Journal of Industrial Microbiology, 4 (1989) 403–408
Elsevier

SIM 00194

Efficacy of bromochlorodimethylhydantoin against *Legionella pneumophila* in industrial cooling water

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Received 14 July 1988
Accepted 10 January 1989

Key words: Bromochlorodimethylhydantion; *Legionella pneumophila*; Industrial cooling water

SUMMARY

Free residual chlorine and bromine can be generated in water from bromochlorodimethylhydantoin (BCDMH). Efficacy of chlorine from inorganic sources has been studied extensively, but there is much less information on the efficacy of bromine against *L. pneumophila*; only a few efficacy studies of organically-derived halogen appear in the literature and the results from different studies conflict or are difficult to interpret. This paper describes the efficacy of halogen from BCDMH against planktonic, pure culture *L. pneumophila* in an industrial cooling water. There was no difference in efficacy between halogen derived from organic or inorganic sources in controlled laboratory experiments. Effective doses in laboratory studies cannot be translated directly to field applications because of significant differences in the microbiology. However, the data suggest that disinfection (> 99.9% reduction in viability within 10 min) of planktonic, pure culture *L. pneumophila* can be achieved with about 1 ppm free residual halogen (expressed as chlorine) from BCDMH in a typical industrial cooling water.

INTRODUCTION

Water treatment programs are often scrutinized carefully whenever there is an occurrence of Legionnaires' disease. The efficacy of cooling-water antimicrobial treatments against *L. pneumophila* continues to be an important area of research in industrial microbiology [2,4,8,11,12,14,15,19,21,24, 25,31]. This paper deals with the antimicrobial efficacy of halogens derived from bromochlorodimethylhydantoin (BromiCide® biocide, Great Lakes Chemical Corporation).

A great deal has been learned about the efficacy of chlorine in water against *L. pneumophila*; less is known about bromine. Field studies have shown effective application of chlorine to prevent amplification of *L. pneumophila* concentration in cooling-

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water systems [2,11,12,16,21,30,31]. Field studies of chlorine and bromine derived from BCDMH have also shown effective control of *L. pneumophila* in cooling-water systems [15], Leinbach, E.D., et al., Abstr. Annu. Meet. Am. Soc. Microbiol. 1985, Q89, p. 272) but another did not [11]. Field studies are, of course, essential in order to assess the efficacy of an antimicrobial treatment in real world conditions. Unfortunately, it is often difficult to control all of the variables in the field to the extent that unbiased comparisons of different treatments can be made. The most serious objection to field study comparisons of antimicrobial treatments is that there are usually inadequate controls for the myriad of variables that can effect the results.

To our knowledge, the laboratory experiments described in this paper are the first direct comparisons of chlorine (from sodium hypochlorite) versus chlorine and bromine (from bromochlorodimethylhydantoin) against *L. pneumophila* in a typical cooling tower water at relevant use-concentrations, water hardness, pH values, and temperature. This study was designed to compare the efficacy of halogen derived from these two sources in controlled, repeatable experiments and therefore, they can be used to help interpret results from previous field studies.

MATERIALS AND METHODS

Cooling Tower Water

The water was collected from the west cooling tower of the Metropolitan Center for High Technology (Detroit, MI). No chemical additives had been used in this tower for at least 2 weeks prior to sampling. The pH of the water was 9.0, temperature was 28°C, and the total hardness was 320 ppm. The total water hardness was adjusted to 500 ppm by the addition of synthetic hard water (Assoc. Official Anal. Chemists, Section 4.027) and measured by the EDTA titration (Assoc. Official Anal. Chemists, Section 4.028). The pH of the water was adjusted with KOH or HCl as required in order to prepare pH 6.0 and 8.5 samples for testing.

Test Culture

A pure culture of *Legionella pneumophila* (flint 1 strain, serogroup 1) was maintained in filter sterilized cooling water (0.22 μm cellulose nitrate filter). Preliminary studies showed that the *L. pneumophila* culture survived well in the cooling tower water at either pH 6.0 or 8.5. The culture was transferred to the cooling tower water at pH 8.5 and allowed to adapt for 5–7 days at 28°C; this precaution was followed because the organism was shown previously to become more resistant to chlorine after adaptation in tap water [14]. On the day of use, the adapted *L. pneumophila* was present at a density of 2.0×10^6 colony forming units/ml (CFU/ml) and had not declined during the adaptation period. Survival of the organism in this and all subsequent experiments was measured by plating in duplicate as droplets according to the method of Neblett [22] onto buffered charcoal yeast extract plus cysteine agar. Inactivation of the antimicrobials in treated samples was achieved with 100 ppm thioglycolic acid before plating. All plates were incubated at 37°C for at least 7 days even though colony formation was essentially complete after approximately 4 days. Selected plates were chosen for examination by epifluorescence microscopy with specific antibody (serogroup 1, Zeus Scientific, Inc.) in order to confirm that the culture was pure.

