The Use of PHMB as a Sanitizer in Domestic Spas

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The control of bacteria in spa water is much more demanding than pool water. The heated water and higher concentrations of both organic load and bacteria shed by bathers can result in unacceptable bacterial growth developing in spa water within a few hours of heavy use. To prevent health affecting bacterial outbreaks, three approaches may be employed: continuous feed of the sanitizer, daily monitoring and addition of the sanitizer, and the use of a stable and non-load dependent sanitizer. Bromine and chlorine require daily monitoring and adjustment. Polyhexamethylene biguanide (PHMB or biguanide) is a non-oxidizing sanitizer that is minimally affected by high bather use. Prior to registration for use in spas by the US EPA the antibacterial performance of PHMB was measured in domestic spas with simulated use levels of one person per 125 gallons for 30 minutes per day. The spas were inoculated with high levels of bacteria implicated in spa related skin rashes (Ps. aeruginosa and Staphylococcus aureus) and those shed in feces (Escherichia coli, and Enterococcus faecalis) plus 2 pints of synthetic bather load per day. Throughout the study period, the PHMB treated spa water had fewer bacteria than allowed by US EPA standards for recreational water as well as fewer bacteria than allowed in drinking water. A sodium dichloro-s-triazinetrione system treated spa exceeded EPA standards for total coliforms on 13 of 14 days and had persistent levels of Ps. aeruginosa. PHMB provided a significant improvement in the control of spa associated bacteria when compared to the sodium dichloro-s-triazinetrione system.

Introduction

Polyhexamethylene biguanide (PHMB) is a polymeric cationic antimicrobial that is used as a sanitizer in recreational water. All chemical treatment systems that make antimicrobial claims must be registered with the US EPA under the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA). PHMB was first registered as a swimming pool sanitizer in 1981. In 1992 PHMB was registered for use in spas. PHMB is presently sold under the brand names of Baquacil® Chlorine Free Swimming Pool Sanitizer and Algistat, Baqua Spa™ Sanitizer and Soft Swim™.

Prior to registration under FIFRA, the efficacy of all chemical treatment systems must be demonstrated. Current efficacy guidelines used for disinfectant/sanitizer approvals date from the mid 1960's. The end–use microbiological guidelines used by the EPA specify that the maximum permissible level of heterotrophic plate count bacteria is 20,000 CFU/100ml, coliforms 2 CFU/100 ml and fecal streptococci 2 CFU/100 ml (subdivision G, EPA guidelines 91–8). These guidelines, however, do not reflect the state of the art understanding of dermal rashes associated with recreational water. During the mid 1970's, *Staphylococcus aureus* was considered a cause of skin rashes in swimming pools (Black *et al.* 1970). The Centers for Disease Control and Prevention (CDC) has linked *Pseudomonas aeruginosa* to numerous cases of folliculitis in poorly maintained systems (Highsmith *et al.* 1985). To reflect these concerns, Zeneca has established an internal maximum permissible level of *P. aeruginosa* at 2 CFU/100 ml and *S. aureus* at 50 CFU/100 ml.

This work was designed to assess the relative stability and antimicrobial efficacy of PHMB against EPA and Zeneca guidelines in spas.

Materials and Methods

Spa Design

Evaluations were conducted in 250 gallon Down East Spring Harbour Fire Lake free standing spas. Water balance parameters used were pH 7.4–7.6, total alkalinity 80–100 ppm, calcium hardness 200–400 ppm, and temperature 100–104°F (39°C +/– 1°C). The water was circulated for 1.5 hours per day at 115 gallons per hour during which the blowers were acti-

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vated for 0.5 hours.

The PHMB was initially dosed at 10 ppm (active) and maintained between 6 – 10 ppm throughout the study. Hydrogen peroxide was used as an oxidizer and added to the water to establish a level of 27.5 ppm. A chelating agent, tetrasodium ethylene–diaminetetraacetic acid was also added to the water at a dose of 15 ppm.

