

Relative Bactericidal Effectiveness of Hypochlorous Acid and Chloroisocyanurates

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Cyanuric acid stabilizes available chlorine by reducing the concentration of photoactive hypochlorite ion through formation of chloroisocyanurates, which are more stable to sunlight. However, laboratory studies have shown that addition of cyanuric acid reduces the effectiveness of available chlorine as a disinfectant against bacteria. No attempt was made to correlate the kill times with the concentration of biocidal species. The present work was carried out to determine if the decrease in kill rate is due solely to a reduction in hypochlorous acid (a powerful disinfectant) concentration or whether chloroisocyanurates possess significant bactericidal properties. A knowledge of all thirteen equilibrium constants for the cyanuric acid-available chlorine system allows calculation of the hypochlorous acid concentration as a function of pH and the concentrations of total isocyanurate and total free available chlorine. A computer program was employed to calculate the concentrations of hypochlorous acid and the various chloroisocyanurates in the published experimental data on the effect of cyanuric acid on the kill time of bacteria by available chlorine. Statistical analysis showed that the disinfection data correlate excellently with the hypochlorous acid concentration and poorly with the concentration of the various chloroisocyanurates. The primary chloroisocyanurate under typical swimming pool conditions is monochloroisocyanurate ion. Although it is an ineffective bactericide, it allows longer term disinfection in outdoor swimming pools compared to unstabilized available chlorine because it serves as a reservoir of hypochlorous acid, releasing it on demand via rapid hydrolysis.

INTRODUCTION

Chloroisocyanurates are solid sources of available chlorine used in bleaching, sanitization, and disinfection. Initially introduced in the late 1950's and early 1960's, they include trichloroisocyanuric acid

(TCCA) tablets and anhydrous and hydrated granular sodium dichloroisocyanurate (SDCC). In addition, cyanuric acid (CA) was marketed as a stabilizer for available chlorine in outdoor swimming pools. The structures of the isocyanurates are shown in Figure 1.

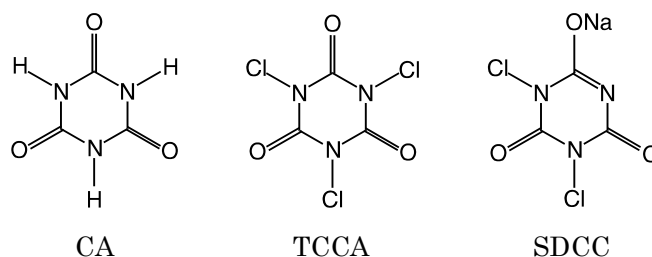
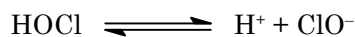


Figure 1. Structures of Isocyanurates

In outdoor unstabilized swimming pools chlorine completely dissipates in about 3 hours, whereas, in the presence of ~50 ppm cyanuric acid, chlorine may persist for several days (Nelson 1967). At typical swimming pool pH, free available chlorine in unstabilized water consists of equilibrium concentrations of hypochlorous acid and hypochlorite ion as shown by the following reaction:



Since ultraviolet radiation from the sun at wavelengths below 290 nanometers (nm) is absorbed by the atmosphere, HOCl is relatively stable to photochemical decomposition because its absorption maximum occurs at 235 nm (see Table 1) and its absorptivity at or above 290 nm is negligible. By contrast, hypochlorite ion is unstable since its broad absorption, centered at 290 nm, extends out to ~350 nm.

Addition of cyanuric acid stabilizes free available chlorine (av. Cl) by formation of chloroisocyanurates, whose hydrolysis is suppressed by excess cyanuric acid, resulting in markedly lower concentrations of HOCl and ClO⁻. The main chloroisocyanurate under swimming pool conditions is monochloroisocyanurate ion (HClCy⁻); dichloroisocyanurate ion (Cl₂Cy⁻) forms to a minor extent. Since the absorption maxima of these ions are

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Species	λ_{\max} nm
HOCl	235
ClO ⁻	290
HClCy ⁻	215
Cl ₂ Cy ⁻	220

Table 1. Absorption Maxima for Main Chlorine Species

well below 290 nm, they are relatively stable to sunlight. Studies with chloroisocyanurate solutions (CA = 26 ppm and av. Cl = 14 ppm) showed maximum stability at pH 6.8 which corresponds to minimal hydrolysis (O'Brien *et al* 1975). Stability decreases as pH is increased due to increased ionization of HOCl to ClO⁻.

