APPLIED MICROBIOLOGY, Nov. 1967, p. 1291-1295

Vol. 15, No. 6
Printed in U.S.A.

Effect of Algicidal Quaternaries on the Germicidal Activity of Chlorine on Swimming Pool Water

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Received for publication 16 May 1967

The Swimming Pool Water Disinfectant Test Method of the Association of Official Analytical Chemists was used to determine the effect of the accepted level of 2 ppm of some commercial quaternary ammonium algicides on the germicidal activity of chlorine. Accurate determinations on the amounts of residual available chlorine in chlorine-quaternary mixtures could not be made by the usual chemical methods. This made it necessary to base all comparisons on the starting concentrations of available chlorine rather than the final concentration as specified in the method employed. No evidence was obtained to support the use of lower concentrations of residual available chlorine for disinfection in the presence of algicidal quaternaries than those commonly recognized as effective by the American Public Health Association. The rate of kill against the gram-positive test organism *Streptococcus faecalis* was faster in quaternary-chlorine mixtures than in the sodium hypochlorite control solutions. The practical significance of this result in the bench method identified cannot be ascertained in the absence of more sensitive and precise chemical procedures for determining concentrations of residual available chlorine in the presence of quaternaries or in actual swimming pool tests.

Various quaternary ammonium compounds are being used to control algae in swimming pools. A number of reports on the use of such compounds for this purpose have been made (2, 3, 6–10, 13). Some of these chemicals, as well as other chemicals also possessing toxic properties to algae, but not accepted for disinfecting water, have been screened for potential value as bactericides in swimming pool water in our laboratory and elsewhere (7, 16). In actual use, swimming pool water is generally treated with a low level of quaternary about 2 ppm, as an algicide along with chlorine or a chlorine-releasing compound that has been recognized by public health authorities to be an effective disinfectant. Antonides and Tanner (3) reported that in both laboratory and field tests low concentrations of a quaternary in conjunction with low concentrations of chlorine effectively controlled both algae and bacteria.

It frequently has been claimed that through the use of quaternary-chlorine mixtures only half as much residual available chlorine needs to be maintained in swimming pool water to obtain a level of bactericidal control equivalent to that provided by available chlorine alone. The primary objective of this study was to determine the validity of this claim.

MATERIALS AND METHODS

Residual available chlorine determinations. Residual available chlorine determinations were made (i) colorimetrically by the orthotolidine method using an Enslow chlorimeter, (ii) by the iodine-starch titration method, and (iii) by the amperometric titration method using a Wallace and Tiernan apparatus.

Quaternaries. Two commercial quaternaries were used throughout this study at the presently accepted algicidal level of 2 ppm. Quaternary no. 1 was *N*-alkyl (C_{14}, 50%; C_{12}, 40%; C_{16}, 10%) dimethylbenzyl ammonium chloride, and quaternary no. 2 was a 50:50 mixture of *N*-alkyl (50% C_{12}, 30% C_{16}, 3% C_{18}) dimethyl ethylbenzyl ammonium chloride and *N*-alkyl (60% C_{14}, 30% C_{16}, 5% C_{12}, 5% C_{18}) dimethylbenzyl ammonium chloride. The quaternary concentrations were prepared by dilution in sterile distilled water from concentrated commercial products.

Quaternary titrations were made by the method of Barr, Oliver, and Stubbings (3), previously used successfully by Ortenzio et al. (14) to study the effect of detergent residues on the disinfecting process.

Test method. The test method used to study the effects of these algicidal quaternaries on the bactericidal activity of chlorine as a swimming pool disinfectant was the Association of Official Analytical Chemists (AOAC) Swimming Pool Disinfectant Method described by Ortenzio and Stuart (15). In brief, this method calls for a sodium hypochlorite

control solution (*p*H 7.5 at 25 C) having an initial concentration of 0.6 ppm of available chlorine and a final concentration of at least 0.4 ppm of available chlorine as determined by iodine-starch titration. This is challenged with 1 million organisms of *Escherichia coli* ATCC 11229 and *Streptococcus faecalis* ATCC 6569 per ml, separately. Appropriately neutralized samples are taken to check for viable organisms after 0.5, 1, 2, 3, 4, 5, and 10 min. Count reduction rates are established by dilution plate counts, and complete kill time, by five confirmatory tube tests at each test interval. In this test, the NaOCl control solution must kill *E. coli* within 30 sec and *S. faecalis* within 2 min for the results on the test solutions to be considered valid.

