DP-MS-82-85

Conf-830387--2

EFFECTIVENESS OF BROMICIDE™ AGAINST LEGIONELLA PNEUMOPHILA IN A COOLING TOWER

by

Carl B. Fliermans and Ray S. Harvey

E. I. du Pont de Nemours & Co.
Savannah River Laboratory
Aiken, SC 29808

DP-MS--82-85

DE83 017508

A paper proposed for presentation at the
83rd Annual Meeting of the American Society for Microbiology
New Orleans, Louisiana
March 6-11, 1983
and for publication in **Applied and Environmental Microbiology**

This paper was prepared in connection with work under Contract No. DE-AC09-76SR00001 with the U.S. Department of Energy.

DISCLAIMER

EFFECTIVENESS OF BROMICIDE™ AGAINST *LEGIONELLA PNEUMOPHILA* IN A COOLING TOWER*

by

Carl B. Fliermans and Ray S. Harvey

E. I. du Pont de Nemours & Co.
Savannah River Laboratory
Aiken, SC 29808

ABSTRACT

Cooling towers are considered to be man-made amplifiers of *Legionella*. Thus the proper maintenance and choice of biocides is important. The only biocide that has thus far been shown to be effective in field tests is the judicious use of chlorination. Perturbation studies were conducted on an industrial cooling tower shown to contain *Legionella*, using 1-bromo-3-chloro-5,5-dimethylhydantoin (Bromicide™, Great Lakes Chemical Corp.). At the manufacturer's recommended concentrations neither the density nor the activity of *Legionella* was affected. At concentrations greater than 2.0 ppm free residual, the Bromicide™ was not effective in reducing *Legionella* to source water concentrations, nor was it effective in reducing the INT activity of the bacterium *in situ*. The data indicate that at concentrations up to 2.0 ppm, Bromicide™ is not effective in these tower studies.

INTRODUCTION

Both man and *Legionella* 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 consequence of the sharing of habitats. The evidence from epidemiological and ecological studies(3,4,6) indicate that man and *Legionella* have shared habitats for a considerable length of time. Cooling lakes and cooling towers have been shown to be amplifiers of *Legionella* and have been implicated in the dissemination of the bacterium(2,11,12). The significance of *Legionella*

* The information contained in this article was developed during the course of work under Contract No. DE-AC09-76SR00001 with the U.S. Department of Energy.

in an amplifier is yet to be fully understood, but it is clear that the mere presence of Legionella in a habitat makes neither a statement as to the quality of that habitat nor the restrictions to be placed on that habitat. It does, however, indicate that one needs to be aware of the potential that is present so the habitat is used in a way that will not propagate Legionella nor cause its dissemination to a susceptible host.

Numerous studies conducted in the laboratory have indicated that a number of biocidal agents are both static and cidal against Legionella(15,18,19,22). In the laboratory, low concentrations of quaternary ammonium compounds, phenolics, glutaraldehyde, formaldehyde, and hypochlorite were effective against L. pneumophila(22). Skaliy et al.(18) demonstrated that biocides containing hypochlorite, and 2,2 dibromonitrilopropionamide, or a combination of quaternary ammonium salts and isopropanol were cidal for L. pneumophila suspended in tap water, while isothiazolone, thiocarbamates, and chlorophenols were less effective. Grace et al.(15) 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 1/25 of the recommended dosage.

A comprehensive study done by Soracco(19) 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 Legionella in the laboratory. Zedler et al.(23) found that organotin compounds were synergistic with the quarternary salts against both gram-negative and gram-positive bacteria.

Work reported by Braun(5) indicated that the use of a biocide containing dithiocarbamates and dithiocarbonate was not effective in controlling the population densities of Legionella in a commercial cooling tower during an 18-month study period. Further work on the use of tributyitin in four evaporative heat exchangers, established that these biocides at concentrations recommended by the manufacturers were not effective in controlling Legionella in selected cooling towers or evaporative condensers.

The results from a series of tests by Braun(5) indicated that Legionella was not removed from the tower by the use of 23.7% n-alkyl (C14,C16,C12,C18) dimethylbenzyl ammonium chlorides and 2.5% bis (tributyltin) oxide at 1 to 3 times manufacturer's recommended levels; moreover, another opportunistic pathogen, Pseudomonas aeruginosa was also not inhibited by the biocidal treatment.