Shortly after the commercialization of chloroisocyanurates, it was shown that cyanuric acid decreases the kill rate of bacteria such as *S. faecalis* and *S. aureus* by available chlorine in distilled water (Andersen 1965). Later studies confirmed these findings (Stewart and Ortenzio 1964; Fitzgerald and DerVartanian 1967; Swatek *et al* 1967). However, no attempts were made to correlate the observed kill times with the concentration of biocidal species.

In the absence of cyanuric acid, the rate of disinfection by free av. Cl decreases with increased pH in accordance with the ionization pattern of HOCl, dem-

onstrating that HOCl is the active biocidal agent and that hypochlorite ion is a poor disinfectant (Fair *et al* 1948). Disinfection by HOCl is believed to be due to diffusion through the cell wall of bacteria followed by oxidative attack resulting in disruption of metabolic processes (Green and Stumpf 1946). The poor bactericidal properties of hypochlorite ion are attributed to slower diffusion through the cell membrane compared to the neutral molecule HOCl.

DISCUSSION

Chloroisocyanurate Equilibria – The various cyanurate equilibria in the cyanuric acid – available chlorine system are shown in Figure 2. The complex equilibrium system consists of six ionization and six hydrolysis reactions, shown horizontally and vertically, respectively. Table 2 lists the reported values of the equilibrium constants. The equilibrium constants for the first 12 reactions were measured at 24–25°C (O'Brien *et al* 1975). Other values of some of the chloroisocyanurate equilibrium constants have also been reported (Brady *et al* 1963; Gardner 1973; Pinsky and Hu 1981). The equilibrium constant for ionization of hypochlorous acid was determined over the 4 to 34 °C temperature range (Morris 1966).

Mathematical Model and Computer Program – A computer program (similar to O'Brien *et al* 1975) was employed for calculation of the concentration of all species in the cyanuric acid – available chlorine system. Material balance yields the following equations for total free av. Cl (Cl_T) and total cyanurate (Cy_T):

Reaction	pK Number	pK
Cl ₃ Cy + H ₂ O ⇌ HCl ₂ Cy + HOCl	1	1.8 ± 0.2
HCl ₂ Cy ⇌ H ⁺ + Cl ₂ Cy ⁻	2	3.75 ± 0.03
HCl ₂ Cy + H ₂ O ⇌ H ₂ ClCy + HOCl	3	2.93 ± 0.07
H ₂ ClCy ⇌ H ⁺ + HClCy ⁻	4	5.33 ± 0.05
H ₂ ClCy + H ₂ O ⇌ H ₃ Cy + HOCl	5	4.07 ± 0.08
H ₃ Cy ⇌ H ⁺ + H ₂ Cy ⁻	6	6.88 ± 0.04
Cl ₂ Cy ⁻ + H ₂ O ⇌ HClCy ⁻ + HOCl	7	4.51 ± 0.09
HClCy ⁻ ⇌ H ⁺ + ClCy ⁻	8	10.12 ± 0.02
HClCy ⁻ + H ₂ O ⇌ H ₂ Cy ⁻ + HOCl	9	5.62 ± 0.05
H ₂ Cy ⁻ ⇌ H ⁺ + HCy ⁻	10	11.40 ± 0.10
ClCy ²⁻ + H ₂ O ⇌ HCy ²⁻ + HOCl	11	6.90 ± 0.11
HCy ²⁻ ⇌ H ⁺ + Cy ³⁻	12	13.5
HOCl ⇌ H ⁺ + ClO ⁻	13	7.54

Table 2. Equilibrium Constants for Reactions in the Cyanuric Acid – Available Chlorine System @ ~25°C

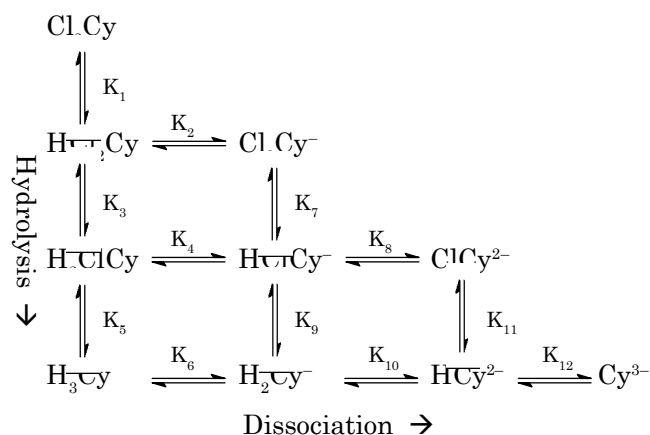


Figure 2 – Chloroisocyanurate Equilibria

$$Cl_T = [H_2ClCy] + [HClCy^-] + [ClCy^{2-}] + 2[HCl_2Cy] + 2[Cl_2Cy] + 3[Cl_3Cy] + [HOCl] + [ClO^-]$$

$$Cy_T = [H_3Cy] + [H_2Cy^-] + [HCy^{2-}] + [Cy^{3-}] + [H_2ClCy] + [HClCy^-] + [ClCy^{2-}] + [HCl_2Cy] + [Cl_2Cy] + [Cl_3Cy]$$