The chlorine spa was maintained using a commercial granular chlorine maintenance program. It consisted of adding the following at start–up: 0.5 oz. lithium hypochlorite concentrate, 2.5 capfuls of Stain and Scale Control, 0.25 capful water clarifier, 1 capful water freshener. About 16 g of chlorinating concentrate (sodium dichloro–s–triazinetrione) was added daily in order to achieve 3 ppm minimum free chlorine residual (expressed as HOCl). On a weekly maintenance basis: 0.5 oz. lithium hypochlorite concentrate, 0.5 capful of Stain and Scale Control, 0.25 capful water clarifier, 1 capful water freshener was added. The identities of the non–chlorine products was unknown.

To simulate typical bacterial load for home spas each spa was inoculated daily with *Escherichia coli* ATCC 25922, *Enterococcus faecalis* ATCC 19433 (formerly *Streptococcus faecalis*), *Pseudomonas aeruginosa* ATCC 27853, and *Staphylococcus aureus* ATCC 25923. Separate cultures of each organism were grown overnight on Tryptic Soy Agar (Difco) at 37°C. The bacteria were harvested in phosphate buffered saline and inoculated immediately after harvest. The inoculum size was adjusted to $2 \cdot 10^6$ CFU which represented typical total bacterial load shed by bathers for a spa of this dimension. The inoculum size was verified by plating on TSA at 37°C for 48 hours. Given the spa volume of 250 gal (946 L), the initial innoculum can be calculated to be approximately 2 CFU/ml.

E. coli and *E. faecalis* are specified as test bacteria in Disinfectants (Water) for Swimming Pool Water (AOAC 1990). *P. aeruginosa* and *S. aureus* were included as these two organisms present significant threat of bacterial induced dermatitis in inadequately maintained spas. All strains were obtained from the ATCC.

To simulate organic load shed by bathers two pints of artificial perspiration was added each day at the time of bacterial inoculation. The quantity of perspiration added reflected human load for spas of the volume. The perspiration consisted of ammonium sulfate, 161.3 mg/L; urea, 277.1 mg/L; uric acid, 3/6 mg/L; creatinine, 23.2 mg/L; creatine, 1.0 mg/L; and casamino acids; 57.1 mg/L (White 1986).

Microbiology

Samples were collected 18 inches below the water surface in sterile containers. The water samples were neutralized using 10 ml of a 0.7% lecithin/2.0% Tween 20 solution per 100 ml of PHMB treated water and 1 ml of a 2% solution of sodium thiosulfate per 100 ml of chlorine treated water. Duplicate neutralized aliquots were filtered using a Milliflex filtration system. Filter membranes were aseptically trans-

Medium	Organism	Incubation Temperature	Incubation Time	Positive Reaction	Maximum Permissible Level per 100 ml
m–TGE	total aerobic count	35°C	42 – 52 hours	any colony present	20,000
m–ENDO	total coliforms	35°C	21 – 27 hours	red with *dark* metallic sheen	2
m–FC	fecal coliforms	44.5 +/–0.2°C	22 – 26 hours	various shades of blue	2
Pseudomonas agar	*Ps. aeruginosa*	40–41°C	48 – 72 hours	red or pink colonies	2
KF agar	fecal streptococci	34–36°C	24 – 48 hours	red or pink centers	2
MSA	*S. aureus*	34–36°C	18–40 hours	yellow to golden colonies and yellow agar	50

Table 1 – Incubation conditions and reactions for bacteriological media

ferred onto appropriate media cassettes. Media and incubation conditions used are listed in Table 1.

