A study of the inhibitory influence of cyanuric acid on disinfection of water in swimming pools indicates that cyanuric acid inhibits the disinfection action of chlorine, the source of which may be either calcium hypochlorite or chlorinated cyanurates. The data indicate further need for detailed field studies under conditions similar to those used in this investigation.

AN EVALUATION OF THE INHIBITORY INFLUENCE OF CYANURIC ACID UPON SWIMMING POOL DISINFECTION

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ADEQUATE protection of swimming pool waters has been of paramount importance for many years to health officials who are charged with responsibility for the safety and health of the public. The use of the chlorine compounds or of chlorine gas has been written into practically every regulation concerned with the disinfection of drinking water. The limitations of chlorine for swimming pool water treatment have been discussed thoroughly over the years—its instability, its capacity for forming amines and other substances which are either less effective disinfecting agents or are irritating to the eyes of bathers—are well known. New compounds which would eliminate these problems have been sought. One group of chemicals which has been proposed for use as swimming pool disinfectants is the chlorinated cyanurates, which made their appearance as sanitizing compounds and household bleaches in 1958. They appeared to be well adapted for swimming pool water disinfectants as they were soluble, neutral in pH, and provided a relatively stable chlorine residual in the presence of sunlight. A number of these products were placed upon the market and include: (1) trichloroisocyanuric acid, (2) dichloroisocyanuric acid, (3) potassium dichloroisocyanuric acid, (4) sodium dichloroisocyanurate.

The literature concerned with these various compounds has been reviewed comprehensively by Anderson[1] who undertook an evaluation of the effect of cyanuric acid on the bactericidal properties of chlorine under laboratory conditions at 20° C, approximately the conditions developed by Butterfield, et al.[2] In this study, Anderson found that at a pH of 7.0 with Staphylococcus aureus as a test organism and a concentration of 0.25 mg/l of free residual chlorine, the time required to effect a 99 per cent kill was 0.5 minutes; 11 minutes when 25 mg/l of cyanuric acid was added to the chlorine solution; 15 minutes when 50 mg/l of cyanuric acid was used;

and 25 minutes at a concentration of 100 mg/l of cyanuric acid. He obtained similar results with Streptococcus faecalis at both pH 7.0 and 9.0. He concluded that "higher chlorine concentrations may be required in the presence of cyanuric acid than in the absence of cyanuric acid to achieve 99 per cent bacterial death in one minute."

The present report deals with one aspect of a larger study carried out to evaluate and extend Anderson's work and is concerned with the effect of three compounds, namely, calcium hypochlorite, trichloroisocyanuric acid, and potassium dichloroisocyanurate, with and without 50 ppm of cyanuric acid, upon three test organisms, Escherichia coli, Streptococcus faecalis, and Staphylococcus aureus, in chlorine demand free water having a pH of 7.2, an alkalinity of 50 mg/l, and at 25° C temperature.

Methods

The basic technic followed for testing the bactericidal effectiveness of the compounds was that of Ortenzio and Stuart[8] with minor variations which will be indicated.

All glassware used was acid cleaned between each test run. This was found to be absolutely essential for accurate results.

The test organisms used included the standard testing strains Escherichia coli No. 11229, Streptococcus faecalis PRD (ATCC 8043), and Staphylococcus aureus FDA 209 (ATCC 6538). The E. coli and Staph. aureus cultures were maintained as prescribed on nutrient agar C and transferred for testing purposes to nutrient agar (BBL) while the Strep. faecalis stock cultures were kept and used on trypticase soy agar (BBL). Initially, the E. coli strain was found to be morphologically rough, and constant reisolation was necessary to obtain and maintain smooth cultures. All bacterial suspensions were filtered through sterile Whatman filter paper. E. coli and Strep. faecalis were used in concentrations of 1,000,000 organisms/1.0 ml and Staph. aureus at 500,000 organisms/1.0 ml. Standardization of suspensions was carried out on a B&L Spectronic 20 spectrophotometer.

Distilled water was prepared fresh each day in a glass still and was sterilized by filtration through a membrane filter. The sterile water was adjusted to a pH of 7.2 (as determined by a glass electrode pH meter) and an alkalinity of 50 mg/l.

The test chemical solution was prepared daily after proper standardization tests were carried out. Cyanuric acid was added in sufficient quantities to provide 50 mg/liter by weight.

