

Novel N-Halamine Water Biocides

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Novel N-halamine biocides are described which could be of importance to the swimming pool and spa industry. A water soluble imidazolidinone compound is discussed which should be competitive with cyanuric acid as a stabilizer for free chlorine. The reaction products of this imidazolidinone in situ, which are monochloro and dichloro derivatives, are biocidal against microorganisms and are algistatic. Thus the material should serve a dual purpose as a chlorine stabilizer and as a preventative of microorganism growth in the water. An insoluble poly-(styrenehydantoin) compound is also discussed which is an excellent contact biocide when brominated and which could serve as a bromine stabilizer for pools and spas. A discussion of the mechanisms of biocidal action and the possible uses of the new compounds is also presented.

There is currently a need for new chemical materials to be used as free chlorine and bromine stabilizers for swimming pools and spas. If such materials could also be employed to provide long-term biocidal action against pathogenic microorganisms and algae, the pool and spa industry would be well served. The most common chlorine stabilizer in use today for outdoor swimming pools is cyanuric acid, the chemistry for which has been thoroughly discussed (Wojtowicz 1996). However, rather large quantities of this material (50 mg/L) must be employed for effective stabilization against photodegradation by sunlight which eventually necessitates draining of the pool. Further-

more, the chloroisocyanurates which form in the pool upon reaction of free chlorine with cyanuric acid are less effective disinfectants than is free chlorine itself (Andersen 1965), and some states, e.g., New York, have banned the use of cyanuric acid in public pools due to possible health risks to bathers. There is really no effective bromine stabilizer available, although bromochlorodimethylhydantoin (BCDMH), itself a biocide, is being used partially in this capacity. Many algicidal and algistatic materials are commercially available, but an effective one which could be used in the dual capacity of stabilizer and algicide or algistat would seem to be a commercializable entity.

Work has been proceeding in these laboratories since 1980 designed to develop new materials for use in water disinfection applications. The work originally focused on a series of water-soluble organic N-chloramines and N-bromamines which were found to be very stable (persistent) in water and which possessed biocidal efficacies against a broad variety of organisms including algae (for example see Worley and Williams 1988). More recently, the emphasis has shifted to insoluble polymeric N-halamine biocides which are effective in cartridge filter applications (Sun *et al.* 1995). This paper will discuss the water-soluble and water-insoluble compounds developed in these laboratories which would be the most beneficial to the swimming pool and spa industry.

Methods

Materials – The syntheses, purification, and structural proofs of the N-halamine compounds to be discussed in this paper have been reported (Worley *et al.* 1991; Worley *et al.* 1992; Worley *et al.* 1996). The chemical structures of the compounds are given in reaction schemes 1–3; abbreviations will be used

