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Sporicidal Properties of Chlorine Compounds: Applicability to Cooling Water for Canned Foods [1,2]

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ABSTRACT

Sporicidal effects of chlorine compounds as measured by many authors are reviewed. Since spore destruction rates and hypochlorous acid concentration appear to be related, the data from the several reports were recalculated in terms of time required for a 90% reduction in spores as a function of hypochlorous acid concentration. From these data a single graph was prepared. Results of the analysis indicate that *Bacillus* spores are more resistant to chlorine than *Clostridium* spores. The sporicidal effect of chlorine solutions increases with (*a*) an increase in free available chlorine, (*b*) a decrease in pH, and (c) an increase in temperature. Numbers of *Clostridium botulinum* and other spore-forming organisms in canning plant cooling water will depend on water quality factors such as the quantity of soil and organic matter, pH, temperature, and chlorine level. Control of these variables to desired levels in cooling water will reduce the probability of post-process infection of low-acid canned foods.

Microbial spoilage of canned foods can occur as a result of either underprocessing or the leakage of viable organisms into the container after heat processing. Post-processing infection is commonly called leaker spoilage and represents an economic loss for the processor and a potential public health hazard.

Cooling water is the primary source of microorganisms causing leaker spoilage. There is a direct correlation between high populations of bacteria in cooling waters and the probability of leaker spoilage (*12*). One way of decreasing this probability is to reduce the microbial load in the cooling water. The most widely used method to control the microbial population in water is the application of chlorine compounds. Bacterial spores are more resistant to chlorine than vegetative cells and, therefore, the number of viable spores in cooling water could be used as an indication of the effectiveness of the chlorination programs (*10*).

Put et al. (*12*) carried out studies in eight canning plants. They found chlorine levels from 0.1 to 2 ppm in the cooling water. *Bacillus* spores were found in the cooling water of seven plants and *Clostridium* spores in four of the eight plants. They found larger relative numbers of *Bacillus* spores than *Clostridium* spores. This condition was especially evident when surface water supplies were used for cooling.

In this report we have identified and reviewed the results of research on the sporicidal properties of chlorine compounds. The application of this review is to cooling waters for low acid canned foods; specifically, to insure that there is minimal public health hazard from post-process leakage of organisms into containers.

BACILLUS SPORES

Most of the work on the sporicidal effects of chlorine compounds has been done using aerobic spore-forming organisms. Two of the early studies on spore destruction by chlorine compounds dealt with a spore-forming organism isolated by Charlton and Levine (*3*) and named *Bacillus metiens.* A composite of the results of studies by Charlton and Levine (*3*) and Rudolph and Levine (*13*) are shown in Table 1. These data for calcium hypochlorite indicate the important role of pH on the sporicidal effect of the chlorine. At a pH of 7.3, a 99% reduction in viable spores was obtained in less than 0.33 min with 1000 ppm of chlorine, while at a pH of 11.3 and 1000 ppm of chlorine 70 min was required for a 99% reduction.

Charlton and Levine (*3*) did studies comparing calcium hypochlorite with Chloramine T; their results are also shown in Table 1. The concentration of Chloramine T in Table 1 are those in the solution as initially prepared. They did not determine the amount of free available chlorine (FAC) released from Chloramine T. The FAC concentration was probably low since chloramines release chlorine very slowly (*11*). As is evident from data in Table 1, chloramine T was not effective in killing spores. Even at high pH values (10 to 11.3), calcium hypochlorite was a more effective sporicide than was Cloramine T at low pH values (6.0 to 8.8). Cousins and Allan (*4*) exposed *Bacillus subtilis* spores to 1000 ppm of Chloramine T at pH 6.5 and reported no sporicidal effect in 4 h, while 100 ppm of FAC from sodium hypochlorite at pH 8.0 reduced the spore population by 99% in 60 min. These data indicate that the FAC released from sodium or calcium hypochlorite is more effective in killing spores than the combined available chlorine of Chloramine T. Chlorine in the combined form should not

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TABLE 1. *Effect of concentration and pH of chlorine compounds on the destruction of* Bacillus metiens *spores*[a]

