APPLIED MICROBIOLOGY, Mar. 1969, p. 415-421

Vol. 17, No. 3
Printed in U.S.A.

Pseudomonas aeruginosa for the Evaluation of Swimming Pool Chlorination and Algicides

G. P. FITZGERALD AND M. E. DERVARTANIAN
Water Chemistry Laboratory, University of Wisconsin, Madison, Wisconsin 53706

Received for publication 23 January 1969

Concentrations of ammonia and the chlorine stabilizer, cyanuric acid, which could be expected in swimming pools decreased the rate of kill by chlorine of the potential pathogen, *Pseudomonas aeruginosa*. The effect of cyanuric acid increased as the concentration of chlorine decreased, a fact of significance from a public health view. Quaternary ammonium algicides had little effect on the kill rate of chlorine, but an organic mercury algicide had a synergistic effect with chlorine when the chlorine activity was stressed by the addition of ammonia or the use of 100 times the normal concentration of bacteria. The effect of natural waters, rain, beaches, and swimming pools on the kill rate by 0.5 mg of chlorine per liter indicated that a treatment time of 1 hr or more was required to kill 99.9% of 10^6 *Pseudomonas* cells per ml. The synergism of chlorine and the organic mercury algicide was also demonstrated with these waters and with sewage treatment plant effluents. The necessity of developing and using laboratory tests which simulate conditions in swimming pools with heavy loads of swimmers, as opposed to tests in chlorine demand-free conditions, is discussed. Samples taken from well-supervised swimming pools when the swimmer load had been especially high required treatment times of 1 to 3 hr to obtain 99.9% kills of the potential pathogen, *P. aeruginosa*, with 0.5 mg of chlorine per liter.

The potentially pathogenic bacterium, *Pseudomonas aeruginosa*, was selected for studies of some of the factors involved in the evaluation of the bactericidal properties of chlorine in swimming pools because of results reported by Keirn and Putnam (13) and Favero et al. (6), in which excessively high numbers of *Pseudomonas* were found associated with certain swimming pool treatments. The significance of exceptionally high bacterial numbers in other pools associated with the presence of the chlorine stabilizer, cyanuric acid (A. P. Black, *in press*), was also of interest.

It is felt that a reemphasis should be made of the factors which affect laboratory evaluations of bactericides and the conditions existing in swimming pools when the maximum effectiveness of a bactericide is desired. To test the relative effectiveness of bactericides, the conditions existing under maximum numbers of swimmers in pools should be duplicated in laboratories, as opposed to the chlorine demand-free systems usually used (2, 10).

The possibility of a synergism between an algicide and a bactericide observed in swimming pools by Black (*unpublished data*) has been evaluated and found possibly to exist.

MATERIALS AND METHODS

Chlorine demand-free, buffered (*p*H 7.3), double-distilled water (10) was used for all dilutions and test waters. Agar slants of King et al. (14) medium A were used to maintain the 2F5 strain of *P. aeruginosa*, obtained from Elizabeth McCoy, Department of Bacteriology, University of Wisconsin. Preliminary test cultures were prepared by adding one or two loops from a slant to approximately 60 ml of King et al. medium A and incubating at 37 C overnight. The next morning the preliminary culture was added to 400 ml of fresh culture medium and stirred at 37 C until optical density measurements at 600 nm indicated that the culture was in the log phase of growth. Samples of about 20 ml were centrifuged for 6 to 7 min, and the supernatant fluids were discarded; the bacteria were resuspended in chlorine demand-free buffer and centrifuged again. Chlorine demand-free conditions meant that less than 10% of the chlorine was lost from a solution containing 0.2 mg of chlorine per liter in 30 min. The washed bacteria were again suspended in chlorine demand-free buffer, and the optical density of the suspension was adjusted to 0.055 to 0.060 (600 nm, 1-cm tubes); 1 ml of such a suspension contained about 10^8 cells and was transferred to 100 ml of test solutions for tests requiring 10^6 cells per ml. Controls and test solutions were maintained at either 20 ± 1 C or

25 ± 2 C during tests. Controls and test solutions were appropriately diluted in sterile, buffered neutralizer (200 mg of sodium thiosulfate per liter) and plated in King et al. medium A. Plates were counted after 48 hr of incubation at 37 C.