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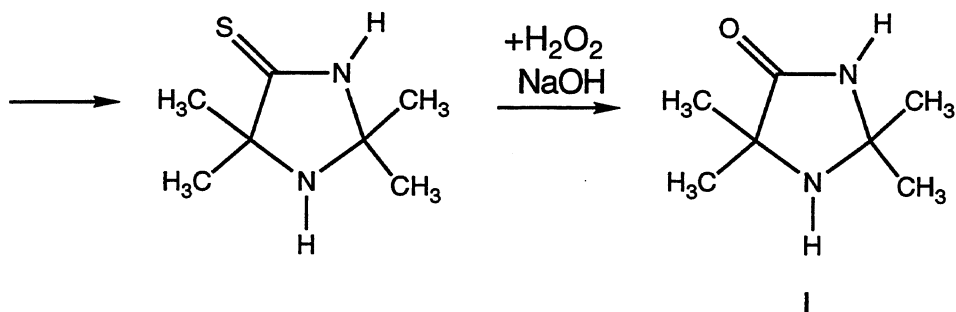
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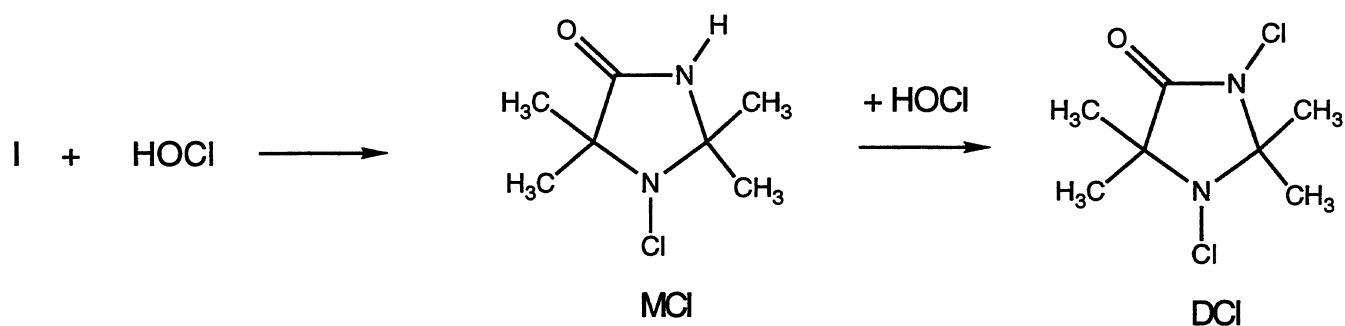
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Scheme 1

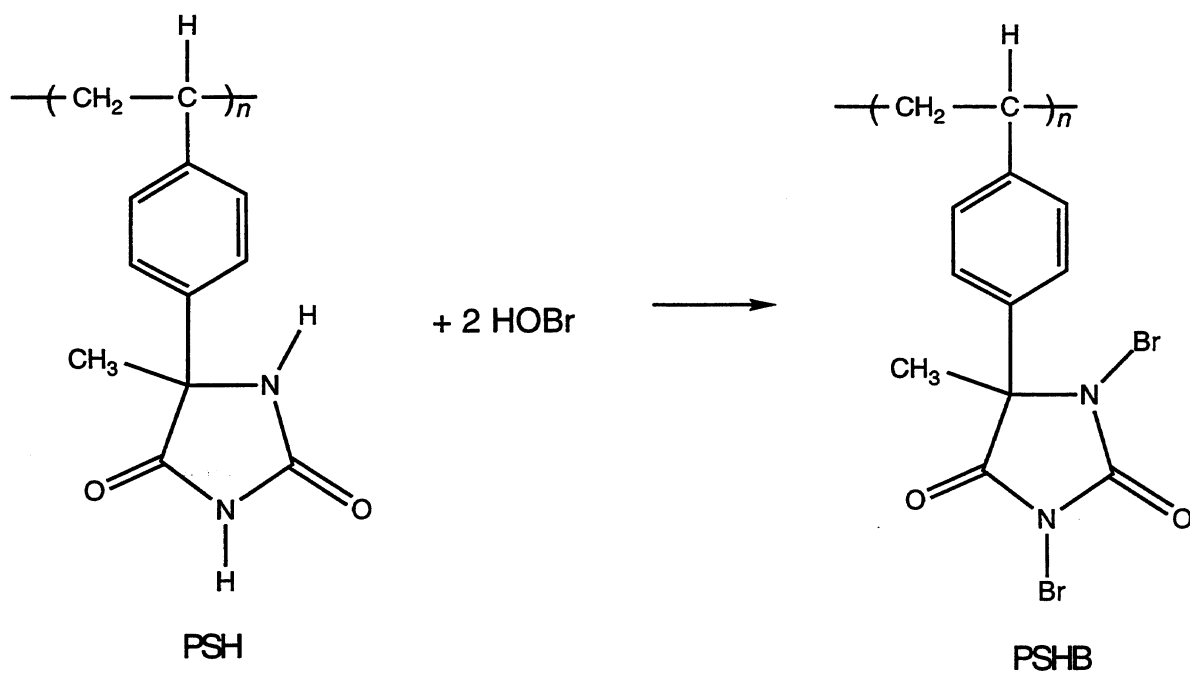
Acetone
Sodium cyanide
Ammonium sulfide
Ammonium chloride