All chlorine measurements were made with an amperometric titrator (Wallace & Tiernan) and both free and total residuals were determined.

Results

The results using E. coli were found to vary depending upon the smoothness of the culture. Chambers[4] discussed some of the discrepancies that had been reported by other investigators using this organism and suggested that the so-called "skips" or irregularities observed were due to the roughness of the culture. He recommended that the bacterial suspensions be filtered through sterile Whatman filter paper prior to use. This was found to be of value, but not as important as frequent subculturing of the test organism to keep and maintain it in the smooth phase.

Under the conditions of the test and in the absence of cyanuric acid, E. coli was killed rapidly by concentrations of calcium hypochlorite, trichloroisocyanuric acid, and potassium dichloroisocyanurate providing 0.11 mg/l of "free" or available residual chlorine (Table 1). The addition, however, of 50 mg/l cyanuric acid to any of these

Table 1—Concentrations of free available residual chlorine (mg/l) needed to obtain 99.999 per cent inactivation of Escherichia coli, Streptococcus faecalis, and Staphylococcus aureus in the absence of cyanuric acid and with 50 mg/l cyanuric acid present for solutions of calcium hypochlorite, trichloroisocyanuric acid, and potassium dichloroisocyanurate, 25°C, pH of 7.2, alkalinity of 50 mg/l

Test organism	Time of exposure	Amount of cyanuric acid present	Source of residual chlorine: calcium hypochlorite	trichloroisocyanuric acid	potassium dichloroisocyanurate
E. coli	30 sec	0.0 mg/l	<0.11	<0.11	<0.11
E. coli	30 sec	50.0 mg/l	0.97	0.80	>0.80
Strep. faecalis	2 min	0.0 mg/l	<0.10	<0.11	0.11
Strep. faecalis	2 min	50.0 mg/l	0.51	0.42	0.43
Staph. aureus	5 min	0.0 mg/l	<0.40	<0.61	0.64
Staph. aureus	5 min	50.0 mg/l	1.64	>0.90	>1.62

compounds reduced the bactericidal effectiveness of each compound so as to either require a higher residual of "free" chlorine or a longer period of time for kill for the same concentration of "free" chlorine (Table 2). The action of potassium dichloroisocyanurate seemed to be bacteriostatic while that of trichloroisocyanuric acid and calcium hypochlorite was bactericidal at higher concentrations. The suggested 99.999 per cent reduction of E. coli within a 30-second contact period is not fulfilled in the presence of 50 mg/l of cyanuric acid at values for free residual less than 0.97 mg/l* (footnote on page 304).

It has been recommended also that a swimming pool water disinfectant be capable of providing a 99.999 per cent inactivation of Strep. faecalis within a

Table 2—Per cent of survivors of Escherichia coli in solutions of calcium hypochlorite, trichloroisocyanuric acid, and potassium dichloroisocyanurate of various concentrations of free available residual chlorine in the presence of 50 mg/l of cyanuric acid for various contact times, 25°C, pH of 7.2, alkalinity of 50 mg/l.

Source of chlorine	Amount of free available residual chlorine mg/l	Time of contact: 30 sec	1 min	2 min	3 min	4 min	5 min
Calcium hypochlorite	0.45	12.272	0.563	0.000			
	0.56	7.375	0.118	0.000			
	0.81	4.222	0.041	0.000			
	0.89	2.071	0.013	0.000			
	0.97	0.000					
Trichloroisocyanuric acid	0.43	30.000	4.272	0.018	0.000		
	0.74	2.909	0.002	0.000			
	0.80	0.000					
Potassium dichloroisocyanurate	0.43	12.909	10.909	0.036	0.025	0.016	0.000
	0.80	5.273	0.070	0.000			

Table 3—Per cent of survivors of Streptococcus faecalis in solutions of calcium hypochlorite, trichloroisocyanuric acid, and potassium dichloroisocyanurate of various concentrations of free available residual chlorine in the presence of 50 mg/l of cyanuric acid for various contact times, 25°C, pH of 7.2, alkalinity of 50 mg/l.

