Washington, D.C.
2. **American Society of Heating, Refrigerating and Air Conditioning Engineers.** 1981. Position paper on legionellosis. American Society of Heating, Refrigerating and Air Conditioning, Washington, D.C.
3. **Band, J. D., and D. W. Fraser.** 1981. Legionellosis (Legionnaires' disease and Pontiac fever), p. 958–971. *In* A. I. Braude (ed.), Medical microbiology and infectious diseases. The W. B. Saunders Co., Philadelphia.
4. **Blackmon, J. A., F. W. Chandler, W. B. Cherry, A. C. England, J. C. Feeley, M. D. Hicklin, R. M. McKinney, and H. Q. Wilkinson.** 1981. Legionellosis. Am. J. Pathol. **103**:429–465.
5. **Christensen, S. W., R. L. Tyndall, J. A. Soloman, C. B. Fliermans, and S. B. Gough (ed.).** Legionnaires' disease bacterium in power plant cooling systems, p. 97. Electric Power Research Institute, Palo Alto, Calif.
6. **Cordes, L. G., and D. W. Fraser.** 1980. Legionellosis: Legionnaires' disease; Pontiac fever. Med. Clin. North Am. **64**:395–416.
7. **Fliermans, C. B., G. E. Bettinger, and A. W. Fynsk.** 1982. Treatment of cooling water systems containing high levels of *Legionella pneumophila*. Water Res. **16**:903–909.
8. **Fliermans, C. B., W. B. Cherry, L. H. Orrison, and L. Thacker.** 1979. Isolation of *Legionella pneumophila* from nonepidemic-related aquatic habitats. Appl. Environ. Microbiol. **37**:1239–1242.
9. **Fliermans, C. B., W. B. Cherry, L. H. Orrison, S. J. Smith, D. L. Tison, and D. H. Pope.** 1981. Ecological distribution of *Legionella pneumophila*. Appl. Environ. Microbiol. **41**:9–16.
10. **Fliermans, C. B., R. J. Soracco, and D. H. Pope.** 1981. Measure of *Legionella pneumophila* activity *in situ*. Curr. Microbiol. **6**:89–94.
11. **Grace, R. D., N. E. Dewar, W. G. Barnes, and G. R. Hodges.** 1981. Susceptibility of *Legionella pneumophila* to three cooling tower microbicides. Appl. Environ. Microbiol. **41**:233–236.
12. **Orrison, L. H., W. B. Cherry, C. B. Fliermans, S. B. Dees, L. K. McDougal, and D. J. Dodd.** 1981. Characteristics of environmental isolates of *Legionella pneumophila*. Appl. Environ. Microbiol. **42**:109–115.
13. **Skaliy, P., T. A. Thompson, G. W. Gorman, G. K. Morris, H. V. McEachern, and D. C. Mackel.** 1980. Laboratory studies of disinfectants against *Legionella pneumophila*. Appl. Environ. Microbiol. **40**:697–700.
14. **Tyndall, R. L., C. B. Fliermans, S. B. Gough, E. Dominque, and B. C. Duncan.** 1983. Isolation of a new *Legionella* species from thermally altered waters. Curr. Microbiol. **9**:77–80.
15. **Wang, W. L., M. J. Blaser, J. Cravens, and M. A. Johnson.** 1978. Growth, survival and resistance of the Legionnaires' disease bacterium. Ann. Intern. Med. **90**:614–618.
16. **Zedler, R. J.** 1961. Organotins as industrial biochemicals. Tin and Its Uses **53**:7–11.