The optical density measurements, used to calculate the dilution of the initial bacterial suspension required to obtain the desired bacterial stock suspension, were adequate to give a range of about ±20% of the desired 10^8 cells per ml. The necessity of washing bacterial suspensions used in chlorine toxicity tests, as reported earlier (10), is demonstrated by the fact that washed *Pseudomonas* (10^6 cells per ml) had a chlorine demand (reduced the concentration of chlorine present) of about 0.04 mg per liter after 2 min in a solution of 0.5 mg of chlorine per liter, whereas unwashed bacteria or the original medium appropriately diluted had a chlorine demand of about 0.4 mg per liter. The chlorine demand of *Pseudomonas* was proportional to the concentration of bacteria, and 10^6 cells per ml had a chlorine demand of about 0.1 mg per liter at 10 min and 0.2 mg per liter at 30 min when tested with solutions originally containing 0.5 mg of chlorine per liter. The viability of the *Pseudomonas* final stock held in buffered, chlorine demand-free water at 20 C was such that there was relatively little loss over 3 or 4 days. Very little difference could be detected between results of early tests carried out at 20 C and tests at 25 C.

RESULTS

Effect of cyanuric acid and ammonia on kill by chlorine. It has been well established that, when chlorine is stabilized with cyanuric acid, longer reaction times are required to cause a kill of a certain percentage of various bacteria than when free, available chlorine is used (2, 10, 18, 19). By using *Pseudomonas* at a test concentration of 10^6 cells per ml at *p*H 7.3, the effects of 0, 25, 50, and 100 mg (per liter) of cyanuric acid on the bactericidal properties of 0.1, 0.25, and 0.5 mg of chlorine per liter were tested. The results are summarized in Fig. 1A as the time required for a 99.9% kill of the bacteria.

At all concentrations of free, available chlorine tested, 1 min or less was required to obtain 99.9% kills under the conditions of these tests. These results are similar to those reported for *Streptococcus faecalis* (10) in that increasing concentrations of cyanuric acid caused increased time required to obtain 99.9% kills. The effect of cyanuric acid was greater as the concentration of chlorine in a test water decreased, a fact which may have significance in swimming pools.

The effect of ammonia on the bactericidal properties of 0.5 mg of chlorine per liter, with 10^6 *Pseudomonas* cells per ml at *p*H 7.3, was tested in the presence of 0, 25, 50, and 100 mg of cyanuric acid per liter. The results of two to four tests of each condition are summarized in Fig. 1B.

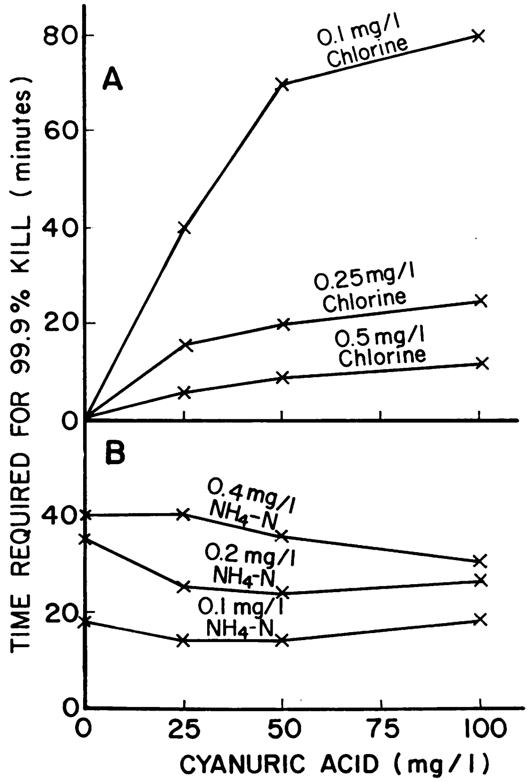

FIG. 1. *Effect of cyanuric acid on the kill of Pseudomonas aeruginosa by (A) different concentrations of free, available chlorine, or (B) combinations of 0.5 of mg chlorine per liter and different concentrations of ammonia.*

