Comparative Algaecidal, Algaestatic, and Bacteriostatic Evaluations of Selected Commercial Algaecides

Fernando del Corral, Ph.D. Betty S. Johnson Buckman Laboratories International, Inc.

Nine commercial algaecides, including Poly(iminoimidocarbonyliminoimidocarbonyl-imino hexamethylene hydrochloride, (PHMB) were evaluated for their algaestatic and algaecidal properties against three axenically grown algal cultures and two bacterial isolates. Typical representative algal species were selected based on their prevalence in recreational water systems, such as the chlorophyte Chlorella pyrenoidosa, the eustigmatophycea Eustigmatos vischeri and the filamentous cyanophyte Phormidium faveolarum (Adamson and Sommerfeld, 1978). Results indicated that five out of nine products had algaestatic properties against C. pyrenoidosa, within the manufacturer's label recommendations. Two of the nine products had algaestatic efficacy within the label use directions, against Phormidium faveolarum. In addition, two out of nine products required significantly higher levels to control Eustigmatos vischeri. Bacteriostatic activity levels were easily achieved against the coliform bacterium Enterobacter aerogenes with low levels of PHMB and polymeric quaternary ammonium chloride (polyquat). The monomeric quaternary ammonium products tested were the most effective against the pigmented Rhodococcus sp. bacterium isolated from a residential pool.

There appears to be a time-concentration relationship between algeacidal and algaestatic effects on the test algal cultures.

Proceedings of the 1st Annual Chemistry Symposium National Spa and Pool Institute – November 1996 Pages 18–25 Copyright © 1997 by NSPI All rights of reproduction in any form reserved.

water systems can be aesthetically displeasing, and if left unchecked, cleaning can be costly and labor intensive. In some cases, algae growth is an indication that a pool is improperly operated and conditions may exist that promote growth of pathogenic bacteria. Algaecides have customarily been utilized with good success as aids to sanitizers in abating potential algal blooms. Currently in the United States there are approximately 360 U.S. E.P.A. registered, nonoxidizing, algaecide formulations for use in recreational water systems (Pestbank 1996). Only two major active classes account for most of the registered formulations. Forty-one (41) percent of all formulations cited are monoquaternary ammonium chloride products, (monoquat) such as alkyl dimethyl benzyl ammonium chloride formulations. Forty-one percent (41%) are formulations of polymeric quaternary ammonium products (polyguat) and thirteen percent (13%) are copper based products. A small fraction (3%) of those comprise combination type products, such as monomeric and polymeric quaternary ammonium chloride and copper formulations. Silver based products comprise a lower percentage (1%) of the total, as well as other types of actives (1%) such sodium tetraborate pentahydrate. There are other non-conventional approaches to algal control such as the use of ammonium sulfate and lanthanum carbonate.

The uncontrolled growth of algae in recreational

There is no current published data or available information depicting the overall activity of new commercial algaecide formulations toward typical algae cultures. The majority of investigations date back to the 50's and 60's (Fitzgerald 1959a; 1959b; 1964; 1968;). More recently, Adamson and Sommerfeld (1980) published investigations relating to the efficacy of commercial algaecides.

The objective of this research study was to evaluate the effectiveness (algaestatic and algaecidal) of nine selected commercial algaecides, including PHMB, against three species of axenically grown cultures of organisms that are typically found in recreational water systems.

Materials and Methods

Algal assays – The algae cultures employed in this study were Chlorella pyrenoidosa (UTEX 26), Eustigmatos vischeri (UTEX 310) and Phormidium faveolarum (UTEX B-427). All algal cultures were purchased from the University of Texas (Austin) algal collection. Algal cultures were maintained in agar slants of modified Allen's media (Starr and Zeikus 1987). For experimental evaluations, cultures were grown in 250 ml Erlenmeyer flasks containing 100 ml Allen's Medium and incubated at $26 \pm 3^{\circ}$ C under cool-white fluorescent illumination of 9,500 lx, with a dark/light cycle of 16/8-hr. For the test assays, test algal cultures were grown for at least 10 days prior to assays, to obtain the desired population density. Algal cells were suspended in a test tubes with small glass beads and shaken for 30 seconds to break-up algal clumps that might be present. Cells were then inoculated to each flask containing Allen's media to equal approximately 10⁴ cells or filaments/mL. Each algal culture was standardized turbidimetrically using a spectrophotometer set at 590 nm.