Scheme 2



Scheme 3



in the remainder of this paper for designation of the compounds as follows: 2,2,5,5-tetramethyl-1,3-imidazolidin-4-one (I), 1-chloro-2,2,5,5-tetramethyl-1,3-imidazolidin-4-one (MCI), 1,3-dichloro-2,2,5,5-tetramethyl-1,3-imidazolidin-4-one (DCI), poly(5-methyl-5-(4'-vinylphenyl)hydantoin (PSH), and poly(1,3-dibromo-5-methyl-5-(4'-vinylphenyl)hydantoin (PSHB).

Biocidal Efficacy Testing – The water-soluble compounds I, MCI, and DCI were tested against the bacteria *Staphylococcus aureus* (ATCC 25923) and *Pseudomonas aeruginosa* (ATCC 27853) in buffered demand-free water (DFW) and in a synthetic demand water (SDW) which contained heavy halogen demand, salts, turbidity, was buffered to pH 9.5, and was held at 4°C; the testing procedures used in these laboratories have been documented elsewhere (Williams *et al.* 1987).

The water-insoluble polymers PSH and PSHB were tested in a filter application against a variety of bacteria, fungi, protozoa, and rotavirus in flowing water. In this paper the data for the bacterium *Klebsiella terrigena* (ATCC 33257), which is the bacterium recommended for testing by the U.S. E.P.A., will be presented. The testing protocols have been reported (Sun *et al.* 1995). In this application 4.93 g of PSHB of mesh size 25–60 was packed into a glass column having an inside diameter of 1.1 cm and a length of 10.2 cm; the empty bed volume was 3.8 mL.

Algistatic Efficacies – Algae testing for the water-soluble compounds MCI and DCI was performed in aerated, illuminated 10 gal aquariums held in the temperature range 21–24°C in the laboratory; a mixture of the algal species *Anabena cylindrica* (UTEX B1611), *Chlorella pyrenoidosa* (UTEX 395), and *Oscillatoria lutea* (UTEX 1814) at a cell density of about 10^7 per mL was employed. Algae concentration was monitored by a Milton Roy Spectronic 301 spectrophotometer set at an absorbance wavelength of 750 nm. A control aquarium containing no biocidal compound was also monitored concurrently. Total chlorine concentrations were measured by standard iodometric titrations; the initial concentrations of the two compounds were each 10 mg/L total chlorine measured as total Cl^+ (this would correspond to 20 mg/L total chlorine measured as Cl_2 , which is the convention in the water treatment industry, albeit a term having no chemical significance as only Cl^+ can inactivate organisms).

Stabilities – Three types of stability experiments relevant to the pool and spa industry for the water-soluble compounds were performed. In one, the compounds MCI, DCI and free chlorine from calcium hypochlorite were each dissolved to a concentration level of 10 mg/L total chlorine, measured as Cl^+ by

iodometric titration, in the synthetic-demand-water (SDW) formulation mentioned earlier. The percent total Cl^+ remaining was then monitored as a function of time. In a second, compound DCI and free chlorine from calcium hypochlorite were each dissolved in 100 mL beakers at a concentration of 10 mg/L total chlorine in 85 mL of demand-free water buffered at pH 7.0, and the solutions were placed in a temperature-controlled water bath (22–24°C) in direct sunlight. The percent chlorine remaining in each beaker was then monitored over a 10 hour period. In the third, three solutions containing 10 mg/L of I, the molar equivalent of cyanuric acid, and only demand-free water at pH 7.0 were placed in direct sunlight, and to each was added 4.1 mg/L free chlorine from calcium hypochlorite. Total chlorine concentrations were measured as a function of time of sunlight exposure.