Compound	pH	Conc. of chlorine[b] (ppm) FAC	Conc. of chlorine[b] (ppm) FAC and CAC	Time to kill 99%[c] (min)
Calcium hypochlorite (*13*)	6.0	25		2.5
	7.0	25		3.6
	8.0	25		5.0
	9.0	25		19.5
	10.0	25		80.6
	10.0	100		42.4
	10.0	500		20.6
Calcium hypochlorite (*3*)	7.3	1000		<0.33
	10.4	100		70.0
	11.3	1000		70.0
Chloramine T (*3*)	6.0	—[d]	1000	900.0
	6.0	—	2000	324.0
	6.0	—	4000	156.0
	8.7	—	2000	3840.0
	8.8	—	4000	1404.0

[a] Data adapted from Charlton and Levine (*3*) and Rudolph and Levine (*13*).

[b] FAC = Free available chlorine; CAC = combined available chlorine; FAC plus CAC is the total available chlorine.

[c] Temperature was at 20 C for calcium hypochlorite test and at 25 C for Chloramine T test.

[d] Indicates amount FAC released was not determined.

be considred for chlorination of cooling water as it is an ineffective sporicide.

Several other papers have been published on the chlorine resistance of various species of *Bacillus* spores. Data from these reports are presented in Table 2. To make the results from the several studies comparable, the time for a 90% reduction in numbers of spores and the concentration of hypochlorous acid were calculated from the available data in each report.

The data in Table 2 indicate that for all spores tested the sporicidal activity of the solution increased with increasing amounts of hypochlorous acid (HOCl). The amount of HOCl in a chlorine solution is a function of the solution pH (*11, 15*). At pH values approaching 7 almost all the FAC is present as HOCl, however at pH values near 10 all the FAC is present as hypochlorite ion (OCl^-). Brazis et al. (*1*) reported that HOCl is 100 times more effective in killing spores than OCl^-.

A search was made for an empirical relationship to visually show the effect of HOCl on spore destruction. As a result Fig. 1 was developed, in which the logarithm of the time for a 90% reduction in the number of spores is shown as a function of the logarithm of the HOCl

TABLE 2. *Summary of data for destruction of* Bacillus *spores by chlorine*[a]

Organism	Chlorine compound	Test temp. (C)	pH	FAC[b] (ppm)	Calculated HOCl (ppm)	Time for 90% reduction (min)
B. cereus (*4*)	NaOCl	21	6.5	50	43	1.5
			8.0	100	25	2.5
B. cereus (*15*)	NaOCl	25	7.0	100	75	0.88
			5.2	150	149	0.18
			7.0	150	113	0.40
			8.0	150	38	1.2
B. coagulans (*9*)	NaOCl	20	4.5	20	20	2.5
			6.8	20	17	6.0
			4.5	10	10	8.5
			6.8	10	8.5	12.0
			7.8	10	3.5	23.0
			4.5	5	5.0	21.0
			6.8	5	4.3	28.0
			7.8	5	1.7	59.0
B. macerans (*11*)	NaOCl	21	6.0	15	14	4.3
			6.5	15	12.9	4.6
			7.0	15	11.3	6.4
			7.5	15	7.5	10.3
			8.0	15	4.0	21.0
B. metiens (*13*)	CaOCl	20	6	25	24	1.25
			7	25	19	1.8
			8	25	6	2.5
			10	25	<1	40.3
			10	100	<1	21.2
			10	500	<1	10.3
B. glodbigii (*1*) (*subtilis*)	—	22	6.2	1.8-1.9	1.7	22.8
			6.2	.11-.24	.1-.2	255.0
			7.2	2.5-2.6	1.6	20.5
			7.2	.15-.34	.1-.2	270.0
			8.6	23.8-24.8	1.7	22.5
			8.6	1.8-2.3	.13-.16	285.0
			10.5	454	<1	31.7
			10.5	21.9-23.1	<1	330.0
B. stearothermophilus (*2*)	NaOCl	25	7	1000	750	1.0
			7	2000	1500	.78