This method has been accepted as official by the AOAC, and is used routinely in our laboratory for regulatory testing. It has also been successfully employed in a number of investigations concerning swimming pool water disinfection (11, 12, 16, 17). The starting concentration of 0.6 ppm of available chlorine was selected as the lowest concentration which could be used to assure a residual of at least 0.4 ppm throughout the test period. This is the minimal permitted residual available chlorine concentration stipulated by the American Public Health Association Committee on Swimming Pool Disinfection (1).

RESULTS

In this study, difficulties were immediately encountered in measuring the amount of residual available chlorine in the chlorine-quaternary mixtures by the iodine-starch titration method specified in the AOAC procedure. End points were neither sharp nor constant, owing, apparently, to some type of interference by the quaternaries with the iodine-starch titration system. Substitutions of the Wallace and Tiernan amperometric titration method and the orthotolidine method for the iodine-starch procedure failed to provide the desired sensitive, reproducible end points at the 10-min exposure interval. In the Wallace and Tiernan amperometric titration apparatus, precision was also considerably less in the presence of quaternaries than in their absence. In the orthotolidine method, sensitivity to changes in available chlorine concentrations was less in the presence of quaternaries than in their absence.

Variations of the type encountered in titration end points with the iodine-starch method and the amperometric method are illustrated in Table 1. Table 1 shows that according to both titration systems there is no significant drop in the available chlorine concentration in the sodium hypochlorite solution alone within 10 min. According to the iodine-starch titrations starting with approximately 0.6 ppm of available chlorine, there is about 0.1 ppm decrease in available chlorine after 10-min exposure to 2 ppm of either of the quaternaries in the absence of test organisms. However, the amperometric titration does not show a decrease of this magnitude. On the average, the decrease is about one-half that found in the iodine-starch titrations, but variations are so great that it is not possible to state with certainty that there is any decrease.

In most instances, there is a close correlation between the two titration methods when they are used to measure the decrease in available chlorine upon addition of *E. coli* as the test organism without quaternary. In the iodine-starch titrations, the residual available chlorine is always above the 0.4 ppm required by the AOAC method

TABLE 1. *Effect of 2 ppm of quaternaries on chlorine demand in AOAC test with NaOCl at pH 7.5*

Sample	Iodine-starch titration					Amperometric titation						
	Available Cl concn (ppm) at start											
	1 .56	2 .60	3 .56	4 .58	Avg .58	1 .63	2 .59	3 .65	4 .63	5 .58	6 .62	Avg .62
Cl control	.55[a]	.60	.57	.58	.58	.61	.60	.64	.63	.59	.65	.62
2 ppm of quaternary 1	.48	.48	.48	.44	.47	.44	.46	.63	.54	.64	.59	.55
2 ppm of quaternary 2	.50	.52	.50	.51	.51	.42	.59	.63	.62	.52	.61	.57
Escherichia coli	.46	.51	.49	.49	.50	.56	.49	.53	.58	.66	.55	.56
E. coli + 2 ppm of quaternary 1	.34	.42	.34	.40	.38	.42	.32	.49	.56	.57	.50	.48
E. coli + 2 ppm of quaternary 2	.11	.43	.28	.40	.31	.40	.39	.48	.56	.52	.51	.48
Streptococcus faecalis	.40	.49	.44	.46	.45	.36	.28	.43	.44	.40	.41	.38
S. faecalis + 2 ppm of quaternary 1	.27	.35	.27	.35	.31	.27	.27	.45	.38	.40	.28	.34
S. faecalis + 2 ppm of quaternary 2	.28	.37	.30	.34	.33	.12	.24	.48	.39	.36	.26	.31

[a] Results show the available chlorine concentration (ppm) after 10 min.