Antimicrobials

Two sources of oxidizing biocide were studied: Sodium hypochlorite solution (5.25% available chlorine) and BCDMH (BromiCide® biocide, Great Lakes Chemical Corporation). Quantification of halogen concentration was by the diethyl-*p*-phenylene diamine (DPD) colorimetric method (*Standard Methods for the Examination of Water and Wastewater*, 16th Ed., 408 E) and the results were expressed as free residual chlorine (FRC) regardless of the source of halogen even though the BCDMH treatments generated both chlorine and bromine. Stock solutions (100 ppm FRC) were prepared in deionized water and the chlorine demand (2 h contact time) of the cooling tower water was determined. The quantity of biocide required to satisfy the demand and leave the desired FRC in 10 mls of cooling tower water was calculated and then dosed. The actual concentration of FRC in the water was determined after 2 h contact. The samples were then inoculated with pure culture *L. pneumophila* so that the initial cell density would be about 1×10^5 CFU/ml. Survival of the organism was determined at regular time intervals by the plating procedure already described.

RESULTS

Table 1 summarizes the results of these disinfection studies. The contact time required to achieve >99.99% reduction in viability of about 10^5 CFU/ml of adapted *L. pneumophila* in a typical cooling tower water depended on pH and concentration of FRC. Only 10 minutes was required when the pH was 6.0 for concentrations higher than about 0.3–0.4 ppm FRC. All concentrations caused a >99.99% reduction in viability at pH 6.0 when contact times were greater than 20 min. Slightly higer concentrations were required for the same efficacy after 10 min of contact at pH 8.5; even the lowest concentrations were effective at pH 8.5 with contact times greater than 20 min. Every concentration tested caused >99.99% loss of viability regardless of pH when the contact time was greater than 30 min.

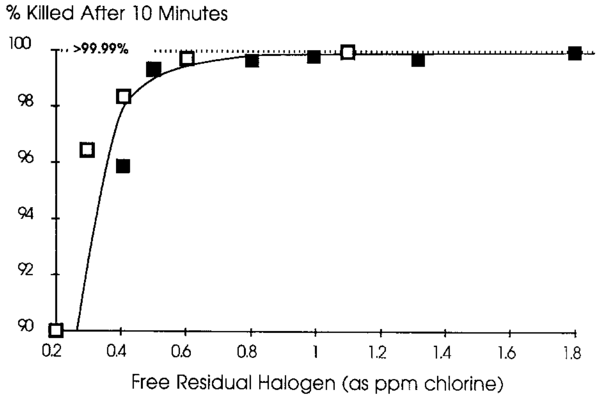

Fig. 1. Disinfection of *Legionella pneumophila* in cooling tower water at pH 8.5 with NaOCl (open symbols) and BCDMH (closed symbols).

Fig. 1 shows the data from the pH 8.5., 10 minute contact time experiments. There was no apparent difference in efficacy of halogen regardless of the source.

DISCUSSION

Bromochlorodimethylhydantoin (Fig. 2) reacts quickly with water to generate powerful antimicro-

Table 1

Efficacy of halogen derived from two different sources, at two different pH values against *L. pneumophila* in a typical cooling tower water

Source	Free Residual Halogen (*ppm as Cl_2*)	% Killed at 10 min		% Killed at 20 min	
		pH 6.0	pH 8.5	pH 6.0	pH 8.5
NaOCl	0.2	97.67	90.00	99.73	99.89
	0.3	99.98	96.47	99.99	99.93
	0.4	99.79	98.33	99.98	99.88
	0.6	>99.99	99.67	>99.99	>99.99
	1.1	>99.99	99.97	>99.99	>99.99
BCDMH	0.4	99.94	95.88	>99.99	99.79
	0.5	>99.99	99.33	>99.99	99.75
	0.8	>99.99	99.60	>99.99	99.95
	1.0	>99.99	99.94	>99.99	>99.99
	1.3	>99.99	>99.79	>99.99	>99.99
	1.8	>99.99	99.98	>99.99	>99.99

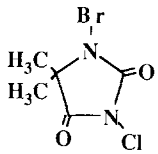

Fig. 2. Bromochlorodimethylhydantoin.

bial agents, predominantly hypobromous and hypochlorous acids.

There are several important technical reasons for the wide-spread use of BCDMH in modern water treatment but, relative to antimicrobial efficacy, the two most significant reasons are the hypohalous acid dissociation constants and the reactivity of the haloamines [1,5,6,9,10,13,17,18,23,26].