m–TGE, m–Endo, and m–FC were purchased from Millipore as 1.8 ml ampoules and used with Milliflex liquid media cassettes. KF and Mannitol Salt Agar (MSA) was purchased from Difco. The m–PA specified in Standard Methods for the Examination of Water and Wastewater (APHA 1992) was developed for chlorinated water samples. This formulation proved difficult to standardize and the results were not reproducible when using non–halogen disinfectants. Instead of m–PA, Difco Tryptic Soy Agar was modified to include the antibiotics and phenol red at the concentrations specified in the m–PA recipe. This media was prepared using the following protocol: TSA was dissolved, 0.08 g of phenol red was added and the pH was adjusted to 6.5. This basal solution was sterilized at 15 psi for 15 minutes. The agar was tempered to 55–60°C and the pH readjusted to 7.1 (+/– 0.1), and dry antibiotics were added per liter of solution: sulfapyridine (174 mg), kanamycin (8.5 mg), nalidixic acid (37 mg), and cycloheximide (150 mg).

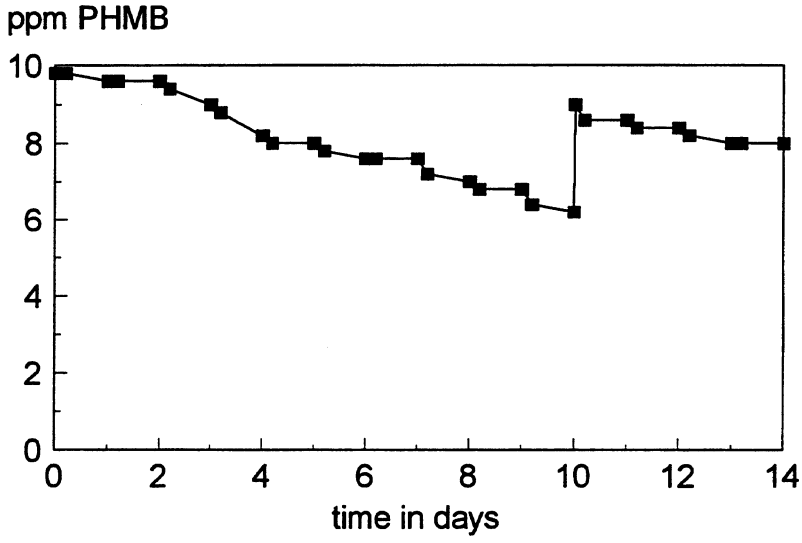

Figure 1 – Concentration of PHMB in treated spa

Chemical Sampling and Testing

The procedure for collecting samples for water balance and disinfectant/sanitizer testing was the same as the bacteriological samples. All chemical parameters were tested using a Baquacil ProLab.