Concentrations of ammonia of 0.1 and 0.2 mg of NH_4-N per liter proportionately affected the bactericidal properties of 0.5 mg of chlorine per liter; 18 and 35 min, respectively, were required for 99.9% kills, as compared to 0.25 min without ammonia. The effect of ammonia concentrations of 0.4 mg of NH_4-N per liter was greater than the effect of 0.2 mg per liter but not proportional to the added amount of ammonia. Concentrations of 25, 50, and 100 mg of cyanuric acid per liter had little effect on the kill rate of 0.5 mg of chlorine plus 0.1 mg of NH_4-N per liter; however, cyanuric acid did reduce the time required for 99.9% kills when tested in the presence of higher concentrations of ammonia. These effects of ammonia on the kill rate of chlorine are similar to those obtained with *S. faecalis* (10), but the magnitude of effect was much greater with the latter bacterium; approximately 100 min was required to kill *S. faecalis* with 0.5 mg of chlorine and 0.1 mg of NH_4-N per liter, as compared to 18 min with *Pseudomonas*. Thus, *Pseudomonas* appears to be less resistant than *S. faecalis* to combinations of chlorine and ammonia.

Effect of algicides. Based on the activity of amines or quaternary ammonium compounds, tests have indicated that the concentrations required to kill the green alga, *Chlorella pyrenoidosa* (Wis. 2005), within the 4-hr treatment time which is used to evaluate swimming pool algicides (9, 11, 12), will kill 99.9% of *Pseudomonas* cells within 6 min, when tested at a concentration of 10^6 cells per ml. The compounds tested were cetyl trimethylammonium bromide (8 mg per liter), lauryl pyridinium chloride (24 mg per liter), Exalgae L. C. (9) (10 mg per liter), and Algistat (9) (28 mg per liter). These products and concentrations did not interfere with the algicidal properties of 0.1 mg of chlorine per liter, and the addition of 25 or 50 mg (per liter) of cyanuric acid had no effect on the bactericidal properties of the mixtures of chlorine and quaternary ammonium products. These results were the same as those found with other bacteria (9, 10, 15). In contrast, the commerical swimming pool algicide, Algimycin-200, based on the activity of an organic mercury compound was not bactericidal, alone or in the presence of 0.1 mg of chlorine per liter, within a 1-hr treatment time when tested with 10^6 *Pseudomonas* cells per ml at the concentration of algicide (3 mg per liter) which has been shown to be algicidal to *Chlorella* within 5 min (9).

Effect of algicides when chlorine activity is placed under stress. Algicide tests reported thus far have been carried out under idealized conditions in the laboratory such that the concentration of washed bacteria used, 10^6 cells per ml, had very little chlorine demand (0.04 mg per liter after 2 min) and such that there was no ammonia or other source of chlorine-demand chemicals added. To try to test conditions which might be more representative of conditions existing in heavily used swimming pools, stresses were placed on the bactericidal properties of 0.4 or 0.5 mg of chlorine per liter by the addition of 0.4 mg of NH_4-N per liter or by using test concentrations of *Pseudomonas* of 10^8 cells per ml. In either case, the chlorine added will not exist as free, available chlorine but will be present as some form of combined chlorine. The effects on the bactericidal properties of chlorine were tested under conditions such that the recommended initial treatment concentrations of the organic mercury algicide and two quaternary ammonium algicides were added. Table 1 gives a summary of the ranges of time required for 99.9% kill of 10^6 *Pseudomonas* cells per ml by combinations of 0.5 mg of chlorine, 0.4 mg of NH_4-N, and the recommended concentrations of the algicides.