With regard to the acid dissociation constants, at 25°C, the pK_a (pH at which an acid is 50% dissociated) of hypochlorous acid is about 7.4 while the pK_a of hypobromous acid is about 8.8 (Fig. 3).

$$HOCl \overset{pK_a = 7.4}{\rightleftharpoons} H^+ + OCl^-$$

$$HOBr \overset{pK_a = 8.8}{\rightleftharpoons} H^+ + OBr^-$$

Fig. 3. Dissociation of hypohalous acids

Antimicrobial activities of the undissociated hypohalous acids are much greater than their respective anions (at relevant use-level concentrations) and therefore, bromine is significantly more effective than chlorine in alkaline systems especially above pH about 8.5 [1,6,18,23].

With regard to the reactivity of the haloamines, nitrogen–chlorine covalent bonds are much stronger than nitrogen–bromine bonds and so, the bromamimes are generally more reactive than the chloramines. For example, monobromamime is about as active against *Escherichia coli* and *Streptococcus faecalis* as are the hypohalous acids while monochloramine is very much less effective [9,23]. Thus, 'breakpoint' bromination is unneccesary and explains the observation that bromine is much more active than chlorine in water with high ammonia or amine concentration.

The effect of pH on effcacy against *L. pneumophila* (Table 1) can be explained by considering the hypohalous acid dissociation constants. About 25.1% of the hypochlorous acid was dissociated to the less active hypochlorite ion at pH 6.0 whereas about 79.4% was dissociated at pH 8.5 (a difference of 54.3%). Similarly, about 6.3% of the hypobromous acid is dissociated at pH 6.0 and about 49.9% is dissociated at pH 8.5 (a difference of 43.6%). This shows that the undissociated hypohalous acids were the most active species against *L. pneumophila* in our experiments.

Alleman et al. have recently compared wastewater disinfection of *Pseudomonas, Escherichia,* and *Streptococcus spp.* with chlorine and bromine [1,6]. These data clearly show that the effect of pH favors the bromine-based disinfection treatments particularly in water containing reduced nitrogen (e.g., ammonium ion). The cooling tower water used in our experiment was undoubtedly much different than the wastewater studied by Alleman. The effect of ammonia on the efficacy of chlorine and bromine against *L. pneumophila* in cooling water has never, to our knowledge, been studied.

The results show that there was no difference in efficacy between halogen regardless of its source in controlled, repeatable laboratory tests with *L. pneumophila* in the cooling tower water tested. Adequate controls are often not possible for field studies and therefore, laboratory results can often help in the interpretation of data and test hypotheses from field work. We acknowledge that laboratory efficacy results should never be extrapolated to predict field results. However, it is suggested that laboratory results can provide a useful tool to test hypotheses generated from field studies. But predictions about efficacy must never be based entirely upon laboratory tests.

Several excellent field studies have documented effective application of chlorine to prevent amplification of viable *L. pneumophila* in recirculating water [12,30,31, J. Nygren, Technical Director, Chem-Treat, Inc., personal communication of unpublished data]. In particular, Fliermans [12] described treatments of cooling systems containing high levels of the organism ($>10^6$ cell/ml by the direct fluorescent antibody technique) with continuous and shock applications of chlorine to prevent amplication of the *L. pneumophila* concentration in the cooling-water circuit.

Field studies that show effective application of BCDMH to prevent amplication of *L. pneumophila* concentration in cooling water also appear in the literature. Kurtz [15] discussed their observations from a two year study. Nineteen out of 239 (8%) of the cooling-water systems in the study were found to be infected with *L. pneumophila* before application of BCDMH. No *L. pneumophila* was isolated after application of 1–2 ppm free residual halogen continuously from BCDMH tablets via an erosion feeder (brominator) after two weeks of treatment or at six-month intervals. The total population of microorganisms was also considerably reduced in these cooling waters. An earlier study by Leinbach et al. (Abstr. Annu. Meet. Am. Soc. Microbiol. 1985, Q89, p. 272) showed effective application of BCDMH to control amplification of *L. pneumophila* under field conditions in a cooling tower.

Fliermans [11] showed data from a field test in which BCDMH did not reduce the viable population of *L. pneumophila* in cooling tower water. Later in the year, the same cooling water system was treated with NaOCl. The viable population of *L. pneumophila* was significantly reduced. We suggest, based on the evidence from field and laboratory studies, that these results were not due to an inherent difference in efficacy of halogen derived from different sources (NaOCl compared to BCDMH) but rather, to the particular circumstances operative during the field study itself. BCDMH was tested during the months between April and October and then, NaOCl was tested in the same system during November. The effect of the season on the overall population of microorganisms and on the physiochemical characteristics of the water was probably significant. For example, the average temperature of the water was nearly 5°C cooler during the NaOCl test.

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