Algaecides employed were those commercially

available within the Greater Memphis, TN. area. Table 1 depicts the type of products tested and their respective active ingredient(s) concentrations and recommended dosages. Algaecides were prepared fresh the day of the assay as 0.05% active stock solutions in sterile deionized water. To compare one chemical to another, all products were assayed as part per million active ingredient (a.i.). Stock solutions were added per flask to make final active concentrations of, 0.5, 1.0, 5.0 and 10.0 ppm (active) for *Chlorella pyrenoidosa* assays, 0.5, 1.0, 5.0, 10.0 and 15.0 ppm for *Phormidium faveolarum* and 5.0, 10.0 and 20.0 ppm (active) for *Eustigmatos vischeri* UTEX 310.

Algaecide tests were conducted in 250 milliliter flasks and incubated as described above. To determine algaestatic properties for each product, inoculated flasks were allowed to incubate for 21 days and visually inspected every 7 days for growth. The lowest concentration which had no visible growth after 21 days was considered to be the algaestatic concentration. To determine the algaecidal concentration of each product, 1.0 mL aliquots were withdrawn from each flask, at 24, 48, and 120 hour intervals (contact time), and filtered under a slight vacuum through a filtering unit with a glass microfiber (47 mm, $1.6 \,\mu m$ particle retention) membrane (Whatman). The membrane was washed twice with fresh sterile Allen's medium to remove excess biocide, transferred to a sterile test tube containing 5 milliliters of Allen's medium, and incubated as described above. Culture test tubes were inspected visually for up to 21 days before being discarded.

Algaecide Label Dosages (ppm ai) Initial / Maintenance			<u>Active ingredient(s) (a.i.)</u>
1.	2.0	0.5	-Alkyl dimethyl benzyl ammonium chloride (10%)
2.	0.23	0.115	-Copper ethanolamine complexes (7.41% elemental copper)
3.	2.72	1.36	-Alkyl dimethylbenzyl ammonium chloride (26%)
			Copper ethanolamine complexes (3% metallic copper)
4.	30-50	30-50	-Poly(iminoimidocarbonyliminoimidocarbonylimino-
			hexamethylene hydrochloride, (PHMB) (20%)
5.	4.0	1.25	-Alkyl dimethyl benzyl ammonium chloride (39.5%)
			Alkyl dimethyl ethyl benzyl ammonium bromide (0.5%)
6.	0.22	0.11	-Copper triethethanolamine complex (7.1% elemental copper)
7.	4.0	0.62	-Alkyl dimethyl benzyl ammonium chloride (30%)
			Alkyl dimethyl benzyl ammonium chloride (10%)
8.	2.0	0.5	-Alkly benzyl dimethyl ammonium chloride (49.5%)
			Dialkyl methyl benzyl ammonium chloride (0.2%)
9.	3.9	1.4	-Poly[oxyethylene(dimethylimino) ethylene- (dimethylimino) ethylene dichloride (60%)

Table 1 – Commercial algaecides, including PHMB, employed in the study

Growth and no growth patterns were assessed visually, and compared to control test tubes containing no algaecide. Algeacidal concentrations were determined as those concentrations which had no growth after transferring the membrane to a culture tube without algaecide. Assays were conducted at least twice.