While chloroisocyanurates analyze as free av. Cl with swimming pool test kits because of rapid hydrolysis, they are not free in the sense of HOCl and ClO⁻. More accurately, they represent potential free av. Cl, releasing HOCl and ClO⁻ on demand.

The concentration of the various cyanurate and chloroisocyanurate species can be calculated from applicable equilibrium constants and the concentrations of H⁺ (calculable from the pH), HOCl, and tricyanurate ion by the following generalized equation:

$$[H_nCl_mCy] = [H^+]^{n+m}[HOCl]^m[Cy^{3-}]\beta_{nm}$$

$$\begin{aligned} [HCy^{2-}] &= [H^+][Cy^{3-}]/K_{12} \\ [H_2Cy^-] &= [H^+]^2[Cy^{3-}]/K_{12}K_{10} \\ [H_3Cy] &= [H^+]^3[Cy^{3-}]/K_{12}K_{10}K_6 \\ [ClCy^{2-}] &= [H^+][HOCl][Cy^{3-}]/K_{12}K_{11} \\ [HClCy^-] &= [H^+]^2[HOCl][Cy^{3-}]/K_{12}K_{11}K_8 = [H^+]^2[HOCl][Cy^{3-}]/K_{12}K_{10}K_9 \\ [H_2ClCy] &= [H^+]^3[HOCl][Cy^{3-}]/K_{12}K_{11}K_8K_4 = [H^+]^3[HOCl][Cy^{3-}]/K_{12}K_{10}K_6K_5 \\ [Cl_2Cy] &= [H^+]^2[HOCl]^2[Cy^{3-}]/K_{12}K_{11}K_8K_7 = [H^+]^2[HOCl]^2[Cy^{3-}]/K_{12}K_{10}K_9K_7 \\ [HCl_2Cy] &= [H^+]^3[HOCl]^2[Cy^{3-}]/K_{12}K_{11}K_8K_7K_2 = [H^+]^3[HOCl]^2[Cy^{3-}]/K_{12}K_{10}K_6K_5K_3 \\ [Cl_3Cy] &= [H^+]^3[HOCl]^3[Cy^{3-}]/K_{12}K_{11}K_8K_7K_2K_1 = [H^+]^3[HOCl]^3[Cy^{3-}]/K_{12}K_{10}K_6K_5K_3K_1 \end{aligned}$$

Table 3 – Expressions for Calculating Concentrations of Isocyanurate and Chloroisocyanurate Species

Where: m and the sum of n + m vary from 0 to 3 and β_{nm} is the reciprocal of the product of the appropriate equilibrium constants. Expressions for calculating the concentration of cyanurate and chloroisocyanurate species are listed in Table 3.

The following working equation was employed:

$$Cl_T = Cy_T N/D + [HOCl](1 + K_{13}/[H^+])$$

Where: N is a 6-term summation representing the various chloroisocyanurates and D is a 10-term summation representing all possible cyanurates. Since [Cy³⁻] occurs as a factor in all terms in both the numerator (N) and denominator (D), it cancels out. The program utilizes a Newton–Raphson convergence technique. For a given reservoir chlorine (Cl_T), total cyanurate (Cy_T), hydrogen ion concentration, and estimated HOCl concentration, the program iterates the HOCl concentration until a solution is obtained, i.e., when the calculated values of Cl_T and Cy_T are equal to the actual values. While the concentration of tricyanurate ion [Cy³⁻] is not known explicitly, it is known implicitly, i.e., once the correct HOCl is obtained, the concentration of tricyanurate ion can be calculated by the following equation: [Cy³⁻] = Cy_T/D.

Species Distribution – A plot of species distribution against pH for water containing ~25 ppm cyanuric acid and ~1 ppm av. Cl is shown in Figure 3. It is seen that at pH 7.5, typical in swimming pools, the species distribution varies as follows:

$$\begin{aligned} [H_2Cy^-] &> [H_3Cy] > [HClCy^-] \gg [HOCl] = [ClO^-] \\ &\cong [Cl_2Cy^-] > [ClCy^{2-}] \end{aligned}$$

By contrast to cyanuric acid, monochloro- and dichloroisocyanuric acids are essentially completely ionized at pool pH to HClCy⁻ and Cl₂Cy⁻.