The results of these tests indicate that the

TABLE 1. *Effect of recommended concentrations of algicides on bactericidal properties of 0.5 mg of chlorine plus 0.4 mg of NH_4-N per liter (10^6 Pseudomonas cells per ml)*

Treatment	Time required for 99.9% kill
	min
Chlorine + NH_4-N	40–55
Chlorine + NH_4-N + Algimycin-200 (3 mg/liter)	20–25
Chlorine + NH_4-N + Exalgae L. C. (5 mg/liter)	45–50
Chlorine + NH_4-N + Algistat (5 mg/liter)	50–55

quaternary ammonium compounds at the concentrations recommended for swimming pool use had little effect on the bactericidal properties of 0.5 mg of chlorine and 0.4 mg of NH_4-N per liter. The treatment time required for 99.9% kills was in the range of 40 to 55 min. In contrast, the recommended concentration of the organic mercury algicide caused a reduction to about 50% of the time required for 99.9% kill under these conditions.

The results of two to five tests in which 10^8 *Pseudomonas* cells per ml were used are summarized in Table 2 as the numbers of bacteria surviving after different treatment times with recommended concentrations of algicides or with such concentrations plus 0.4 mg of chlorine per liter.

The various algicides tested at recommended levels for initial swimming pool use had little bactericidal activity for the first hour when used alone against 10^8 *Pseudomonas* cells per ml. However, after 4- and 24-hr treatment times, the quaternary ammonium products caused a decrease to about 50% of the controls, and the organic mercury product caused a decrease to about 15% of untreated controls. Chlorine at an initial concentration of 0.4 mg per liter caused a sharp decrease within 30 min, and the effect continued such that there were only about 6,000 cells per ml after 24 hr. The effects of the quaternary ammonium products on the bactericidal properties of 0.4 mg of chlorine per liter were not additive and might be considered to reduce slightly the effectiveness of the added chlorine. The combination of chlorine and organic mercury product was very significantly more effective than chlorine alone under these conditions. In other tests in which the bactericidal properties of combinations of 0.4 or 0.8 mg of chlorine per liter and either 1 or 3 mg (per liter) of the organic mercury algicide were measured, with 10^8 *Pseudomonas* cells per ml, similar results were obtained. However, a concentration of 1 mg per liter of the algicide was less effective

TABLE 2. *Bactericidal effects of recommended concentrations of algicides and chlorine plus algicides on 10^8 Pseudomonas cells per ml*

Treatment[a]	No. (per ml) of surviving bacteria after treatment times of			
	0.5 hr	1 hr	4 hr	24 hr
None	94,000,000	81,000,000	92,000,000	93,000,000
Exalgae L. C. (5 mg/liter)	98,000,000	98,000,000	71,000,000	56,000,000
Algistat (5 mg/liter)	86,000,000	84,000,000	50,000,000	47,000,000
Algimycin-200 (3 mg/liter)	92,000,000	71,000,000	54,000,000	14,000,000
Chlorine	10,000,000	4,000,000	60,000	6,000
Chlorine + Exalgae L. C. (5 mg/liter)	30,000,000	15,000,000		750,000
Chlorine + Algistat (5 mg/liter)	7,000,000	4,000,000	250,000	70,000
Chlorine + Algimycin-200 (3 mg/liter)	2,000,000	15,000	2,000	150

[a] Chlorine added at 0.4 mg/liter.

than 3 mg per liter. Cell counts after 1 hr were: for 0.8 mg of chlorine per liter, 155,000; for chlorine plus 3 mg (per liter) of algicide, 2,000; for chlorine plus 1 mg (per liter) of algicide, 7,000.

Tests with natural waters. Since relatively high bacterial loads or the presence of ammonia caused changes in the bactericidal properties of chlorine in laboratory tests with buffered chlorine demand-free, double-distilled water, tests were also carried out in various natural waters associated with swimmers. As a preliminary test, however, buffered single-distilled water was used to demonstrate that the ammonia or other chlorine-demand chemicals present would influence the results of tests of the bactericidal properties of chlorine even when washed bacteria were used. Chlorine at a concentration of 0.5 mg per liter in buffered single-distilled water required 30 to 60 min to cause a 99.9% kill of 10^6 *Pseudomonas* cells per ml, whereas 0.5 mg of chlorine plus 3 mg of the organic mercury algicide per liter caused a 99.9% kill in 16 to 30 min. Therefore, if tests are to be run in natural waters, dilutions of the waters or bacteria should be made with chlorine demand-free water so that the chlorine demand of distilled or buffered water used as a diluent will not affect the results.