Bacterial assays - The bacteriostatic properties of each commercial algaecide product, including PHMB, were determined impedimetrically with the use of the Bactometer (Vitek Systems) as a method of enumeration. Previously determined growth curves were used to standardize Bactometer impedance measurements against cfu/ml. Enterobacter aerogenes ATCC 13048 was employed to screen each product for bacteriostatic efficacy. The culture was maintained in tryptone glucose extract agar (TGEA) slants at room temperature (25°C). The bacterial culture was grown in TGEA plates for 24 hours at 37°C prior to the assay. Bacterial cells were then harvested and suspended in minimal salts solution. Bacterial cell density was adjusted turbidimetrically with the aid of a McFarland-1 standard (approximately 1.0 x 10⁶ cfu/ ml). The assay was conducted in a low nutrient minimal salts solution composed of: dextrose (0.01 gm), peptone, (0.01 gm)., ammonium nitrate, (1.0 gm), magnesium sulfate, (0.25 gm), and 0.25 grams of calcium chloride per liter of distilled water. The minimal salts medium was adjusted to a pH of 7.0 (Trisbuffer). The biocides were made into 0.05% active ingredient in sterile deionized water. Measured aliquots of the stock solution were then added to Erlenmever flasks containing minimal salts solution to contain 0.1, 0.5, 1.0, 10.0, 50.0 and 100 ppm active ingredient per test bottle. After 24 hours contact time at 37°C, a 25 microliter aliquot was aseptically transferred to the Bactometer test modules containing 1.0 milliliter of modified Wilken's Chalgren medium (del Corral, F, B. S. Johnson, and V. M. King: An improved screening method to evaluate bacteriostatic properties of compounds, manuscript in-progress).

A pink colored biofilm deposit, obtained from a residential pool in June, 1996 was brought to our laboratory for microbiological analysis. The deposit was found adhered to pool equipment, stairs and walls. A small sample of the deposit was aseptically removed and viewed under a microscope (wet mount). In addition, the sample was also stained with the Gram and Lactophenol cotton blue (Manual of Clinical Microbiology, 5th Ed.). After bacteriological confirmation, the sample was streaked for bacterial, fungal and algal isolation (standard streak plate technique). Using several selective media (R2-A, MaConkey, Pseudomonas isolation agar, Mannitol salt agar, acidified Potato dextrose agar) and a non-selective medium (TGEA) were employed to characterize the microbial content. A pink pigmented organism and numerous other non-pigmented organisms were isolated on TGEA. The pigmented isolate grew best at room temperature. The organism was maintained in TGEA and further characterized by standard microbiological techniques at room temperature. The bactericidal efficacy of each product was determined quantitatively, after 24 hours contact time in minimal salts solution (pH 7.0) containing active ingredient at the concentrations listed above. The number of surviving colonies after treatment were enumerated by serial 10fold dilutions in sterile deionized water. Dilutions were plated in TGEA agar and incubated for 36-28 hours at room temperature.

Algaecide #	MIC (ppm ai)	M 24 hrs	IAC Range (ppm a Contact Time 48 hours	ai) 120 hours
1	1.0	>1.0 ≤ 5.0	>1.0 ≤ 5.0	>1.0 < 5.0
2.	10.0	>10.0	>10.0	>10.0
3.	5.0	>1.0 ≤ 5.0	>1.0 ≤5.0	>1.0 ≤ 5.0
4.	≤0.5	≤0.5	<u>≤</u> 0.5	≤0.5
5.	1.0	>5.0	≥1.0 <5.0	≥1.0 <5.0
6.	10.0	>10.0	>10.0	>10.0
7.	≤0.5	>1.0 <5.0	≤0.5	≤0.5
8.	≤0.5	>1.0 <5.0	>1.0 <5.0	≤0.5
9.	<0.5	>1.0 <5.0	<0.5	< 0.5

> greater than, \geq greater than or equal to, < less than, \leq less than or equal to

Table 2 – Minimal algaestatic (MIC) and algaecidal concentrations (MAC) obtained with *Chlorella pyrenoidosa* UTEX 26 Algaecide concentrations tested (0.5, 1.0, 5.0, and 10.0 ppm active).