[a] References in parentheses after species name

[b] Free available chlorine

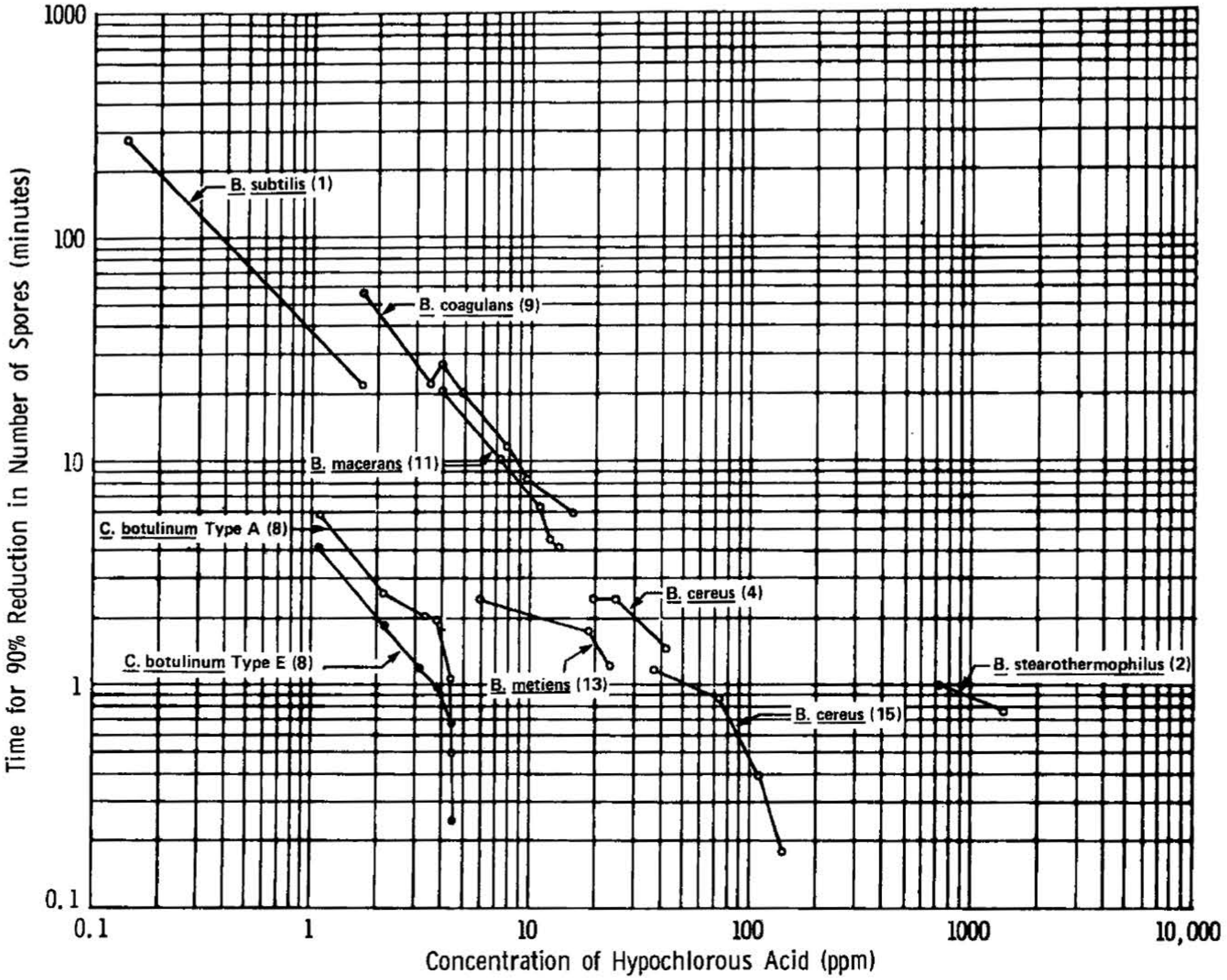

Figure 1. *Summary of data on the sporicidal effect of chlorine solutions expressed as the time to reduce the spore population by 90% as a function of the concentration of hypochlorous acid in parts per million. Numbers in parentheses refer to references.*

concentration. The data in Table 2 are all displayed graphically in Fig. 1.

CLOSTRIDIUM SPORES

Only a few reports have been published on the resistance of *Clostridium* spores to chlorine. Tonney et al. (*14*) found all bacterial spores to be 10 to 1100 times more resistant to chlorine than vegetative cells, and as a group the aerobic spore-formers to be more resistant than the anaerobic spore-formers.