The natural waters which have been used to suspend 10^6 *Pseudomonas* cells per ml for bactericidal tests were adjusted to *p*H 7.3 after the original bacterial flora debris had been removed by membrane filtration. Some of these waters contained chemicals which reduce the chlorine concentration when they are treated with chlorine. (Actual measured concentrations of total available chlorine, after the addition of chlorine to give a theoretical concentration of 0.5 mg of chlorine per liter, were 0.1 mg per liter in the beach waters.) The results obtained with different waters are summarized in Table 3.

It is apparent from these data that the ammonia or other chlorine-demand chemicals present in such sources of water will affect the bactericidal properties of 0.5 mg of chlorine per liter, with at least 1 hr required to cause a 99.9% kill of the 10^6 *Pseudomonas* cells per ml. It is of interest to point out that, in four samples of rain water tested, the results of bioassays indicated that the rain contained chlorine-demand compounds equivalent to 0.1 to 0.4 mg of NH_4-N per liter. The very long reaction times required with the beach waters (4 hr or more) are probably related to chlorine-demand chemicals derived from plankton rather than derived from swimmers, but the 1-hr reaction time required for the pool sample must be related

TABLE 3. *Effect of different waters on the bactericidal properties of chlorine and chlorine plus algicide, with 10^6 Pseudomonas per ml at pH 7.3*

Test water[a]	Range of time required for 99.9% kill
	hr
Single-distilled	
Chlorine	0.5–1
Chlorine (0.5) + Algimycin-200 (3)	0.25–0.5
Rain	
Chlorine (0.1)	2
Chlorine (0.1) + Algimycin-200 (3)	0.5–1
Chlorine (0.5)	1
Chlorine (0.5) + Algimycin-200 (3)	0.25–0.5
Vilas Park Beach	
Chlorine (0.5)	4
Chlorine (0.5) + Algimycin-200 (3)	1–1.5
Willows Park Beach	
Chlorine (0.5)	>4
Chlorine (0.5) + Algimycin-200 (3)	1–1.5
Midview Motel pool	
Chlorine (0.5)	1
Chlorine (0.5) + Algimycin-200 (3)	0.25–0.5

[a] Numbers in parentheses give concentrations (milligrams per liter).

to the high numbers of swimmers present at the time of sampling. The increase, with the addition of the organic mercury algicide, in the bactericidal properties of chlorine that was noted in laboratory waters was also demonstrated in these natural waters.

In the sampling of swimming pool waters for bactericidal tests with *Pseudomonas*, it was interesting to note the variations found in the "quality" of the waters. A sample collected from one open-air pool at 7:30 PM after a hot, sunny day required 30 to 60 min to obtain a 99.9% kill of added *Pseudomonas* with 0.5 mg of chlorine per liter, whereas a sample collected from the same pool at 1:00 PM on a very cool day required only 1 min or less of treatment with 0.5 mg of chlorine per liter for a 99.9% kill. Also, at an indoor pool, samples of which usually required only 1 to 4 min for 99.9% kills, a sample collected at 7:30 PM after a very hot day required more than 3 hr for a 99.9% kill. Therefore, there are times with even well-supervised swimming pools when the bactericidal properties of 0.5 mg of chlorine per liter will be stressed to the point that long contact periods will be required to kill a potential pathogen.

The results of the above tests of waters from which the original bacterial flora had been removed stimulated us to investigate the bactericidal effects of chlorine, or chlorine plus the organic mercury algicide, on the "native" bacterial flora of various waters. In these tests the original total plate count (1) was compared to total counts after various treatments (Table 4).