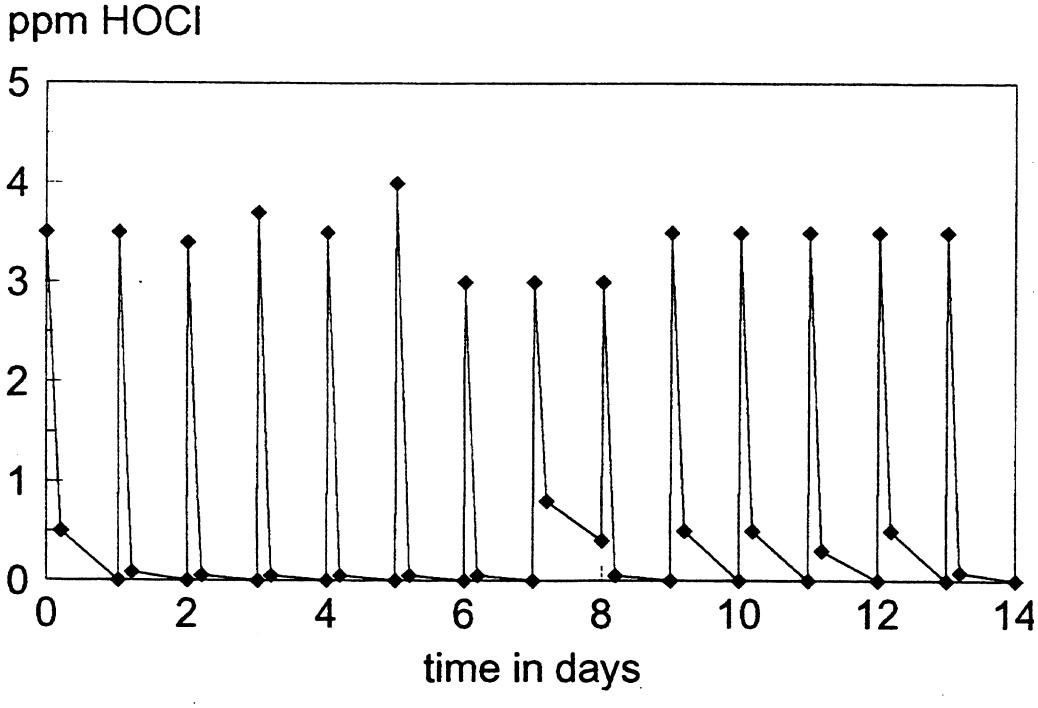

Figure 2 – Concentration of HOCl in treated spa

Results

The rate of depletion of PHMB and HOCl in spas is shown in Figures 1 and 2, respectively. The concentration of PHMB dropped at an average rate of 0.33 ppm (active ingredient) per day. On day 10 the PHMB level was topped up to maintain the concentration in the target range of 6 to 10 ppm. This pattern of consumption was slightly less than the average loss of 0.4 ppm in swimming pools. The stability of PHMB is in sharp contrast to that of HOCl that was rapidly depleted by the organic bather and

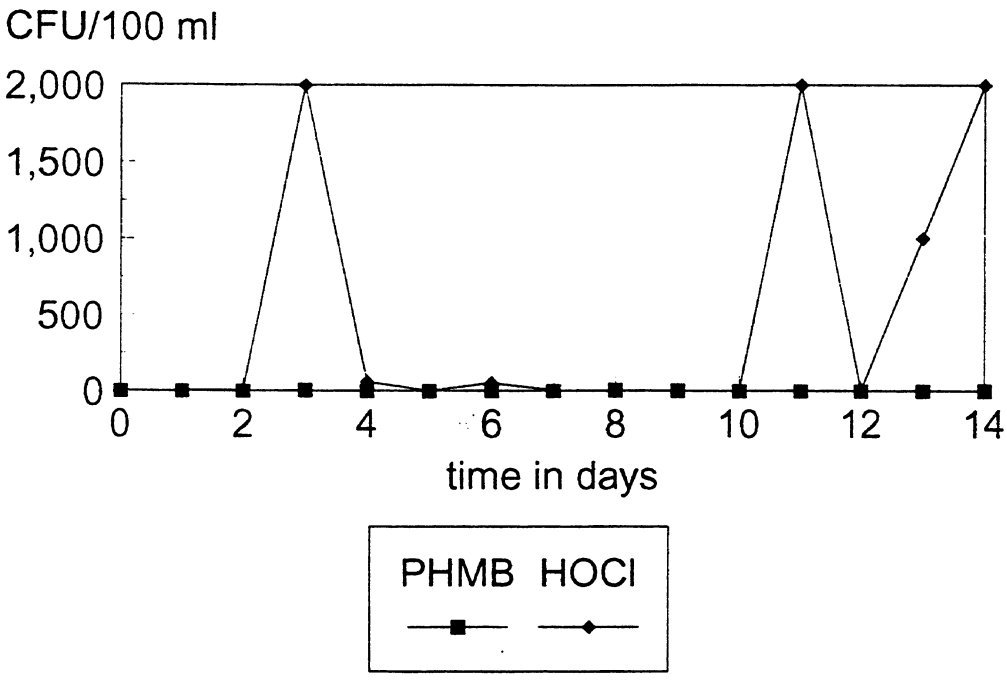

Figure 3 – Coliform plate counts of PHMB and HOCl treated spas

bacterial load. The pattern of depletion is a result of the interaction of HOCl with nitrogenous materials in the bather load and subsequent formation of chloramines. During the course of the study sufficient chlorine was added on a daily basis to reach breakpoint and return the free available chlorine level to 3.5 ppm.