The obvious discrepancy between laboratory findings and the field data are in part due to the fact that it is very difficult to maintain a residual in the cooling tower situation that corresponds

to the laboratory testing. Gawel and Huddleston(14) have reported that the initial concentration of quaternary ammonium salts of 20 ppm in a cooling tower was less than 1 ppm within three hours of treatment. Thus, it appears that at the present time, the described organic biocidal treatment is not effective in removing Legionella from the studied cooling systems.

Previous studies have demonstrated that the judicious use of chlorine is effective in decreasing the electron transport activity of Legionella pneumophila as well as removing the organism from cooling towers and air wash systems(10). Because Bromicide™ has been suggested as an effective biocide for cooling towers(16), and because Bromicide™ has some of the desirable characteristics of chlorine, field tests were conducted to evaluate the effectiveness in reducing both the density and activity of L. pneumophila in a commercial mechanical draft cooling tower.

MATERIALS AND METHODS

Cooling Tower

The studied cooling tower is an induced mechanical draft which receives makeup water from 200-foot-deep wells in the Tuscaloosa Aquifer. The tower is limited to a 4-cycle operation which provides 400 µmho water at a pH of 6.0 to 7.5 for cooling the heat induction equipment. The tower is constructed of zinc-coated steel, and cools 1000 gpm from 50° to 31°C at a wet bulb temperature of 25.7°C. Bromicide™, an oxidizing biocide, and Wrico H-9921™ (Wright Chemical Co.,), a molybdate based inhibitor, were injected into the circulating water to control biofouling and corrosion, respectively. Suspended solids are maintained below 200 ppm through the use of side stream filters.

Bromicide™ Treatment

Bromicide™, 1-bromo-3-chloro-5, 5-dimethylhydantoin, was obtained in cylindrical stick form from Great Lakes Chemical Corporation (W. Lafayette, Indiana) along with the manufacturer's recommended feeder for the size cooling tower to be studied. Halogen residuals were maintained at manufacturer's recommended concentration of 0.2 to 0.5 ppm or increased as experimental protocol dictated. Free halogen residuals were measured using Standard Methods(1).

Legionella Measurements

The presence of *L. pneumophila* in the studied cooling tower was confirmed by guinea pig infectivity studies, fatty acid composition and cultural isolations as previously described(1,7,10,11,17,21). Water samples were collected aseptically from the cooling tower and immediately treated with a tetrazolium chloride dye in order to measure electron transport activity of *L. pneumophila* under the *in situ* test conditions(13). Water samples were incubated for 1 hr, fixed with formaldehyde, and concentrated by continuous flow centrifugation(11,12). As previously described, densities of *L. pneumophila* serogroups 1-4 were measured by epifluorescence microscopy using serogroup specific antibodies for direct fluorescent antibody technique(11,12) and cellular electron transport activity by transmitted bright field microscopy(13).

Chlorination

Following Bromicide™ treatment at manufacturer's recommended levels and at elevated concentrations, the tower was chlorinated with calcium hypochlorite. The levels and the time period of chlorination were 72 hours at 1.5 ppm free residual followed by daily doses of 0.8 ppm free residual for one hour as had been established for our other cooling towers and air wash systems(10).

RESULTS

The data in Table 1 summarize 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 other deep water wells, ca. 200 feet below the water table (Fliermans, unpublished results), and demonstrate the presence and the low-level activity of *Legionella* 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 fluctuate with time and biocidal treatment while such parameters are relatively constant over time in the well makeup water (Table 2). Previous data indicate that the levels of *L. pneumophila* in the ground water of deep wells is always less than the densities in surface waters, and that cooling towers receiving makeup water from underground sources have *L. pnuemophila* densities substantially below towers receiving surface makeup water from lakes, rivers or streams(8,9).

Once the Bromicide™ treatment began, the free halogen residual was maintained between .2-.5 ppm. This residual was continuous without interruption during a one-month study. The data in Table 2

demonstrate that neither the densities nor the INT activity of L. pneumophila were affected by the manufacturer's recommended concentrations of Bromicide™. Having established a reasonable baseline as to the effectiveness of the Bromicide™, the concentrations were increased so that levels were approximately 10 times the manufacturer's recommended concentrations. Such high levels are not suggested to be used continuously in the tower nor are such levels cost effective when compared to established chlorination procedures. The data (Table 2) further indicate that the increased levels of Bromicide™, 1.5 to 2.1 ppm 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(10).