The swimming pool sample had a native population of bacteria which was either quite resistant to 0.5 mg of chlorine per liter, or the ammonia or other chlorine-demand chemicals which also affected results with *Pseudomonas* (Table 3) affected the action of chlorine against these bacteria. The fact that bacteria found in such a pool cannot be readily killed by 0.5 mg of chlorine per liter, along with the fact that the kill rate of a potential pathogen such as *Pseudomonas* was also affected, may have some public health implications.

The chlorination of sewage plant effluents is a well-established procedure (17), and these results (Table 4) indicate that 1 and 5 mg of chlorine per liter are effective. The addition of the organic mercury algicide increased the rate of kill by 1 mg of chlorine per liter but had little if any effect when added with 5 mg of chlorine per liter, a very successful treatment concentration.

The bacteria of a hide brine vat were relatively difficult to kill with chlorine, either because of the type of organisms present or the tremendous amount of organic contaminants present. The addition of the organic mercury algicide caused only minor improvements.

TABLE 4. *Bactericidal properties of chlorine and chlorine plus algicide on the bacterial flora of various waters*

Source and original total plate count (no./ml)	Treatment[a]	Bacteria surviving (no./ml)	
		16 min	60 min
Midview Motel pool (7/26/68) (300,000)	Chlorine (0.5)	370	280
Madison sewage plant effluent (6/25/68) (1,500,000)	Chlorine (1)	108,000	21,000
	Chlorine + Algimycin-200 (3)	7,400	200
	Chlorine (5)	750	150
	Chlorine (5) + Algimycin-200 (3)	400	200
Madison sewage plant effluent (6/29/68) (2,300,000)	Chlorine (1)	TMTC[b]	57,200
	Chlorine (1) + Algimycin-200 (1)	79,000	16,500
	Chlorine (1) + Algimycin-200 (3)	23,000	1,400
	Chlorine (5)	3,200	100
	Chlorine (5) + Algimycin-200 (1)	3,200	200
	Chlorine (5) + Algimycin-200 (3)	950	250
Milwaukee hide brine vat (6/25/68) (130,000)	Chlorine (10)		63,000
	Chlorine (10) + Algimycin-200 (3)		40,000
	Chlorine (20)		30,000
	Chlorine (20) + Algimycin-200 (3)		22,000
Milwaukee hide brine vat (6/29/68) (160,000)	Chlorine (100)		18,000
	Chlorine (100) + Algimycin-200 (3)		16,000
	Chlorine (500)		16,000
	Chlorine (500) + Algimycin-200 (3)		16,000

[a] Numbers in parentheses give concentrations (milligrams per liter).
[b] Too many to count.

DISCUSSION

P. aeruginosa was selected for study in order to determine whether a potential pathogen would react in the same manner as the previously used *S. faecalis* (10) and to determine why swimming pools treated with cyanuric acid (6) or quaternary ammonium-based algicides (13) developed high *Pseudomonas* populations. It was felt that a large variety of organisms have been used to evaluate bactericides and swimming pool practices (2, 6, 10, 13, 15, 18, 19) and that the usual dispersion of results, ranging from susceptible to resistant, has been recorded. Inasmuch as there was doubt as to which organism was best to use for such studies, an organism was selected which had been observed in swimming pools and which could represent actual harmful organisms in swimming pools as opposed to an organism representing the usual interior or exterior bacterial flora of humans.

An effort was made to present techniques and data which could be reproduced in any laboratory by careful attention to details and use of readily available chemicals to represent problems that might be related to field studies of swimming pools. The necessity of washing bacteria free of chlorine-demand products from culture media and using demand-free test waters has again been pointed out by the data showing that 10^6 unwashed *Pseudomonas* cells per ml had a chlorine demand of 0.4 mg per liter, whereas washed bacteria had a demand of one-tenth this amount. Also, treatment times of 0.5 to 1 hr were required to kill 99.9% of 10^6 *Pseudomonas* cells per ml, as compared to less than 1 min when tests were carried out in demand-free waters; this was indicated by the results of bactericidal tests of 0.5 mg of chlorine per liter with buffered, single-distilled water that had not been chlorinated overnight and boiled to remove chlorine-demand chemicals.