TABLE 2. *Effect of quaternary 1 and quaternary 2 at 2 ppm on the germicidal activity of various concentrations of available chlorine as NaOCl at pH 7.5 against Escherichia coli*

Sample	Titratable Cl concn (ppm) at start	Bacterial count per ml of test water after exposure								Complete kill time (min)[a]
		0 sec	30 sec	1 min	2 min	3 min	4 min	5 min	10 min	
NaOCl control	.61	1.28×10^6	<10	<10	<10	<10	<10	<10	<10	0.5
NaOCl with quaternary 1	.6	1.20×10^6	<10	<10	<10	<10	<10	<10	<10	0.5
NaOCl with quaternary 1	.5	1.20×10^6	560	<10	<10	<10	<10	<10	<10	1
NaOCl with quaternary 1	.4	1.13×10^6	110	10	<10	<10	<10	<10	<10	2
NaOCl with quaternary 1	.3	1.14×10^6	1,500	300	50	<10	<10	<10	<10	3
NaOCl with quaternary 2	.6	0.93×10^6	<10	<10	<10	<10	<10	<10	<10	0.5
NaOCl with quaternary 2	.5	1.28×10^6	<10	<10	<10	<10	<10	<10	<10	1
NaOCl with quaternary 2	.4	1.12×10^6	TNTC[b]	30	<10	<10	<10	<10	<10	2
NaOCl with quaternary 2	.3	1.12×10^6	TNTC	30	10	<10	<10	<10.	<10	3

[a] Five replicate tube subculture check.
[b] Too numerous to count.

TABLE 3. *Effect of quaternary 1 and quaternary 2 at 2 ppm on the germicidal activity of various concentrations of available chlorine as NaOCl at pH 7.5 against Streptococcus faecalis*

Sample	Titratable Cl concn (ppm) at start	Bacterial count per ml of test water after exposure								Complete kill time (min)[a]
		0 sec	30 sec	1 min	2 min	3 min	4 min	5 min	10 min	
NaOCl control	.61	1.09×10^6	2,320	20	<10	<10	<10	<10	<10	2
NaOCl with quaternary 1	.6	1.15×10^6	<10	<10	<10	<10	<10	<10	<10	1
NaOCl with quaternary 1	.5	1.0×10^6	240	270	<10	<10	<10	<10	<10	2
NaOCl with quaternary 1	.4	1.0×10^6	770	350	120	20	<10	<10	<10	4
NaOCl with quaternary 1	.3	1.0×10^6	650	300	100	10	10	<10	<10	5
NaOCl with quaternary 2	.6	0.91×10^6	<10	<10	<10	<10	<10	<10	<10	0.5
NaOCl with quaternary 2	.5	0.91×10^6	150	50	<10	<10	<10	<10	<10	2
NaOCl with quaternary 2	.4	1.0×10^6	TNTC[b]	30	10	<10	<10	<10	<10	4
NaOCl with quaternary 2	.3	0.93×10^6	TNTC	800	500	10	<10	<10	<10	5

[a] Five replicate tube subculture check.
[b] Too numerous to count.

with both test organisms. However, with *S. faecalis* even without quaternary the amperometric titrations show occasional decreases below the minimal level of 0.4 ppm of available chlorine specified in the procedure. If the amperometric data are more accurate than the iodine-starch titration method, it would seem that frequently encountered difficulties in obtaining the 2-min kill time with the gram-positive test organism in the control specified for this method can be attributed to the high chlorine demand of this test culture suspension and the resulting drop in the available chlorine concentration below the required 0.4 ppm level. In the presence of quaternaries 1 and 2, the available chlorine concentrations with both test organisms tended to fall below the required minimal concentration of 0.4 ppm at the 10-min exposure interval with either the iodine-starch or amperometric titration methods. This suggests that bacterial cells exposed to quaternaries may absorb available chlorine more readily than unexposed cells, but it has not been clearly established that these low titration values may not be simple reflections of interferences with the two titration systems by the quaternary salts present.