PSH as a Bromine Stabilizer – Two 3 L solutions each containing about 5 mg/L free bromine (measured by iodometric titration as Br^+), prepared by oxidation of sodium bromide by potassium peroxy monosulfate, were circulated through two glass filters containing 1.6 g PSHB and 6.5 g sand, respectively, by use of identical laboratory water pumps. The reservoirs containing the solutions were continuously irradiated by ultraviolet photons; the filters were wrapped in aluminum foil to prevent direct exposure of PSHB and the sand to UV radiation. The free bromine contents of each reservoir were monitored as a function of time.

Results

Biocidal Efficacies – Biocidal efficacy data for the water-soluble compounds MCI and DCI are given in Table 1. It can be seen that DC generally required less than 10 minutes to effect a 6-log reduction of *S. aureus* and *P. aeruginosa* in demand-free water. The monochloro compound MC required much longer contact times, and both compounds needed considerably longer contact times in the synthetic-demand water, which provides the most difficult challenge for any disinfectant (high pH, low temperature, heavy halogen demand). Nevertheless, both compounds eventually provided a 6-log reduction of the bacteria given sufficient contact time. Thus, although the primary role of I will be as a chlorine stabilizer in forming a mixture of MCI and DCI *in situ*, some biocidal action will also result. It should be noted that I itself does not inactivate microorganisms.

The data in Table 2 show that the polymer PSHB is an excellent biocide in a filter application. The U.S. E.P.A. requirement for disinfection of *K. terrigena* is a 6-log reduction from a starting concentration of 10^7 CFU/100 mL. The polymer PSHB provided a 7-log reduction at a variety of pH's, temperatures, and in DFW and SDW in a few seconds contact time. The

Compound	pH	T(°C)	Water Quality ^a	Conc. mg/L	Organism ^b	% Inactivation at 10 min	Time for 99.9999% Inactivation (min)
DC	4.5	22	DFW	5	SA	>99.9999	9.1
MC	4.5	22	DFW	5	SA	96.3	60
DC	7.0	22	DFW	5	SA	99.1	29
MC	7.0	22	DFW	5	SA	4.4	588
DC	9.5	22	DFW	5	SA	>99.9999	5.4
MC	9.5	22	DFW	5	SA	50.2	454
DC	9.5	4	SDW	10	SA	68.1	240
MC	9.5	4	SDW	10	SA	0.0	2857
DC	7.0	22	DFW	5	PA	>99.9999	4.2

^aDFW = demand free water; SDW = synthetic demand water (see text).

^bSA = *S. aureus*; PA = *P. aeruginosa*.

Table 1 – Inactivation of Bacteria by Water-Soluble N-Chloramine Compounds

Water Quality	pH	T(°C)	Flow Rate (mL/sec)	Contact Time (sec) ^b	Log Reduction ^c
DFW	7.0	25	4.00	0.95	7
DFW	9.0	25	4.17	0.91	7
DFW	4.5	25	4.00	0.95	7
DFW	7.0	15	4.54	0.84	7
DFW	9.0	15	2.86	1.33	7
DFW	4.5	15	1.85	2.05	7
DFW	7.0	4	1.67	2.28	7
DFW	9.0	4	1.67	2.28	7
DFW	4.5	4	1.67	2.28	7
SDW	9.0	4	1.72	2.21	7

^aDFW = demand free water; SDW = synthetic demand water (see text).

^bEmpty bed volume (3.8 mL)/flow rate.

^cInitial concentration was 10⁷ CFU/100 mL.

Table 2 – Inactivation of *K. terrigena* by PSHB in Flowing Water

contact times were computed by dividing the empty-bed volume of the PSHB filter by the flow rate. A filter containing unbrominated PSH of the same particle size provided no reduction in viable *K. terrigena*, indicating that PSHB truly kills the bacterium rather than merely filtering it out. A filter containing a chlorinated PSH also provided a 7-log reduction, although the contact times needed were somewhat longer (4.76 seconds for SDW). Thus, it can be concluded that the halogenated PSH compounds will function in an outstanding manner in a water-filter application.