The differences in stability of the two systems result in different microbiological levels 24 hours after use. The heterotrophic plate counts of the HOCl treated spa were consistently above 4,000 CFU/100 ml whereas the highest count noted in the PHMB spa was 135 CFU/100 ml on day seven and was 0 CFU/100 ml on seven of fourteen days.

Both PHMB and HOCl failed to provide complete control of coliforms (Figure 3). The plate counts are shown on the same scale to compare relative efficacy. On day four and six the coliform counts in the PHMB spa were 8 and 5 CFU/100 ml, respectively. HOCl coliform counts passed EPA guidelines only on day nine and exceeded 2000 CFU/100 ml on three days.

Both PHMB and HOCl treated spas were free of *S. aureus* 24 hours after inoculation (Figure 4). Considering the high coliform and *P. aeruginosa* counts in the HOCl spa it is likely that the *S. aureus* was inhibited by bacterial competition or bacteriocin production rather than by direct antimicrobial treatment.

Pseudomonas aeruginosa was the most persistent bacteria in the HOCl spa. On 11 of 14 samples

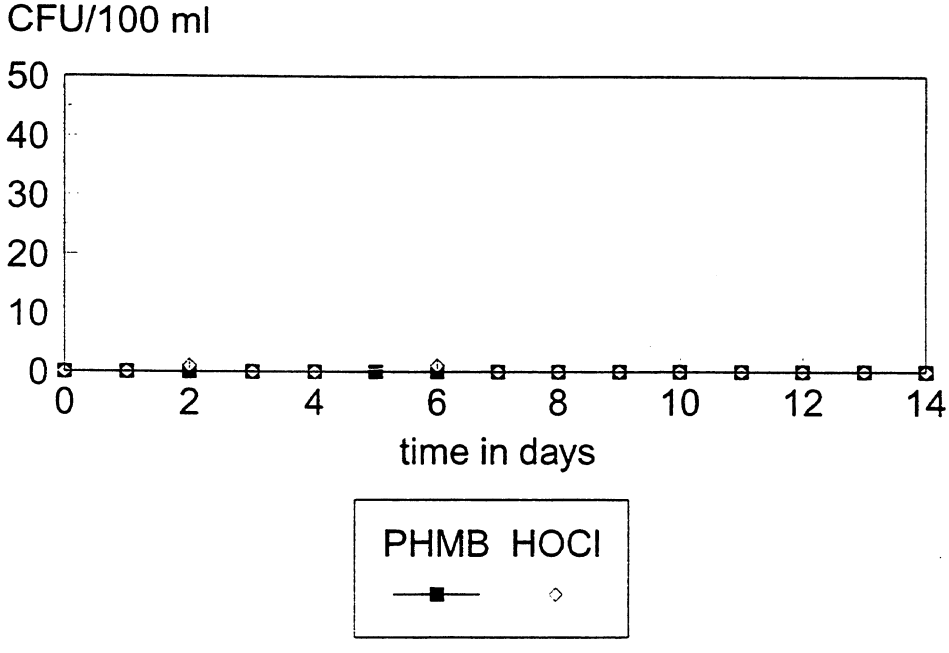

Figure 4 – *S. aureus* plate counts of PHMB and HOCl treated spas

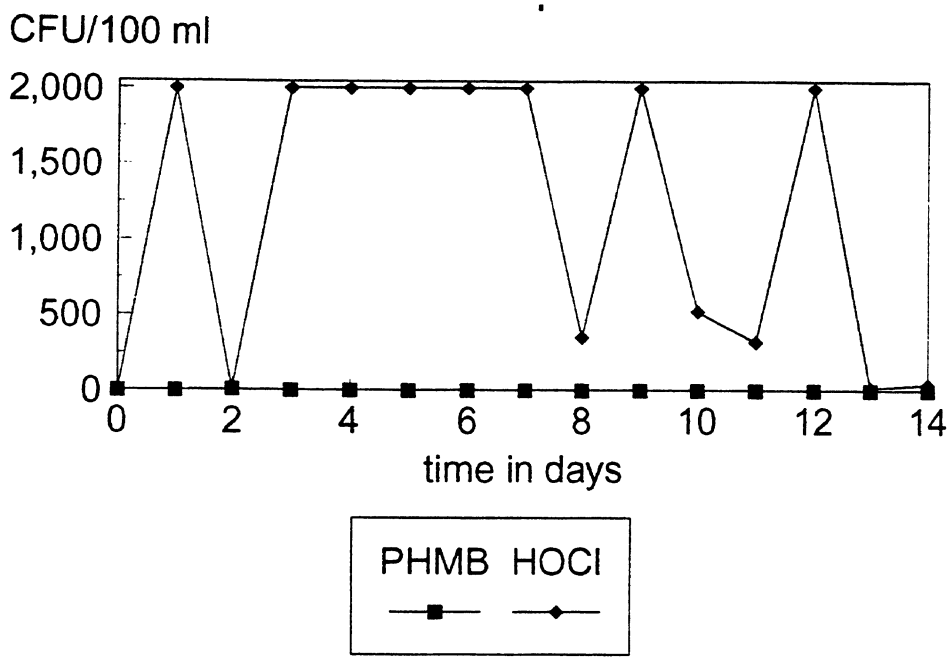

Figure 5 – *P. aeruginosa* plate counts of PHMB and HOCl treated spas

the population exceeded 2 CFU/ml (Figure 5). The cyclic pattern seen with *P. aeruginosa* and with the coliforms are generally out of phase. This suggests that ecological population shifts between these two dominant groups were occurring.

Discussion

The mode of action of PHMB is similar to that of quaternary ammonium compounds that rupture the plasma membrane and cause cell death as a result of the loss of cytoplasmic contents. The mode of PHMB consists of a three stage process of initial electrostatic attraction, adsorption onto the cytoplasmic outer membrane leaflet, and the disruption of the outer leaflet by the insertion of hydrophobic linker groups. The key step in this process is the attraction of the cationic PHMB molecule to the negatively charged cell surface (Broxton *et al.