The laboratory study of the effects of cyanuric acid on the bactericidal properties of different concentrations of chlorine has confirmed observations in many swimming pools (6; A. P. Black, *in press*). Probably the most important result reported was that at lower chlorine concentrations cyanuric acid caused longer times to be required for the kill of bacteria. Thus, at the time when the greatest numbers of swimmers would be present and the available chlorine would be minimal, the effect of cyanuric acid would be to further lower the effectiveness of what little chlorine was present. It should be pointed out, however, that in some areas with intense sunlight available chlorine probably could not even be present without the stabilizing effect of cyanuric acid. The need to stabilize a bactericide in order to preserve at least a small degree of its effectiveness points out the need for a more realistic approach to the selection of bactericides for swimming pools (13).

Further evidence of the poor qualities of chlorine as a swimming pool bactericide is reflected by the data obtained with combinations of chlorine and ammonia. Natural products from swimmers will decrease the effectiveness of chlorine just when the greatest bactericidal effectiveness is most important. Therefore, the possible synergism of the organic mercury algicide and chlorine reported here, and reported by Black (*unpublished data*), for the organic mercury algicide and iodine should be of interest to public health authorities.

When a study is made of the effectiveness of products such as bactericides or algicides, it is of prime importance to define the actions demonstrated. This is pointed out by studies of the concentrations of algicides required to kill representative algae versus the recommended use concentrations on the product labels. There can be up to eightfold differences between the latter and former (7–9, 11, 12). In the studies reported here, the concentrations of algicides required to kill algae with a 4-hr contact time are used because field studies had indicated that initial concentrations of amine and quaternary ammonium compounds would probably not persist in filtered swimming pools for longer periods (7, 8). The results with the quaternary ammonium compound tested indicated that they would probably have little significance in swimming pool chlorination studies. It is of interest to point out that the concentration of one of the quaternary ammonium algicides (Exalage L. C.) required to kill *Chlorella* within 4 hr (10 mg per liter) has been used to obtain algal cultures free of bacteria by using 1-hr treatments (11). The relationship between the appearance of unusually high numbers of *Pseudomonas* in pools treated with quaternary ammonium algicides (13) has not been explained by the data obtained in this study.

The necessity of developing and using laboratory tests which simulate the conditions in swimming pools with heavy loads of swimmers, as opposed to the tests in chlorine demand-free conditions in laboratory glassware, is pointed out by the contrasting results reported here. We have used more than usual numbers of bacteria, to represent the effects of an increase in living organisms associated with an increase in the number of swimmers, and the addition of ammonia, to represent the natural products shed by swimmers which could affect the bactericidal properties of chlorine. The concentrations of ammonia used in this report (0.1 to 0.4 mg of NH_4-N per liter) were well within the concentrations found in swimming pools by various workers (2–4, 16). Under either stress condition, the concentration of quaternary ammonium compounds to be expected in swimming pools would have little if any effect on the bactericidal properties of chlorine under stress. However, the possibility of synergistic activity between the organic mercury product and chlorine has been suggested by the results summarized in Tables 1 through 4.

When the bactericidal properties of chlorine were tested in various waters other than the chlorine demand-free, buffered, distilled water of laboratories, it was found that increasing periods of time were required to cause a 99.9% kill of 10^6 *Pseudomonas* cells per ml: from the 0.5 to 1 hr more required in buffered, single-distilled laboratory water, rain water, and some pool waters, to 4 hr required in beach waters. It is considered very significant that samples from some swimming pools, both outdoor and indoor, contained chlorine-demand chemicals which at certain times are associated with periods of high swimmer loads but few or no products affecting the bactericidal properties of chlorine associated with periods of very low swimmer loads. It has been shown that the water of pools with few or no swimmers and continuous chlorination approached the characteristics of the chlorine demand-free waters of laboratories. Thus, the importance of when samples are taken for bacteriological examinations cannot be overemphasized.