Algistatic Efficacies – Both MCI and DCI caused a steady and substantial decline in absorbance at 750 nm indicative of a decline in algae concentra-

tion over a period of 16 days. However, when the concentration of MCI or DCI reached zero in the aquariums, the absorbance began to increase again, indicating that MCI and DCI are probably algistatic rather than algicidal. It was also observed in a separate experiment that as long as MCI or DCI were present in a measurable concentration in water solution, algae could not establish growth. Thus, it can be concluded that the two N-chloramines prevent the growth of algae.

Stabilities – The relative stabilities of DCI, MCI, and free chlorine in SDW are shown in Figure 1. The compound MCI is remarkably stable to loss of chlorine in the water containing heavy halogen de-

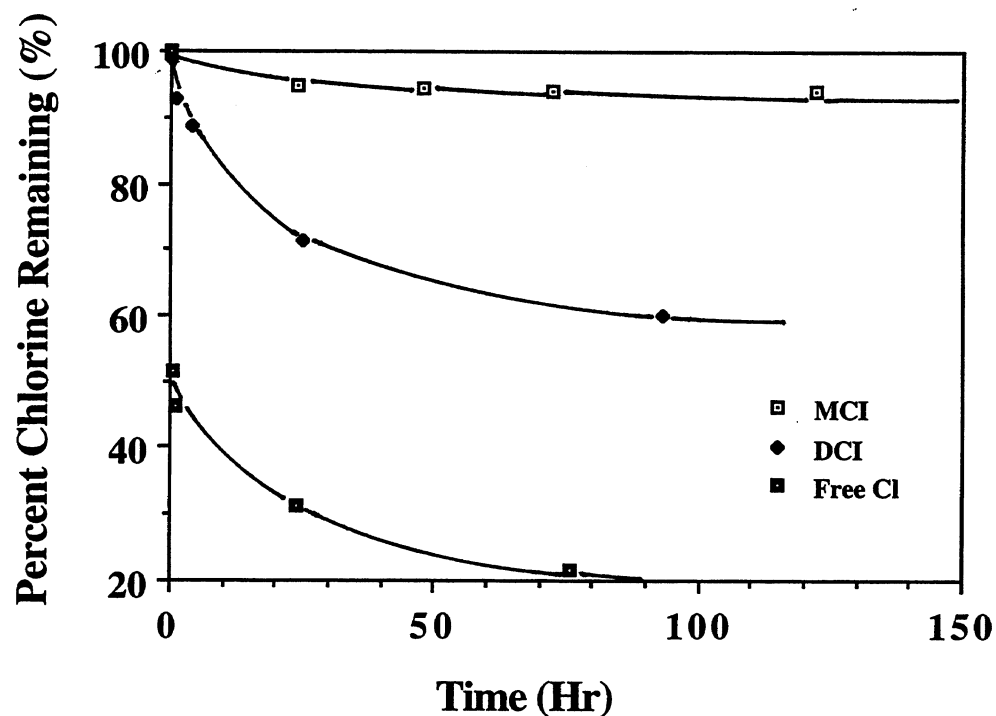


Figure 1 – A Comparison of the Stabilities of MCI, DCI, and Free Chlorine in Synthetic Demand Water