* 1984). The positive charges on the PHMB molecule are evenly spaced along the backbone in the biguanide groups. Because of this, the overall activity of the molecule is not compromised by the presence of interfering extraneous organic and nitrogenous materials commonly found in recreational water.

Although the specific mode of action of HOCl remains in question, it is well recognized the most efficacious form of the molecule is the fully protonated hypochlorous acid form. The direct oxidation of membrane components by HOCl as well as the ability of small uncharged molecules, such as HOCl, to cross the differentially permeable plasma membrane and directly attack cytoplasmic targets makes it difficult to separate the primary and secondary effect of chlorine (Dukan 1996). The reduced efficacy of the OCl^- ion is a likely a result of its reduced oxidative capacity and inability to cross the membrane. Intact cytoplasmic membranes are good electrical insulators at physiological concentrations. The propensity of HOCl to react with inorganic and organic nitrogen groups necessarily means that it will not remain in the most biocidal form in the recreational waters with high bather loading, such as spas. This pattern is illustrated by the rapid depletion of free chlorine in spas after use and the subsequent development of high bacterial levels in the water column.

When used properly, and according the label directions, both PHMB and HOCl can maintain hygienic levels of bacteria in spas. The primary differentiater of the two compounds is their stability in the presence of organic material. PHMB requires fewer daily adjustments to the active concentration level and may provide improved control of microbial levels to less fastidious spa users.

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