Source of chlorine	Amount of free available residual chlorine mg/l	Time of contact 30 sec	1 min	2 min	3 min	4 min	5 min
Calcium	0.51	1.666	0.001	0.000			
hypochlorite	0.61	0.045	0.000				
	0.65	0.018	0.000				
Trichloroiso-	0.42	0.500	0.000				
cyanuric acid	0.69	0.138	0.000				
Potassium	0.43	2.000	0.333	0.000			
dichloroiso-	0.82	1.467	0.045	0.000			
cyanurate	1.04	0.933	0.007	0.000			

two-minute contact period.* In the present study all three compounds were able to provide this degree of inactivation within the concentrations of free residual chlorine used (Table 1). The addition of cyanuric acid again reduced the rapidity of bactericidal action (Table 3) and the results were similar to those obtained with E. coli. The bacteriostatic effect of potassium dichloroisocyanurate was marked. Strep. faecalis, which investigators have reported to be more resistant than E. coli to chlorine compounds, appears to be more rapidly destroyed by the three compounds in the presence of 50 mg/l of cyanuric acid than does E. coli under similar test conditions. No explanation can be offered for this behavior at the present. Studies into the effects of the chlorinated cyanurates upon bacterial metabolism are suggested.

Staph. aureus proved to be a most interesting test organism. No official recommendations have been proposed for the length of time needed to obtain a 99.999 per cent kill using this organism. An arbitrary designation of five minutes is used in this study (Table 1). The details of the results may be seen in Figures 1, 2, and 3, and Tables 4 and 5. In the absence of cyanuric acid, calcium hypochlorite and trichloroisocyanuric acid are effective in killing this organism within a five-minute contact period at concentrations of "free" chlorine residuals between 0.4 and 0.6 (mg/l) while potassium dichloroisocyanurate appears to be bacteriostatic and slow acting. When cyanuric acid is present, both the concentration of "free" chlorine residual and the length of time to bring about 99.999 per cent bacterial reduction are increased markedly.

Discussion

These data provide valuable information about the disinfection of swimming pool water. However, complete interpre-

* A 99.999 per cent reduction of E. coli in 30 seconds and of Strep. faecalis in two minutes are the criteria used by the Pesticides Regulation Division, Agricultural Research Service, US Department of Agriculture in evaluating the bactericidal efficiency of commercial disinfectant preparations recommended for use in swimming pool water disinfection. These criteria have also been adopted by the National Sanitation Foundation Advisory Committee for Swimming Pool Water Treatment Chemicals and/or Processes.

tation cannot be made without accompanying field test data. This study is limited to the extent that the disinfecting action studied is the result of single applications of chemicals in test solutions. The effect of hydrolysis products of previously applied chemicals upon the disinfectant was not, and could not be, evaluated.

The data of the disinfecting action of calcium hypochlorite with and without 50 mg/l of cyanuric acid can be in-

Figure 1—Per cent of surviving organisms of Staphylococcus aureus for varying concentrations of free available residual chlorine from calcium hypochlorite with and without 50 mg/l of cyanuric acid—pH=7.2; alkalinity=50 mg/l