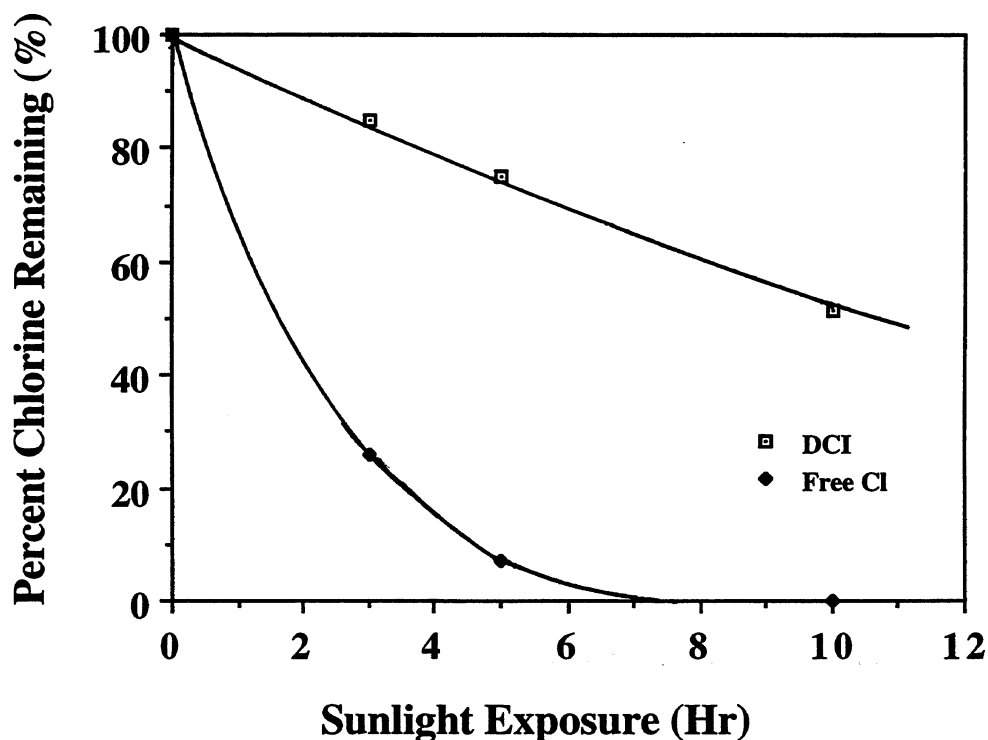


Figure 2 – A Comparison of the Stabilities of DCI and Free Chlorine in the Presence of Direct Sunlight

mand, losing only 6% of its chlorine over a period of 122 hours. Compound DCI lost 40% of its chlorine over 93 hours, and free chlorine declined by 76% in only 21 hours. The free chlorine curve in Figure 1 levels at about an 80% loss because at that point the loss of chlorine from stable chloramines formed from reaction of the free chlorine with halogen demand in SDW is then being observed as total chlorine; the free chlorine is certainly exhausted. Thus MCI and DCI are considerably more stable than free chlorine in the SDW solution.

Figure 2 compares the loss of DCI with that of free chlorine in DFW at pH 7.0 in the presence of direct sunlight. Whereas free chlorine reaches a half life in well under 2 hours, DCI requires 10 hours to achieve loss of a half life. Compound MCI was not studied in this experiment, but it should be more stable to sunlight than is DCI. In a related experiment (see Methods section) in which solutions containing I, cyanuric acid, or no stabilizer were chlorinated *in situ* by free chlorine and placed in direct sunlight, it was found that I stabilized the chlorine more efficiently than did an equivalent concentration of cyanuric acid by a factor of 4. Thus, it may be concluded that compound I is an effective chlorine stabilizer, the products being MCI and DCI.

PSH as a Bromine Stabilizer – In the experiment described in the Methods section in which water irradiated by UV photons was continuously circulated through identical filters containing the polymer PSH and sand, it was found that

PSH stabilized free bromine (by forming PSHB *in situ*) by a factor of 17 relative to sand. All free bromine was depleted from the sand-filtered solution within 8 hours, but 1.3 mg/L Br⁺ still remained in the PSHB-filtered solution after 50 hours. Thus, it can be concluded that the PSH polymer can be an effective stabilizer for free bromine in pools and spas when used in a circulating filter application.