DISCUSSIONS

Bromicide™ is a biocidal treatment for cooling water systems(16). The stated advantage of Bromicide™ 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 microbiocidal compounds(20). Additionally, 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 Bromicide™ would be an effective replacement compound for chlorine and could be effective against Legionella. But as with literature studies and laboratory experiments, such findings do not always translate into similar field results(19). Such is the case with Bromicide™. The data demonstrate that Legionella was not readily removed from the studied cooling tower when Bromicide™ was used at manufacturer's recommended concentrations, nor was the Bromicide™ effective at concentrations up to 10 times the recommended levels.

The data do not indicate whether Bromicide™ had a static effect on Legionella, that is, keeping the levels reduced by continuous treatment. It is clear that chlorination as previously described(10) is effective in not only reducing the levels of INT active cells in the L. pneumophila population, but also is effective in reducing the levels of L. pneumophila to below source water concentrations.

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, such as in cooling towers, should be tested for effectiveness under expected operating conditions.

REFERENCES

1. American Public Health Association 1980. Standard methods for the examination of water and waste water. 15th Ed., American Public Health Association, Washington, DC.

2. American Society of Heating, Refrigerating and Air Conditioning Engineers, 1981. Position Paper on Legionellosis.

3. Band, J. D., and Fraser, D. W., Legionellosis (Legionnaires' Disease and Pontiac Fever), In Medical Microbiology and Infectious Diseases, Braude, A. I., Ed., W. B. Saunders Co., Philadelphia, PA, 1981.

4. Blackmon, J. A., Chandler, F. W., Cherry, W. B., England, A. C., Feeley, J. C., Hicklin, M. D., McKinney, R. M., and Wilkinson, H. Q., 1981. Legionellosis, American Journal of Pathology, 103: 429-465.

5. Braun, E. B., Factors affecting the distribution, seasonal response and control of Legionella pneumophila in aquatic environments, Ph.D. Thesis, Rensselaer Polytechnic Institute, Troy, NY, 1982.

6. Cordes, L. G. and Fraser, D. W., 1980. Legionellosis: Legionnaires' Disease; Pontiac Fever, Medical Clinics of North America, 64: 395-416.

7. Electrical Power Research Institute, Legionnaires' Disease bacterium in power plant cooling systems, Christensen, S. W., Tyndall, R. L., Soloman, J. A., Fliermans, C. B., and Gough, S. B., 1982. Eds. 97.

8. Fliermans, C. B., 1982. Growth of Legionella pneumophila in diffusion chambers. Abstracts of the Annual Meeting of the American Society for Microbiology, p. N-14.

9. Fliermans, C. B., Ecological Niche of Legionella pneumophila. In Legionellosis. S. M. Katz. Ed., Critical Reviews in Microbiology. CRC Press, Cleveland, OH (in press).

10. Fliermans, C. B., Bettinger, G. E., and Fynsk, A. W., 1982. Treatment of cooling water systems containing high levels of Legionella pneumophila, Water Research, 16: 903-909.

11. Fliermans, C. B., Cherry, W. B., Orrison, L. H., and Thacker, L., 1979. Isolation of Legionella pneumophila from nonepidemic-related aquatic habitats. Applied and Environmental Microbiology, 37: 1239-1242.

REFERENCES, Contd.

12. Fliermans, C. B., Cherry, W. B., Tison, D. L., Smith, R. B., and Pope, D. H., 1981. Distribution of Legionella pneumophila. Applied and Environmental Microbiology, 41: 9-16.

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

14. Gawel, L. J. and Huddleston, R. L., 1972. The biodegradability of low concentrations of certain quaternary ammonium antimicrobials by bacteria. Presented at the American Oil Chemists National Meeting, Los Angeles, CA.

15. Grace, R. D., Dewar, N. E., Barnes, W. G., and Hodges, G. R., 1981. Susceptibility of Legionella pneumophila to three cooling tower microbiocides, Applied and Environmental Microbiology, 41: 233-236.

16. Macchairola, N. T., Johnson, C. H., and Hornack, R. S., Bromicide™ - a new biocide for cooling water systems. Presented at Cooling Tower Institute TP-2194, Houston, TX, Jan. 1980.