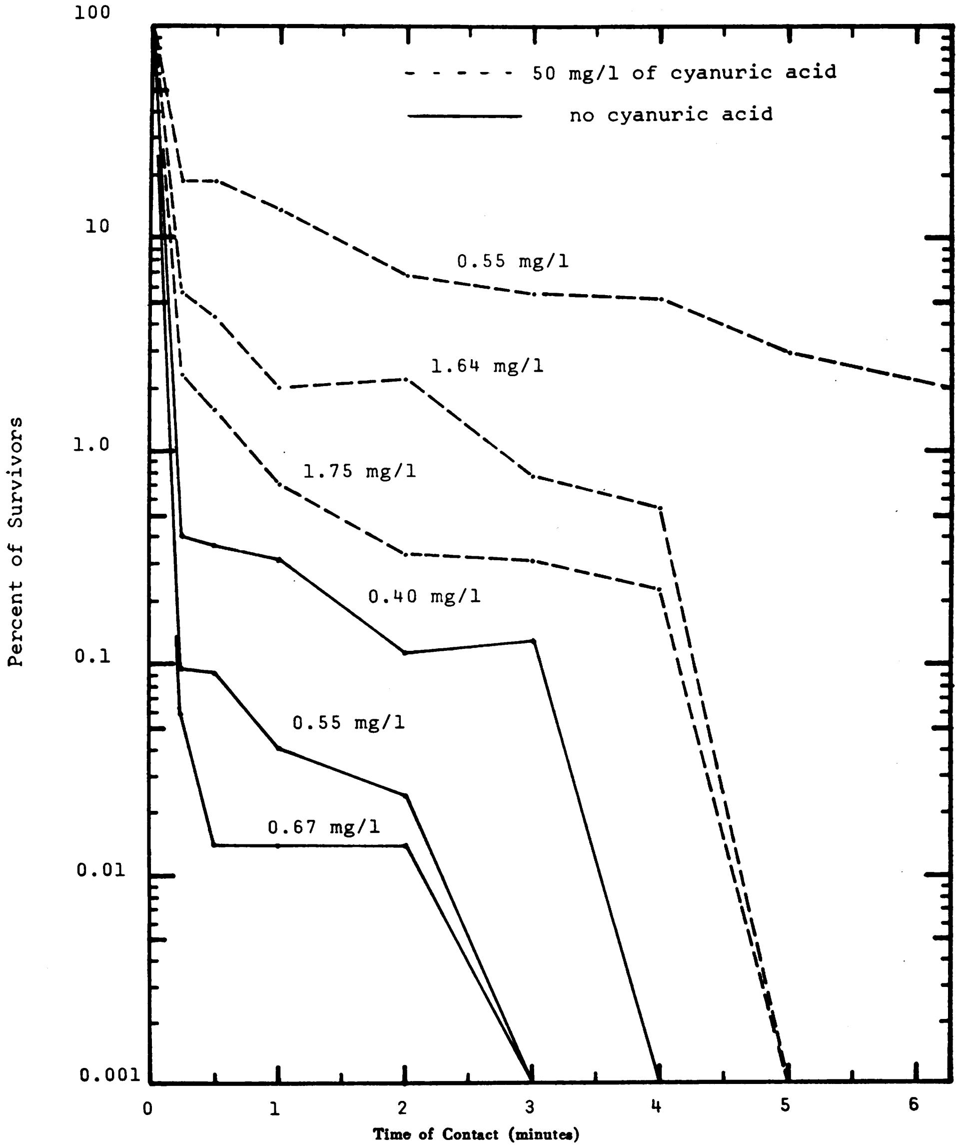
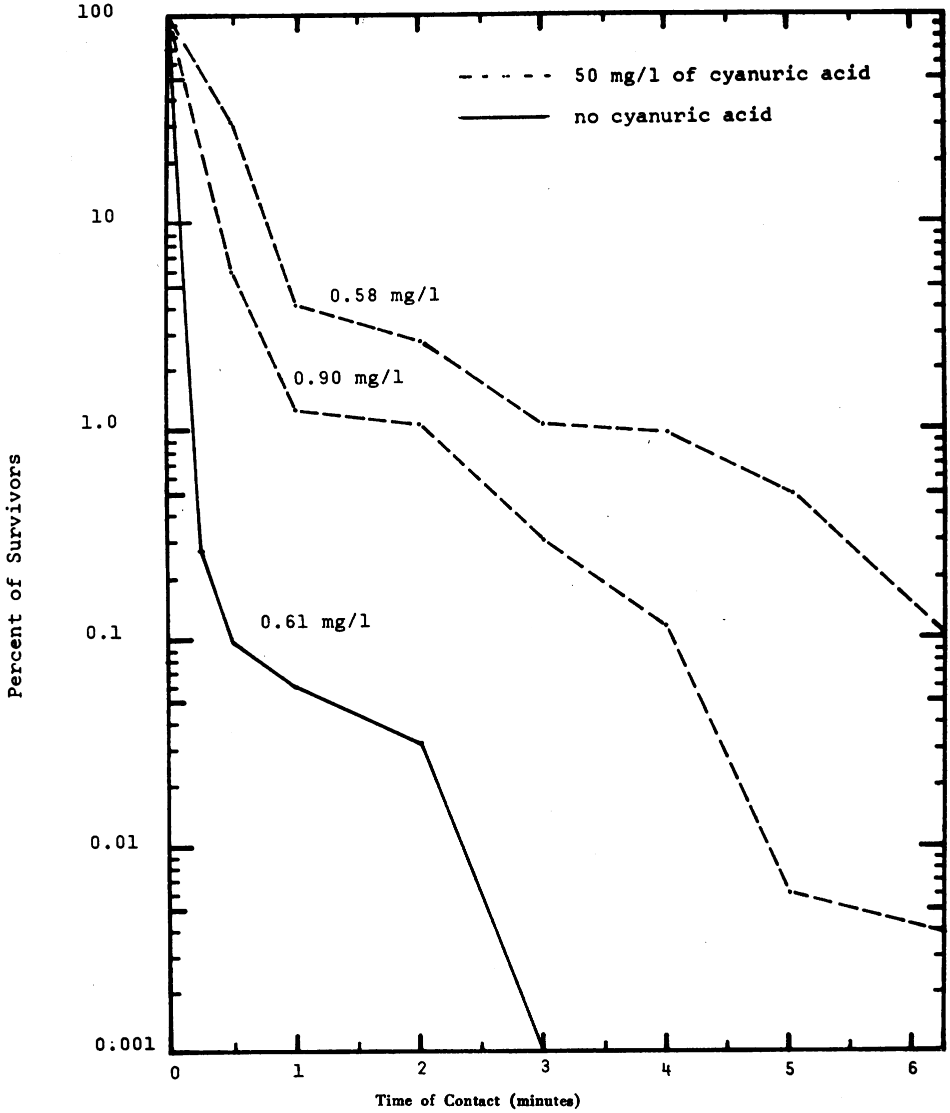