Discussion

Why are the water-soluble N-chloramines MCI and DCI stable over extensive periods to loss of chlorine, and what is their mode of action against microorganisms? In our opinion the answer to these questions may be found in the strength of the N-Cl chemical bond. If chlorine dissociates from these compounds as free chlorine (Cl⁺ as in HOCl after combination with water), a negative charge will be left behind on nitrogen. Methyl groups are known to be electron-donor moieties, and as such, destabilize developing negative charge. Thus the N-Cl bond in MCI is extremely strong, the nitrogen residing between carbons containing four methyl groups. The second N-Cl bond in DCI is somewhat weaker because it is adjacent to an electron-withdrawing carbonyl group which stabilizes developing negative charge, but it is still strong relative to most chloramines because of the two methyl groups on the other adjacent carbon. Thus, it is evident why MCI is more stable to loss of free chlorine than is DCI. When free chlorine reacts with I *in situ*, a mixture of MCI and DCI results; when DCI loses one chlorine, it forms MCI.

Since even DCI releases almost no free chlorine (its first hydrolysis equilibrium constant is only $2.6 \cdot 10^{-8}$ (Worley *et al.* 1991)), its mechanism of biocidal action is not the same as that of free chlorine. Free chlorine acts by HOCl crossing the cytoplasmic membrane of a cell, with Cl⁺ causing oxidation of the cell and subsequent cell death. Prior work in these laboratories has established that the stable N-halamine compounds themselves are the biocidal agents (Williams *et al.* 1988). We believe that the N-halamine molecule penetrates the cell wall and then releases Cl⁺, which oxidizes the cell.

The mechanism of action of the polymer PSHB is yet different. Being insoluble, it functions as a biocide of essentially infinite concentration. Organisms collide many times with PSHB molecules as they pass through the filter. Eventually one or more Br⁺ entities penetrate the cell wall and dissociate from the polymer, followed again by oxidation and cell death. Some bromine is also liberated by PSHB into the water, which then functions in the same manner as free bromine in inactivating the cells.

How can the new N-halamine compounds addressed in this work best benefit the swimming pool and spa industry? In the case of the water-soluble

compound I, the two primary uses would be as a chlorine stabilizer in that I binds free chlorine to form MCI and DCI and as an algae preventative in that both MCI and DCI are algistatic. We have found that I can be used in much lower concentration (about 5 mg/L) than cyanuric acid (50 mg/L) to protect free chlorine from sunlight, so even though I may be more expensive to produce than cyanuric acid, it may be more cost effective. Its cost should be competitive with that of other algicides and algistats on the market. The chemical structures of I, MCI and DCI are similar to that of the hydantoin BCDMH, which has been approved for use in recreational water, so it is likely that these new compounds will also gain approval. The insoluble polymer PSH has an obvious use as a bromine stabilizer for pools and spas when placed in a cartridge filter. Its equilibrium release of free bromine at ambient temperature is about 2 mg/L measured as Br⁺. It should thus function in a time-release mode, i.e., it binds free bromine and releases it upon demand. In addition, PSHB has been shown to be an excellent biocidal material. The fact that PSHB is a combination of poly(styrene) and a bromodimethylhydantoin, both of which are nontoxic and enjoy regulatory approval, should indicate that it also will be safe for use in pools and spas. The material should be cost effective since only about one-half pound will be necessary for the filter in an average 500 gallon spa; yet the necessary addition of bromine will be much less frequent. Furthermore, we have found that upon exhaustion of the bromine from PSHB, that it can be regenerated *in situ* by simply flowing a charge of free bromine through it.

Conclusions

The water-soluble compound I should be a cost-effective competitor of cyanuric acid for chlorine stabilization. Its chlorinated products MCI and DCI will function as algae preventatives and persistent biocides against pathogenic microorganisms. The insoluble polymer PSH employed in a cartridge filter mode will serve as a bromine stabilizer and as an excellent contact biocidal material.

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