Great importance should be attributed to the fact that in stressed pool waters a potentially pathogenic organism, such as *P. aeruginosa*, was not readily killed by chlorine alone. The reports of chlorine-resistant bacteria being found in swimming pools (5) cause one to wonder whether pathogenic organisms could also survive in pools. The latter possibility is, of course, feasible if the reason for bacteria being present in pools with relatively high levels of chlorine is that the presence of ammonia or other chlorine-demand chemicals lowered the toxicity of the chlorine present. One method of determining such situations would be to require that, whenever the bacteria load of swimming pool waters is checked, samples of membrane-filtered pool waters should be tested with standardized concentrations of bacteria and chlorine to detect the presence or absence of products affecting the bactericidal effectiveness of chlorine. With results of such tests available, the search for new bactericides might be stimulated, or the necessity of combinations of chemicals might be shown.

ACKNOWLEDGMENT

The technical aid of Mrs. S. L. Faust is gratefully acknowledged.

LITERATURE CITED

1. American Public Health Association. 1965. Standard methods for the examination of water and wastewater, 12th ed. American Public Health Association, New York.
2. Andersen, J. R. 1965. The effect of cyanuric acid on chlorine's killing power. Swimming Pool Data and Reference Annual 32:86–97.
3. Brown, J. R., D. M. McLean, and M. C. Nixon. 1963. Swimming pools and bathing places in Southern Ontario: chemical and microbiological studies during 1962. Can. J. Public Health 54:121–128.
4. Bussy, I. J. De. 1949. Growth and control of algae in open-air swimming pools, p. 1–78. Report No. 1. Research Institute for Public Health Engineering, The Hague.
5. Farkas-Himsley, H. 1963. The emergence of chlorine-resistant bacteria in chlorinated swimming pools and their sensitivity to hypochlorite-hypobromite solutions. Can. J. Public Health 54:45.
6. Favero, M. S., C. H. Drake, and G. B. Randall. 1964. Use of staphylococci as indicators of swimming pool pollution. U.S. Public Health Rept. 79:61–70.
7. Fitzgerald, G. P. 1962. Bioassay for algicidal chemicals in swimming pools. Water Sewage Works 109:361–363.
8. Fitzgerald, G. P. 1963. Field test on the duration of algicides in swimming pools. J. Environ. Health 25:319–325.
9. Fitzgerald, G. P. 1968. Compatibility of swimming pool algicides and bactericides. Water Sewage Works 115: 67–71.
10. Fitzgerald, G. P., and M. E. DerVartanian. 1967. Factors influencing the effectiveness of swimming pool bactericides. Appl. Microbiol. 15:504–509.
11. Fitzgerald, G. P., and S. L. Faust. 1963. Factors affecting the algicidal and algistatic properties of copper. Appl. Microbiol. 11:345–351.
12. Fitzgerald, G. P., and S. L. Faust. 1963. Bioassay for algicidal *versus* algistatic chemicals. Water Sewage Works 110: 296–298.
13. Keirn, M. A., and H. D. Putnam. 1968. Resistance of staphylococci to halogens as related to a swimming pool environment. Health Lab. Sci. 5:180–193.
14. King, E. O., M. K. Ward, and D. E. Raney. 1954. Two simple media for the demonstration of pyocyanin and fluoresin. J. Lab. Clin. Med. 44:301–307.
15. Koski, T. A., L. F. Ortenzio, and L. S. Stuart. 1967. Effect of algicidal quaternaries on the germicidal activity of chlorine on swimming pool water. Appl. Microbiol. 15:1291–1295.
16. McLean, D. M., J. R. Brown, and M. C. Nixon. 1962. Recirculating and "overflow-refill" public swimming pools: a chemical and microbiological appraisal. Can. J. Public Health 53:9–16.
17. Rhines, C. E. 1966. The fundamental principles of sewage chlorination. Water Sewage Works 113:97–101.
18. Robinton, E. D., and E. W. Mood. 1967. An evaluation of the inhibitory influence of cyanuric acid upon swimming pool disinfection. Am. J. Public Health 57:301–310.
19. Stuart, L. S., and L. F. Ortenzio. 1964. Swimming pool chlorine stabilizers. Soap Chem. Specialties 40(8):79–82, 112–113.