Figure 2—Per cent of surviving organisms of Staphylococcus aureus for varying concentrations of trichloroisocyanuric acid measured as free available residual chlorine with and without 50 mg/l of cyanuric acid—pH=7.2; alkalinity=50 mg/l



terpreted meaningful, as disassociation of calcium hypochlorite in water does not yield chemicals that interfere with subsequent additions of the hypochlorite, assuming that pH and alkalinity values are maintained at relatively constant values. The results of this study suggest that 50 mg/l of cyanuric acid retard the disinfecting action of calcium hypochlorite by a factor of 4 or more, depending upon which bactericidal index is used. Specifically, these data denote that to obtain a 99.999 per cent of kill of E. coli in 30 seconds, the "free" chlorine residual value must be increased more than eightfold when 50 mg/l of cyanuric acid is present in the water as compared to the amount of "free" chlorine residual needed when no cyanuric acid is present. To get a 99.999 per cent kill of Strep. faecalis in two minutes, approximately a fourfold increase in "free" chlorine is necessary; and to obtain a 99.999 per cent kill of Staph. aureus in five minutes, approximately a three- or fourfold increase seems to be needed.

Figure 3—Per cent of surviving organisms of Staphylococcus aureus for varying concentrations of potassium dichlorisocyanurate measured as free available residual chlorine with and without 50 mg/l of cyanuric acid—pH=7.2; alkalinity=50 mg/l