Because of the lack of certainty in determining residual chlorine concentrations in the presence of quaternaries by the available chemical and physical methods, comparisons drawn at this time have been on the starting concentrations of available chlorine rather than the concentrations found at the conclusion of the 10-min exposure times. Tables 2 and 3 show the effects of the two algicidal quaternaries at a concentration of 2.0 ppm on the germicidal activity of chlorine. From two to four tests were made at each concentration to determine the complete kill times.

Table 2 shows that quaternary no. 1 at 2 ppm did not have a beneficial effect on the germicidal

activity of sodium hypochlorite against *E. coli*; 0.6 ppm of available chlorine was still required to equal the 0.5-min kill time of the control with 0.6 ppm of available chlorine. As less chlorine was used, the kill times became progressively longer.

Table 2 also shows that with quaternary 2 a kill time against *E. coli* equal to that of the 0.6 ppm of available chlorine control as sodium hypochlorite was provided by 0.6 ppm of available chlorine, but not at lower concentrations.

Table 3 shows that quaternary 1 at 2 ppm with a starting concentration of 0.6 ppm of available chlorine did have a beneficial effect against *S. faecalis* in that it killed in 1 min, whereas the chlorine control at 0.6 ppm required 2 min.

Table 3 also shows that quaternary 2 at 2 ppm with a 0.6 ppm available chlorine starting concentration also had a beneficial effect against *S. faecalis* in that it killed the test organism in 0.5 min, as compared with the control which required 2 min. At a starting concentration of 0.5 ppm, the kill time was still equivalent to the 2-min kill time of the control, but this was not so at 0.4 ppm.

DISCUSSION

Certain difficulties arise when the Swimming Pool Water Disinfectant Test Method of the AOAC is used to determine the effect of algicidal quaternaries on the germicidal activity of chlorine. The iodine-starch titration method specified in the AOAC does not accurately and precisely measure decreases in available chlorine concentrations in solutions containing quaternary ammonium salts. Chemical or physical methods for measuring low concentrations of residual available chlorine are needed which are less susceptible to interference from algicidal chemicals and other compounds commonly found in swimming pool water. This is especially important in view of the fact that chemical methods must be depended upon to monitor the biological quality of water in swimming pool operations.

The iodine-starch titration end point is extremely difficult to determine once a quaternary is added to water, and results are of dubious value since some type of interference apparently occurs. Other workers have reported consistent results in determining available chlorine residuals in swimming pool water with the Wallace and Tiernan amperometric titration apparatus, but this device was also found to be susceptible to some type of interference from quaternaries, as judged by the wide variations in titration end points when low concentrations of these chemicals were present. With the orthotolidine method using the Enslow chlorimeter, a low degree of sensitivity to changes below the level of 1 ppm of available chlorine was accentuated by the presence of quaternaries, limiting its usefulness. Black and Whittle (5) recently stressed the limitations of the orthotolidine method and described a new colorimetric test which has not as yet besn used in our laboratory

Although control of algae through use of quaternaries may reduce the chlorine demand of swimming pool water, no substantial evidence was found to show that their presence reduces the required residual available chlorine concentration of 0.4 ppm for disinfecting specified by the American Public Health Association. The practical value of the beneficial effects shown by the two quaternaries in terms of more rapid killing times against the gram-positive test organism *S. faecalis* in this test is not known. If the available chlorine residuals in the quaternary-chlorine mixtures were as low as might be indicated by the figures shown in Table 1, the faster killing times observed against the gram-positive test organism based on the starting concentrations of available chlorine would actually represent minimal expectancies, which would be exceeded in an actual swimming pool operation where the residual available chlorine concentration was maintained through use of an automatic feeder at 0.4 ppm or above. Thus, this observation may have special significance from a practical public health standpoint.

With the quaternary-chlorine mixtures used here, it was clearly shown that a bactericidal effect equivalent to that provided by the sodium hypochlorite control could not be obtained by starting solutions containing half as much available chlorine in the mixture.

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