Dye and Mead (*6*) evaluated the effect of chlorine on eight strains of *Clostridium* spores. Their results for spores exposed to 5 ppm of free available chlorine (pH 8.3) at 10 C are shown in Table 3. *Clostridium welchii* (*perfringens*) spores were the most resistant; *Clostridium bifermentans* and *Clostridium caloritolerans* spores were the least resistant. Chloramine T at 200 ppm (pH 9) was not very effective in reducing the number of any of the *Clostridium* spores tested. There was less than a 2-log reduction of any of the eight strains in a 2-h test period. Also *B. subtilis* spores were subjected to 100 ppm chlorine at a pH of 9.8 and found to be considerably more resistant than any of the *Clostridium* spores tested. Results of Dye and Mead confirm the work of Tonney et al. (*14*) that *Clostridium* spores have less resistance to chlorine than *Bacillus* spores (Fig. 1).

TABLE 3. *Effect of 5 ppm free available chlorine (pH 8.3) on destruction of* Clostridium *spores at 10 C*

Strain	Time to kill 99% (min)[a]
C. welchii 6719	>60
C. tertium	12
C. histolyticum 10	60
C. histolyticum 503	10
C. bifermentans	9
C. sporogenes	17
C. caloritolerans	6

[a] Determined from data of Dye and Mead (*6*).

Clostridium botulinum organisms in canning plant cooling waters are a public health hazard. The post-processing entry of a single *C. botulinum* organism into a food container is a deadly problem (*7*). The work of Tonney et al. (*14*) was one of the earliest published reports on the resistance of *C. botulinum* spores to chlorine compounds. They reported the concentration of free chlorine required to kill the spores in 15-30 sec. Their results for *C. botulinum* Type A, Type B, and a third, unidentified type were 15, 17.5, and 25 ppm of chlorine, respectively. The initial number of spores was not clearly reported in this paper. The temperature at which the test was done and the pH of the chlorine solution were also lacking, so any meaningful comparisons with other studies cannot be made.

Dozier (*5*) subjected *C. botulinum* spores to sodium hypochlorite solutions at concentrations of 4,500 and 5,000 ppm for 1 h and found it to be ineffective. The tests were done at 20 and 37 C, but no information was given regarding the pH or the amount of free residual chlorine.

Recently, Ito et al. (*8*) conducted a thorough study on the effectiveness of commercial germicides on spores of *C. botulinum* Types A, B, and E. Results of their tests at 25 C are shown in Table 4. These results indicate that calcium hypochlorite, sodium hypochlorite, and gas chlorinated water are approximately of equal effectiveness in causing a 4-log (99.99%) reduction in numbers of viable spores. It can be seen that the resistance of Type A and Type B spores are similar and that Type E spores have approximately one-half the resistance of Type A and Type B spores. This difference in resistance by spore type is also true for the two chlorine-detergent compounds tested, but these compounds were not as effective in reducing the number of viable spores as calcium hypochlorite, sodium hypochlorite, and gas chlorinated water. Ito et al. also evaluated the effect of pH on the germicidal efficiency of calcium hypochlorite. The results are presented in Table 5 and indicate that as pH increases, the rate of destruction decreases. The results are not surprising since chlorine is more effective as a sporicidal agent at acid pH values where hypochlorous acid predominates (*11*).

TABLE 4. *Effect of concentration of chlorine compounds on destruction of* Clostridium botulinum *spores (Types A, B, and E) at 25 C*[a]

Compound[b]	Conc. of compound (ppm)	Type	Time for 99.99% kill (min)
Calcium hypochlorite	4.5	A	8.0
Calcium hypochlorite	4.5	B	7.4
Calcium hypochlorite	4.5	E	4.0
Sodium hypochlorite	4.5	B	8.0
Sodium hypochlorite	4.5	E	3.8
Gas chlorinated water	4.5	A	8.2
Gas chlorinated water	4.5	B	8.0
Gas chlorinated water	4.5	E	4.6
Dichloro(s) triazinetrione	10.0	A	15.0
Dichloro(s) triazinetrione	20.0	B	>15.0
Dichloro(s) triazinetrione	10.0	E	7.2
Trichloro cyanuric acid	4.5	A	17.0
Trichloro cyanuric acid	5.0	E	9.0

[a] Data from Ito et al. (*8*).