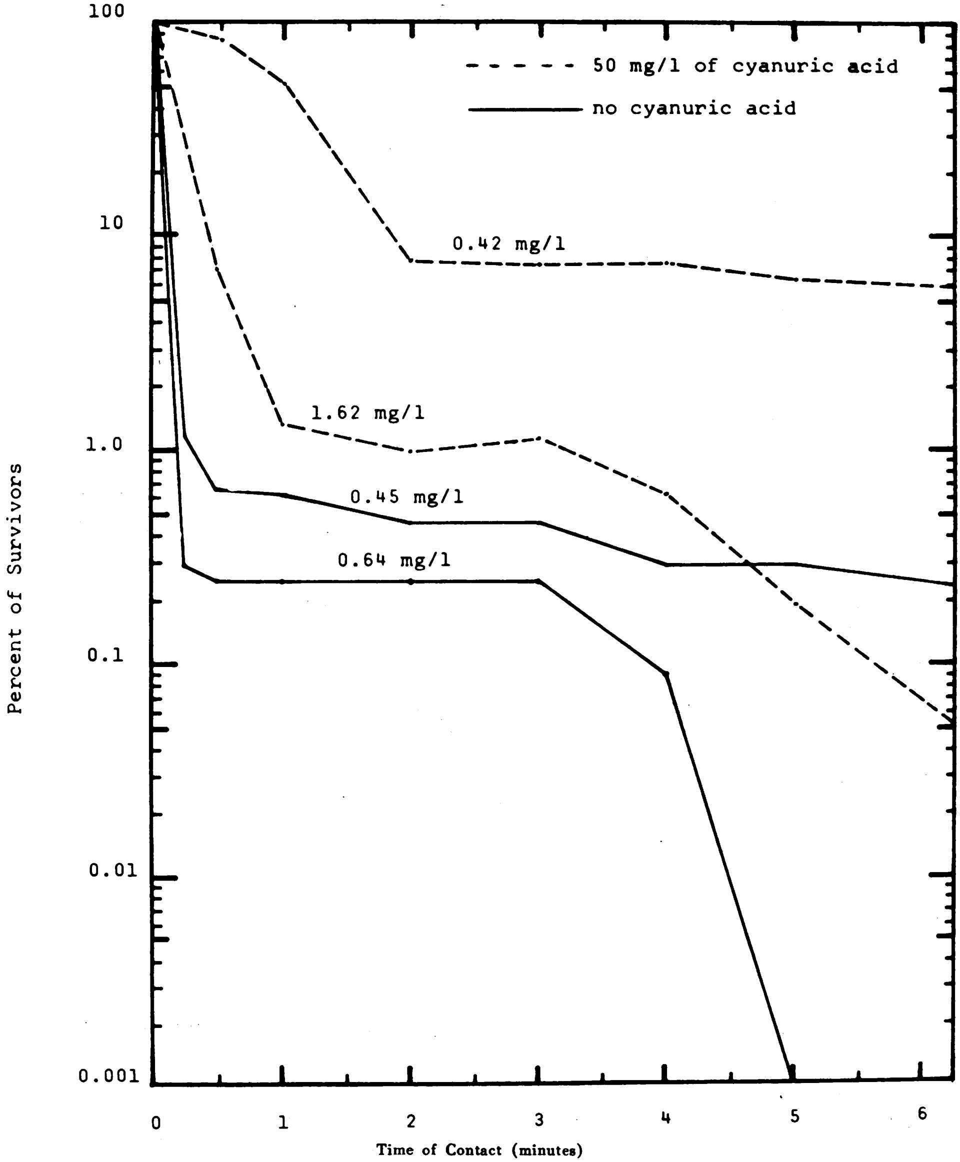


Table 4—Per cent of survivors of Staphylococcus aureus in solutions of calcium hypochlorite, trichloroisocyanuric acid, and potassium dichloroisocyanurate of various concentrations of free available residual chlorine with no cyanuric acid present for various contact times, 25°C, pH of 7.2, alkalinity of 50 mg/l

Source of chlorine	Amount of free available residual chlorine mg/l	Time of contact 30 sec	1 min	2 min	3 min	4 min	5 min	10 min
Calcium hypochlorite	0.40	0.357	0.310	0.114	0.129	0.000		
	0.55	0.093	0.040	0.024	0.000			
	0.67	0.014	0.014	0.014	0.000			
Trichloroiso-cyanuric acid	0.61	0.095	0.060	0.031	0.000			
Potassium dichloroiso-cyanurate	0.45	0.642	0.619	0.452	0.452	0.285	0.285	0.116
	0.64	0.246	0.246	0.246	0.246	0.086	0.000	

Interpretation of the data of the disinfecting action of trichloroisocyanuric acid and potassium dichloroisocyanurate with and without 50 mg/l of cyanuric acid present is limited to generalities. Also, some of the observations made, and the evaluations of them, may be of academic interest only.

These data suggest that 50 mg/l of cyanuric acid exert a similar retarding effect upon the disinfecting powers of trichloroisocyanuric acid and potassium dichloroisocyanurate as they do upon calcium hypochlorite. The magnitude of the delaying action is difficult to state as end-points were not reached in three of the six conditions studied.