Results

Tables 2–4 depict the results obtained with the algal cultures; *Chlorella pyrenoidosa, Phormidium faveolarum* and *Eustigmatos vischeri* and eight commercial algaecides, including PHMB.

Results obtained with Chlorella pyrenoidosa, (Table 2) show that products #4 (PHMB), #5 (monoquat), #7 (monoquat), #8 (monoquat), and #9 (polyquat) were algaestatic within the suggested maintenance label recommendations for each product. The remaining products [#1 (monoquat), #2 (copper), #3 (monoquat/copper), and #6 (Copper)] were also algaestatic against C. pyrenoidosa, but higher levels were required, ranging from twice to 100 times more than the recommended dosage. Algaecidal effects were achieved with PHMB (#4) in 24 hours at low concentrations (<0.5 ppm). The dosages of the products that were algaestatic within recommended dosages also exhibited algaecidal effects; however, longer contact times were required (48 hours-120 hours).

Among the products that were not algaestatic within the use levels recommended were two copper based products (#2 and #6) which failed to be algaecidal at the highest concentration tested (10.0 ppm).

Based on our laboratory results, only PHMB and two monomeric quaternary ammonium products (#3 and #5) showed inhibitory effects (MIC) against Phormidium faveolarum within the recommended manufacturers (maintenance) addition rates. Other products fell outside those levels for this particular algae species, including product #3, a copper based product which had label claims as an effective algaecide against Cyanobacteria ("black algae"). Higher active levels were required for other products (twoto greater than 100 times more) to inhibit Phormidium faveolarum. Most products that were not algaestatic at use levels (#1, #7, #8 and #9) required higher active levels to achieve algaecidal effects on Phormidium faveolarum. Only two copper based products required significant higher active levels for algaecidal effects than those levels evaluated (>15.0 ppm).

Based on the results obtained with the algae Eustigmatos vischeri, four products [#1 (monoquat), #6 (copper), #7 (monoquat), #8 (monoquat), and #9 (Polyquat)] clearly required higher concentrations from those recommended to inhibit *E. vischeri*. Inhibitory levels of PHMB were greater than those evaluated in this assay against *E. vischeri*. The algaecidal concentrations needed for most algaestatic products interestingly fell within the same active ranges for 7 out of 9 products evaluated (#1, #2, #3, #5, #6, #7 and #8). For products #2 (copper) and #5 (monoquat) longer contact times were required for algaecidal effects on *E. vischeri*.

Based on our results, only PHMB and the polymeric quaternary ammonium product had significantly lower bacteriostatic values than those recommended in the label. The copper based product #2 was least effective against the coliform bacterium *Enterobacter aerogenes*.

The final evaluation presented in this research study was the bactericidal efficacy of commercial algaecide products against a pigmented pool isolate. Based on our microbiological observations, the pigmented isolate was presumptively characterized as a Rhodococcus equi strain. These type of bacterial strains are known to possess a pink caretenoid pigment. The pink-pigmented species was catalase positive, endospore negative, gamma hemolytic (5% sheep blood agar), acid fast negative, gram variable bacillus (diptheriod), no branching filaments and contained a caretenoid-like pink coloration. Additional biochemical tests were difficult to perform, due to the inability of the organism to grow in biochemical test substrates. Our observations are only presumptive and additional research is needed to confirm the taxonomy of this pool isolate.