[b] 1×10^4 spores/ml, pH 6.5 phosphate buffer.

The effect of temperature of the calcium hypochlorite solution on time for a 99.99% kill of *C. botulinum* Types A, B, and E spores was also investigated by Ito and co-workers. Their results are presented in Table 6. The time for 99.99% kill decreased with increasing temperature; the kill-time at 25 C was only 0.3 to 0.4 of the kill-time at 15 C.

TABLE 5. *Resistance of* Clostridium botulinum *spores types A, B, and E to calcium hypochlorite solutions at various pH values at 25 C*[a]

pH[b]	Time for 99.99% kill (min) Type A	Type B	Type E
3.5	2.1	3.7	1.0
5.0	4.3	6.2	2.8
6.5	7.8	7.8	4.0
7.0	8.5	8.7	5.0
7.5	10.6	11.2	7.6
8.0	24.0	26.6	17.0

[a] Data from Ito et al. (*8*).

[b] 4.5 ppm free available chlorine, 1×10^4 spores/ml.

TABLE 6. *Resistance of* Clostridium botulinum *spores types A, B, and E to calcium hypochlorite solutions at various temperatures*[a]

Temperature[b] (C)	Time for 99.99% kill (min) Type A	Type B	Type E
5	35.0	40.0	24.0
15	15.0	20.0	10.0
25	6.0	6.0	4.0

[a] Data from Ito et al. (*8*).

[b] 4.5 ppm free available chlorine, 1×10^4 spores/ml, pH 6.5 phosphate buffer.

Ito et al. (*8*) also reported that organic debris, such as peptone, will combine with the free available chlorine, reducing the amount of free chlorine in the solution, ultimately to the point where it is ineffective. Therefore, in a commercial application such as a cooling canal, chlorine must be added continuously so that the design free available chlorine level is maintained continuously.

Data from Ito et al. for *C. botulinum* Type A and Type E are also shown in Fig. 1. It can be observed in Fig. 1 that 2 ppm of HOCl will reduce a population of *C. botulinum* spores by 90% in 2 to 3 min, whereas 20 to 50 min are required for a 90% reduction in the number of *Bacillus* spores.

DISCUSSION

An objective of this project was to not only review research regarding sporicidal effect of chlorine but to develop data to predict spore destruction as a function of chlorine level. With the exception of the data of Cerf et al. (*2*) the data in Fig. 1 appear to form an overall pattern. If we disregard the data for *B. stearothermophilus* (*2*), then for both *Bacillus* and *Clostridium* spores, at the low effective HOCl concentration, the logarithm of the time for 90% reduction is a linear function of the logarithm of the HOCl concentration. As the HOCl concentration increases to where the time for a 90% reduction is 1 to 2 min, the rate of destruction increases. Figure 2 is a modification of the graph in Fig. 1 to include lines which we believe represent general conditions for destruction of *Bacillus* and *Clostridium* spores as a function of HOCl concentration.