In all probability, there is little practical significance to the data concerning the ability of the two chlorinated cyanurates evaluated to kill the test organisms in solutions containing no cyanuric acid. Swimming pool water disinfection is, or should be, a continual process with the disinfectant being added to virtually the same water repeatedly. Compounds formed by chemical reactions between the disinfectant and the water and other dissolved chemicals remain in the swimming pool water and are removed usually by dilution only. It has been postulated that, under field conditions with continuous application of a chlorinated cyanurate, the disinfecting action obtained will be more similar to the laboratory results obtained from the combined effect of the chlorinated cyanurate and an amount of cyanurate acid (probably between 25 mg/l and 100 mg/l) than of the chlorinated cyanurate alone. This reasoning seems to be acceptable as there appears to be sufficient data to suggest that the products of hydrolysis of potassium dichloroisocyanurate and trichloroisocyanuric acid are similar to those formed when cyanuric acid is dissolved in water.

These data indicate the need for detailed field studies to be conducted using the same disinfectants and under similar conditions of use as those employed in this study. This additional information would be of material aid in formulating more concise conclusions about the disinfecting action of the chlorinated cyanurates and of the inhibitory effects of cyanuric acid upon these compounds and calcium hypochlorite.

The findings of this study suggest the need for revising standards of the minimum values of residual disinfectant which must be maintained in swimming pool water if cyanuric acid is used as a stabilizing agent with elemental chlorine and hypochlorite solutions. If chlorinated cyanurates are found to be acceptable when evaluated as to toxicity, safety of use, compatability with other chemicals commonly used in swimming pool water treatment, and so on, standards should be developed for these compounds based upon their demonstrated bactericidal efficiencies. The provisions of the "Suggested Ordinance and Regulations Covering Public Swimming Pools"[5] might be revised as follows: Paragraph 24.1 which now reads in part: ". . . When chlorine is used, a free chlorine residual of at least 0.4 ppm shall be maintained throughout the pool whenever it is open or in use. . . ." might be changed to read in part ". . . If no stabilizing chemical such as cyanuric acid is used and the source of residual chlorine is from elemental chlorine or a hypochlorite solution, a free chlorine residual of at least 0.4 ppm shall be maintained throughout the pool whenever it is open or in use. If cyanuric acid is used as a stabilizing agent of residual chlorine, or if the source of residual chlorine is from a chlorinated cyanurate, a chlorine residual of at least 1.5 ppm shall be maintained throughout the pool whenever it is open or in use. . . ."

Conclusion

From the data collected in this research and reported herewith, the following general conclusions are drawn:

1. Calcium hypochlorite in solutions containing no cyanuric acid is an efficient bactericide as measured by a laboratory procedure developed by the US Department of Agriculture and utilizing E. coli, Strep. faecalis, and Staph. aureus as test organisms.
2. Trichloroisocyanuric acid and potassium dichloroiosocyanurate solutions containing no additional cyanuric acid have similar bactericidal characteristics as calcium hypochlorite under the conditions evaluated except that slightly higher concentrations are needed to destroy Staph. aureus.
3. It was found that 50 mg/l of cyanuric acid produced pronounced retardation of the bactericidal efficiencies of solutions of calcium hypochlorite, trichloroisocyanuric acid, and potassium dichloroisocyanurate such that a

Table 5—Per cent of survivors of Staphylococcus aureus in solutions of calcium hypochlorite, trichloroisocyanuric acid, and potassium dichloroisocyanurate of various concentrations of free available residual chlorine in the presence of 50 mg/l of cyanuric acid for various contact times, 25°C, pH of 7.2, alkalinity of 50 mg/l.

Source of chlorine	Amount of free available residual chlorine mg/l	Time of contact						
		30 sec	1 min	2 min	3 min	4 min	5 min	10 min
Calcium hypochlorite	0.55*	18.181	13.454	6.727	5.454	5.272	2.909	0.636
	1.64	4.363	2.000	2.181	0.764	0.545	0.000	
	1.75	1.531	0.694	0.327	0.306	0.224	0.000	
Trichloroiso-cyanuric acid	0.58	29.811	3.962	2.642	1.075	0.981	0.490	0.000
	0.90	5.849	1.245	1.075	0.302	0.115	0.006	0.000
Potassium dichloroiso-cyanurate	0.42†	85.417	52.083	7.500	7.500	7.292	6.250	4.617
	1.62	6.939	1.306	0.969	1.102	0.612	0.184	0.000

* 0.000 per cent of survivors was attained in 15 minutes.
† After 20 minutes of contact there still remained 0.625 per cent of survivors.

four- to eightfold increase in the amount of "free" available residual chlorine may be necessary to attain the same degree of inactivation of the same organisms in the same interval of time.

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