All monomeric quaternary ammonium chloride products tested were bactericidal against the *Rhodococcus sp.* at low levels (5.0 ppm). The kill rates were in the magnitude of 99.0–99.9% reductions in 24 hours contact time (data not shown). The polyquat was bacteriostatic at all concentrations tested (5.0, 25.0 and 50.0 ppm). One copper based product (#2) was totally ineffective, while the other (#6) showed lower bactericidal levels (72.9%–91.0% reduction). The monomeric quaternary ammonium chloride products were the most effective against the *Rhodococcus sp.*

Discussion

Our results show that there is a wide range in efficacy levels between the different algaecide formulations tested and in the degree of effectiveness against different algal species. This is consistent with previous laboratory studies which showed variations or degrees of efficacy for algaecide formulations against single algal species and mixed bacterial populations (Fitzgerald 1959a; 1968). Investigations demonstrated that not all quaternary ammonium formulations were algaestatic (one of two) at the recommended label dosages against the cyanophytes, Phormidium minnesotense and Plectonema, sp. (Adamson and Sommerfeld 1980), In our investigations we found that most of the products (5 out of 9) evaluated were effective (algaestatic) within the recommended addition rates against the chlorophyte, Chlorella pyrenoidosa. However, only 2 monomeric quaternary ammonium formulations, (#3 and #5) out of the 5 tested, and PHMB were algaestatic at the recommended dose against the Cyanobacteria Phormidium faveolarum (commonly known as "black algae").

The ineffective quaternary ammonium formulation in the Adamson and Sommerfeld (1980) study contained copper triethanolamine (7.1% as elemental copper) and 2.5% alkyl dimethyl dichloride benzyl ammonium chloride. In the present work, product #3, a formulation of complexed copper (3% as metallic copper) and 26% Alkyl dimethylbenzyl ammonium chloride was algaestatic against the cyanophyte species, *Phormidium faveolarum* (Table 3). Possible explanations for such variability in efficacy could be due to resistance to the algaecide formulations or on potentiations based on active ingredient ratios, such as amounts of copper to quat ratios or even the the type of copper source.

According to Adamson and Sommerfeld (1980), the copper/quat formulation was not effective in controlling *Phormidium minnesotense*, *Plectonema* sp. or Oocysts sp., however, it was able to control the xanthophyte, Pleurochloris pyrenoidosa (typically known as "mustard algae"). In our experiments we were able to conclude that only the polymeric quat was unable to inhibit the growth of Eustigmatos vischeri (Table 4) within the recommended allowable maintenance dosages. The remaining products with the exception of PHMB had activity levels at or below 5.0 ppm (active). We erroneously assumed that most products could not control this algae species at low active levels. Experiments are underway to determine the overall effectiveness of these products against Eustigmatos vischeri.

Our observations also indicated that among the readily available commercial algaecides, only a few

		MAC Range (ppm ai) Contact Time		
Algaecide #	MIC (ppm ai)	24 hrs	48 hours	120 hours
1.	5.0	>5.0 <15.0	>5.0 <15.0	>5.0 <15.0
2.	>15.0	>15.0	>15.0	>15.0
3.	1.0	1.0 <15.0	1.0 <15.0	1.0 <15.0
4.	< 0.5	>15.0	>15.0	>15.0
5.	1.0	>1.0<15.0	>1.0<15.0	>1.0<15.0
6.	>15.0	>15.0	>15.0	>15.0
7.	1.0	>1.0< 15.0	>1.0<15.0	>1.0<15.0
8.	1.0	>1.0<15.0	>1.0<15.0	>1.0<15.0
9.	10.0	>15.0	>15.0	>15.0

> greater than, \geq greater than or equal to, < less than, \leq less than or equal to

Table 3 – Depicts minimal algaestatic concentrations (MIC) and algaecidal (MAC) ranges required to control *Phormidium faveolarum* UTEX B-427 Algaecide concentrations tested (0.5, 1.0, 5.0, 10.0 and 15.0 ppm active)