17. Orrison, L. H., Cherry, W. B., Fliermans, C. B., Dees, S. B., McDougal, L. K., and Dodd, D. J., 1981. Characteristics of environmental isolates of Legionella pneumophila. Applied and Environmental Microbiology, 42: 109-115.

18. Skaliy, P., Thompson, T. A., Gorman, G. W., Morris, G. K., McEachern, H. V., and Mackel, D. C., 1980. Laboratory studies of disinfectants against Legionella pneumophila, Applied and Environmental Microbiology, 40: 697-700.

19. Soracco, R. J., Effectivenes of cooling tower biocides against algae and Legionella pneumophila and mode of action of Bis (tributyltin) oxide, Ph.D. Thesis, Rensselaer Polytechnic Institute, Troy, New York, 1981.

20. Tison, D. W., Primary productivity, heterotrophy, metabolic indicators of stress and interactions in algal-bacterial mat communities affected by a fluctuating thermal regime, Ph.D. Thesis, Rensselaer Polytechnic Institute, Troy, NY, 1980.

21. Tyndall, R. L., Fliermans, C. B., Gough, S. B., Dominque, E., and Duncan, C., 1983. Isolation of a new Legionella species from thermally altered waters. Applied and Environmental Microbiology, 45: 536-545.

REFERENCES, Contd.

22. Wang, W. L., Blaser, M. J., Cravens, J., and Johnson, M. A., 1978. Growth, survival and resistance of the Legionnaires' Disease bacterium, Annals of Internal Medicine, 90: 614-618.

23. Zedler, R. J., 1961. Organotins as industrial biochemicals, Tin and Its Uses, 53: 7-11.

TABLE 1

Sampling Data from Cooling Tower Treated with Bromocide™ at Manufacturer's Recommendations for the Removal of L. pneumophila

Sample Location	Date	Temp. °C	Cond. (μmho/cm²)	D.O. (ppm)	pH	Free Halogen Residual (ppm)	Legionella pneumophila/liter				% INT Positive Cells
							Knoxville	Togus	Bloomington	Los Angeles	
TNX-CT	9/8/81	19.6	134	9.10	6.53	0	3.00×10^6	BD*	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**
	12/14/81	19.5	95	6.10	6.84	0	2.40×10^6	BD	BD	BD	38
BROMOCIDE™ 0.2 - 0.5 ppm begun 1/14/82											
	1/18/82	19.3	236	10.16	7.18	0.3	BD	2.51×10^6	BD	BD	40
	2/23/82	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

* BD = below detectable limits of 9.1×10^3/liter.
** NT = not tested.

TABLE 2

Sampling Data from Cooling Tower Treated with Bromocide™ at Concentrations Higher than Manufacturer's Recommendation

Sample Location	Date	Temp. °C	Cond. (μmho/cm²)	D.O. (ppm)	pH	Free Halogen Residual (ppm)	Legionella pneumophila/liter				% INT Positive Cells	Guinea Pig Isolation
							Knoxville	Togus	Bloomington	Los Angeles		
Makeup H_2O	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	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	3.82×10^5	NT**	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 H_2O	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

* BD = below detectable limits of 9.10×10^3/liter.
** NT = not tested.

TABLE 3

Sampling Data from Cooling Tower Treated with Chlorine at Concentrations Shown to be Effective in Other Towers

Sample Location	Date	Temp. °C	Cond. (μmho/(cm²)	D.O. (ppm)	pH	Free Halogen Residual (ppm)	Legionella pneumophila/liter				% INT Positive Cells	Guinea Pig Isolation
							Knoxville	Togus	Bloomington	Los Angeles		
Makeup H_2O	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	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*	BD*	22	-
	11/17/82	13.7	270	10.2	6.73	2.2	5.46×10^4	2.73×10^5	BD	BD	12	-
	11/17/82	18.8	420	8.9	7.00	1.8	BD*	1.37×10^5	BD	BD	5	-
	11/18/82	16.1	570	9.3	7.38	1.6	BD	1.37×10^5	BD	BD	5	-
	11/18/82	20.5	542	8.5	7.45	2.0	9.10×10^3	1.09×10^5	BD	BD	5	NT**
Makeup H_2O	11/18/82	20.6	156	4.50	6.72	0.0	1.91×10^5	1.89×10^5	BD	BD	40	-

* BD = below detectable limits of 9.10×10^3/liter.
** NT = not tested.