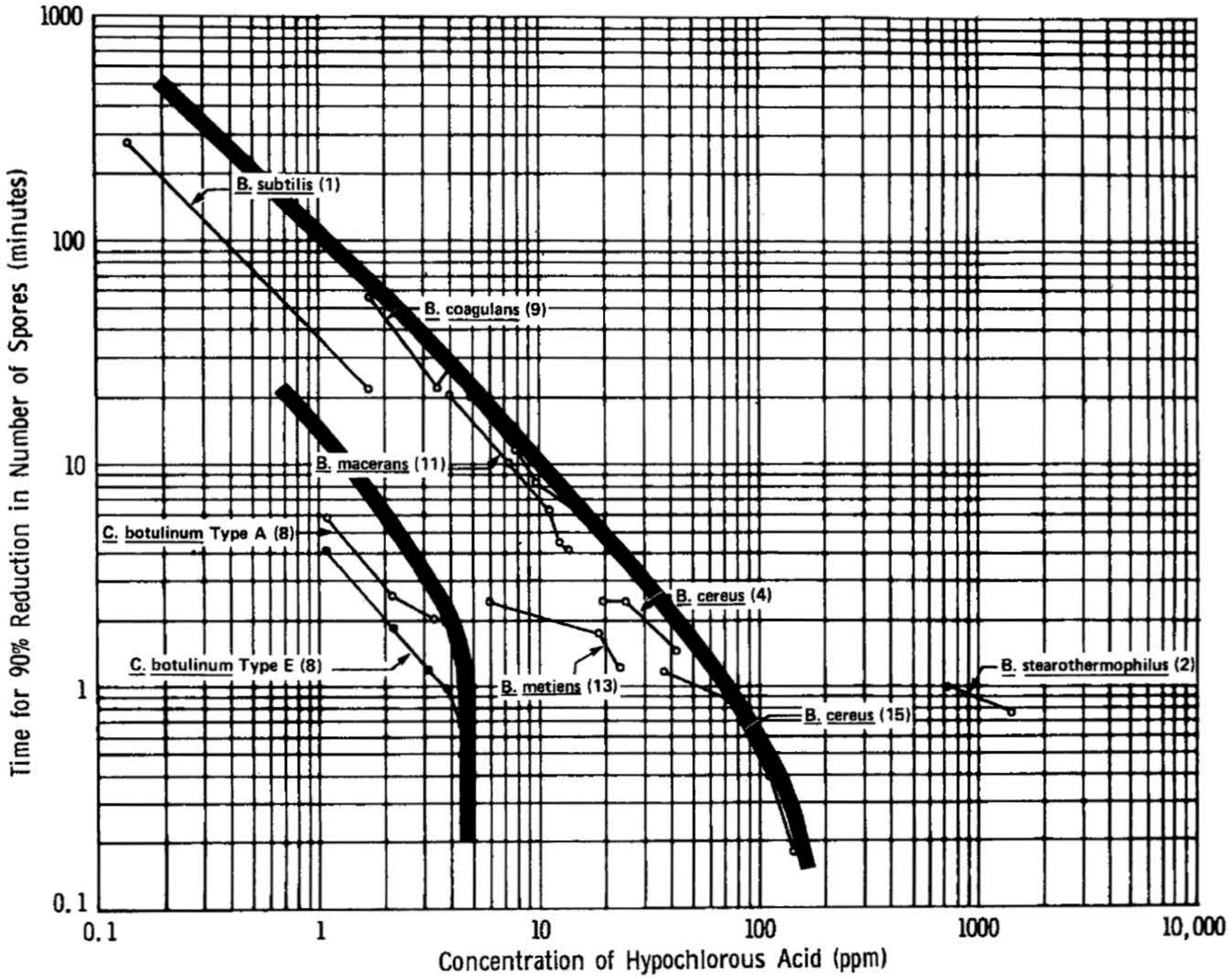

Figure 2. *Suggested relationship between the time to reduce the bacterial spore population by 90% and the concentration of hypochlorous acid (ppm) for* Bacillus *and* Clostridium *spores.*

When chlorine compounds are added to water, so there is free available chlorine present, the solution is both bactericidal and sporicidal. Vegetative bacterial cells are more easily killed than spores; and *Clostridium* spores are more easily killed than *Bacillus* spores. The lethal effect of the chlorine in solution increases with: (*a*) an increase in the free chlorine concentration in the solution, (*b*) a decrease in pH, and (*c*) an increase in temperature.

The relative microbiological quality of the water in the canning plant cooling system will be a function of the quality of the water that is added to the system. Quality factors are: the amount and source of soil and organic matter that are added to the water, pH, temperature, and chlorine level. From the information in Fig. 2 and the literature cited therein it is anticipated that the predominant flora will be resistant *Bacillus* spores when free chlorine levels of 2 to 5 ppm with a pH in the range of 7 to 7.5 are maintained in the cooling water.

The public health hazard from the post-process leakage of *C. botulinum* spores into cans of low acid food should be extremely small if the cooling water is properly chlorinated, the pH level is controlled, and the addition of soil or any other outside source of *C. botulinum* spores is eliminated. Since *C. botulinum* will not likely multiply in cooling water or in a cooling canal containing chlorinated water, only the introduction of large numbers of *C. botulinum* spores into improperly chlorinated cooling water will create a public health hazard.

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