		MAC Range (ppm ai) Contact Time		
Algaecide #	MIC (ppm ai)	24 hrs	48 hours	120 hours
1.	≥ 1.0 < 5.0	≥ 1.0 < 5.0	≥ 1.0 < 5.0	≥ 1.0 < 5.0
2.	≥ 1.0 <5.0	>10 < 20.0	>10 < 20.0	≥ 1.0 <5.0
3.	≥ 1.0 < 5.0	≥ 1.0 < 5.0	≥ 1.0 <5.0	≥ 1.0 <5.0
4.	>20.0	>20.0	>20.0	>20.0
5.	≥ 1.0 <5.0	>5.0 <10.0	>5.0 <10.0	≥ 1.0 <5.0
6.	≥ 1.0 <5.0	≥ 1.0 <5.0	≥ 1.0 <5.0	≥ 1.0 <5.0
7.	≥ 1.0 <5.0	≥ 1.0 <5.0	≥ 1.0 <5.0	≥ 1.0 <5.0
8.	≥ 1.0 <5.0	≥ 1.0 <5.0	≥ 1.0 <5.0	≥ 1.0 <5.0
9.	>20.0	>20.0	>20.0	>20.0

> greater than, \geq greater than or equal to, < less than, \leq less than or equal to

Table 4 – Minimal algaestatic (MIC) and algaecidal (MAC) range required to control *Eustigmatos vischeri* UTEX 310 Algaecide concentrations tested (1.0. 5.0, 10.0 and 20.0, and ppm active)

products were algaecidal within label use rates. Against the chlorophyte *Chlorella pyrenoidosa*, PHMB was algaecidal at significantly lower concentrations than those recommended and was among the most effective against the species. Other products such as #7 (monoquat) and #9 (polyquat), were algaecidal only after 48 hours contact time at lower concentrations than those recommended on the label. Only one product (#5, monoquat) required at least 5 days at higher dosage rates before it produced an algaecidal effect on the chlorophyte (Table 2).

Our observations indicate that most of the products tested in this evaluation are not algaestatic at use levels. These observations are in accordance with the observations of Adamson and Sommerfeld (1980) which found that among all algaecides tested, only a chlorine based product was found to be algeacidal at the specified recommended use levels.

It is therefore critical to maintain recommended active levels of algaecide to keep at least algaestatic conditions that prevent algal blooms from developing. Early investigations (Fitzgerald 1959b, 1960. 1963) have indicated that several factors work toward lowering algaecide levels under normal swimming pool operating conditions. These factors include product adsorption, such as cationic products coming in contact with anionic materials (i.e., algae, soil, etc.) or absorption to filter media. In addition algaecide loss can occur by precipitation. Water quality factors, such as hardness, play an important role in such removal mechanisms. Hardness levels in pool water have been implicated to cause copper based products to precipitate when carbonates or bicarbonates are present and pH values are greater than 5.0. (Fitzgerald 1959). Fitzgerald found a relationship between hardness levels and algal test media versus regular distilled water. This study revealed that for Allen's media which contained a hardness value of 253 ppm (calcium carbonate), the copper products tested were taken out of solution more readily than when prepared in the softer distilled water. This is a possible cause of concern in that ideal recommended hardness levels for recreational water systems range between 200-400 ppm. (Pool and Spa Operators Handbook, 1990). Although our experiments evaluated all algaecides, including copper products in an artificial environment (Allen's media), considered to contain a high level of hardness, these levels fall within normal pool chemistry parameters. Hardness levels, whether in artificial media or in pool water, can play a critical role in the substantivity of algaecides.

Furthermore, it has been reported that hardness levels may also play a critical role in lowering the efficacy of quaternary ammonium compounds (Chambers *et al.* 1955). Contrary to those observations, studies performed with several polymeric quaternary ammonium chloride formulations (Hollis and Jaquess 1993) indicated that hardness levels of 900

Proceedings - NSPI Chemistry Symposium (1996)

ppm as calcium carbonate did not affect (lower) the bactericidal efficacy of selected polymeric quats against *Vibrio cholera*.

Other factors that can play a role in the efficacy of algaecides are incompatibilities with other pool chemicals or sanitizing systems (i.e. ozone), residual sanitizer levels and nutrient levels in the pool water. Regardless of the prevailing environment in a recirculating pool or spa, algaecide levels are an important factor in preventing spontaneous algal blooms. It is essential that residual algaecide measurements be made periodically to ensure the proper level of product is present.

Another observation worth noting is that most, if not all algaecide products have some ability to kill or inhibit prokaryotic organisms (Table 5). This finding has been known for some time, and agree with the work of Fitzgerald (1959a), which demonstrated the bactericidal properties of 8 commercial algaecides against a mixed bacterial population. This property of most algaecides is beneficial only if the product can exert bacteriostatic or bactericidal properties within the initial or maintenance use dosage rates. Our investigations revealed that a pigmented Rhodococcus equi strain isolated from a pool biofilm deposit was very susceptible to monomeric quaternary ammonium compounds and to PHMB at recommended dosage levels. It is important to note that this observation was made with a suspension of organisms and was not representative of the actual in-situ conditions in which the isolate was found (adherent biofilm). Organisms associated in biofilm deposits tend to be more resistant to the action of biocides. LeChevalier et al. (1988) observed that biofilm bacteria were 150 to 3,000 times more resistant to hypochlorous acid (free chlorine, pH 7.0) than were unattached cells. For this reason, it is important to brush or scrub all pool sur-

Algaecide #	MIC (ppm ai)
1.	> 1.0 < 10.0
2.	> 50.0 < 100
3.	>10.0 < 50.0
4.	0.5
5.	>1.0 < 10.0
6.	>10.0 < 50.0
7.	>1.0 < 10.0
8.	>10.0 < 50.0
9.	0.5

Table 5 – Bacteriostatic efficacy range of eight commercial algaecides, including PHMB against *Enterobacter aerogenes* ATCC 13048. faces (walls, stairs, etc.) to detach adherent biofilm formations which often are impervious to the action of disinfectants and algaecide products.

Conclusion

The use of in-vitro laboratory studies to investigate the anti-microbial properties of commercial products is an important complement to field studies. All laboratory data should be taken in strict context and can only be employed as a cursory appraisal of actual field conditions. Laboratory data obtained in this study cannot be fully extrapolated to field conditions, but can be employed as a first tier assessment of the capabilities of each product under identical reproducible conditions. From the studies basic assumptions can be made about the spectrum of activity and dosage approximations of the products. The amount of chemical required to kill algae depends on the type of product, length of exposure, type of algae species, severity of the infestation and also residual sanitizer levels.

Water quality (Chamber *et al.* 1955) as well as filter media have also been implicated in the overall efficacy of microbicides (Fitzgerald 1959b, 1960 and 1963).

To kill algae requires an algaecidal dose. Inhibition or algaestatic conditions will not kill algae, but merely keep them in stasis, as long as the chemical is maintained at the proper dose. Our experiments have revealed that printed label use dosages, in most cases, do not kill algae, but will prevent or abate algal growth.

Few chemical companies today have on-going research and development programs that actively search for new pool algaecides. One of the main reasons is the cost and time associated in obtaining registration with the U.S. E.P.A. The only choice for most companies has been to formulate or re-formulate existing actives, in order to optimize the efficacy of the product and at the same time attempt to offer a more effective and versatile treatment for the end user.

To work within these constraints, the recreational water industry and chemical manufacturers need a better understanding of the factors controlling algal proliferations in recreational water systems, including chemical and biochemical factors affecting algaecide performance and physical conditions that foster algal adherence to surfaces. Having an understanding in these areas will enable better formulations with site specific targeting approaches.

Finally, it is important to mention the impact that test methods (methodology) has on product performance in a laboratory setting. The U.S. E.P.A. has set guidelines (Schneider 1992) to evaluate algaecide formulations in a laboratory setting. These guidelines are based on the early research of Fitzgerald and Faust (1963). The guidelines have been in place at the Agency's Chemical and Biological investigations Branch (Benefit and Field Studies Div.) to help determine the efficacy of algaecide formulations and are part of the agency's enforcement task force. It has been our experience working with the techniques described by Fitzgerald (1962, 1964) that improvements could be made to more closely resemble in-use conditions. Testing algaecides in a synthetic pool water medium instead of rich algae culture media might improve the overall reproducibility of test results.

References

- Adamson and Sommerfeld, (1978). "Survey of swimming pool algae of the Phoenix area and metropolitan area." *Journal of Phycology*. 14: 519– 521.
- Adamson and Sommerfeld, (1980). "Laboratory comparison of the effectiveness of several algicides on isolated swimming pool algae." *Applied and Environmental Microbiology*. Vol. 39(2): 348–353.
- Chambers, C. W. Kabler, P. W., Bryant, A.R., Chambers., L. A. and Ettinger, M. B. (1955).
 "Bactericidal efficiency of. Q.A.C. in different waters." *Public Health Reports* (U.S.) 70: 545-553.
- Fitzgerald, G. P. (1959a). "Bactericidal and Algicidal Properties of some Algicides for Swimming Pools." *Applied Microbiology*. 7(4): 205–211.
- Fitzgerald, G. P. (1959b). "Loss of Algicidal Chemicals in Swimming Pools." Applied Microbiology 8:269– 274
- Fitzgerald, G. P. (1960). "Loss of algicidal chemicals in swimming pools." *Applied Microbiology*. 8: 269– 274.
- Fitzgerald, G. P. (1962) "Bioassay for algicidal chemicals in swimming pools." Water and Sewage Works. 109: 361-363
- Fitzgerald, G. P. (1963). "Field tests on the duration of algicides in swimming pools." Journal Environmental Health 25:319-325.
- Fitzgerald, G. P. and S. L. Faust, (1963) "Bioassay for algicidal and algistatic chemicals." Water and Sewage Works. 110:296-298.
- Fitzgerald, G. P. (1964). "Factors in the Testing and Application of Algicides." *Applied Microbiology*. 12(3) 247-253.
- Fitzgerald, G. P. and S. L. Faust, (1964). "Techniques for economical comparisons of swimming pool algicides." *Water Sewage Works* 112: 271-275.
- Fitzgerald, G. P. (1968). "Compatibility of Swimming Pool Algicides and Bactericides." Water and Sewage Works. 115: 65-71.

Fitzgerald, G. P (1971) Algicides. Literature Review

#2. The University of Wisconsin Water Resource Center. Eutrophication Information Program.

- George C. Hollis and P. Jaquess (1993). Ionene polymers as microbicides. U.S. Patent Pending
- Lechevallier, M. W., C. D. Cawthon, and R. G. Lee. (1988). "Inactivation of Biofilm Bacteria." *Applied* and Environmental Microbiology. 54:(1) 2492– 2499.
- Manual of Clinical Microbiology, 5th Ed. (1991). Ed(s), A. Balows, W. J. Hausler, K. L. Hermann, H. D. Isenberg, and H. J. Shadomy. American Society for Microbiology
- Pool and Spa Operators Handbook (1990), Lester Kowalski (ed). National Swimming Pool

Handbook p. 50.

- Pest-Bank Pesticide Product Data (1996). [CD-ROM]. West Lafayette, In: Center for Environmental and Regulatory Information System (producer). Available: Silverplatter.
- Richard C. Starr and Jeffrey A. Zeikus, (1978). "UTEX- The culture collection of algae at the University of Texas, Austin." *Journal of Phycology* vol. 23 pp. 36-37.
- Schneider, B.A. (1982) Pure culture Technique for evaluating algicides. In Pesticide Assessment guidelines, Subdivision G. Product Performance. U.S. E.P.A. 540/9-82-026