Figure 3 – Species Distribution in the Cyanuric Acid-Available Chlorine System at 25°C
 CA ~25 ppm,
 av. Cl ~1 ppm

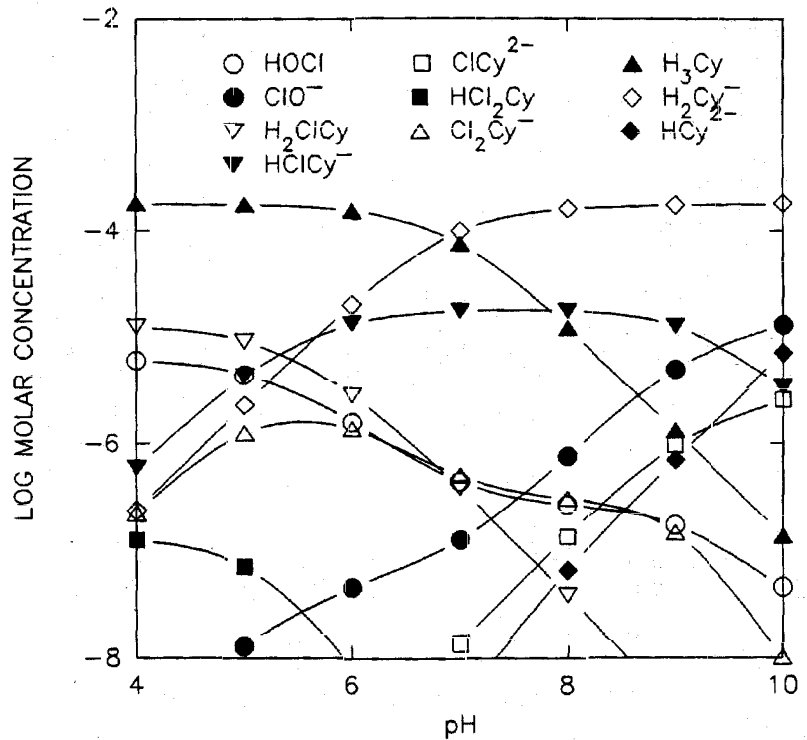
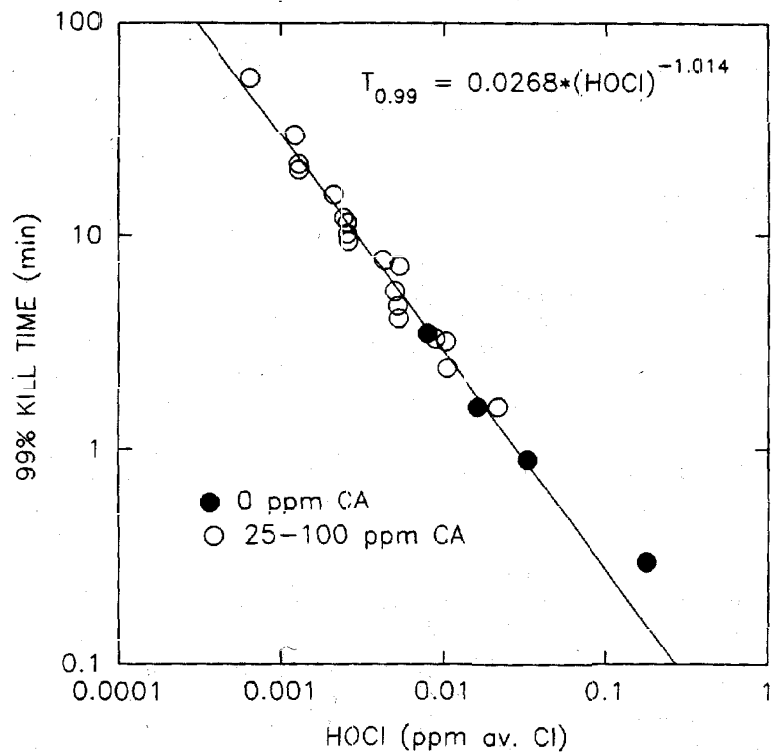


Figure 4 – 99% Kill Time at 20°C v.s. HOCl Concentration at 25°C (Organism: *S. Faecalis*)



The presence of HOCl, in concentrations approximating the calculated values, was verified by room temperature vacuum distillation of dilute chloroisocyanurate solutions at pH 7.5. The HOCl-enriched distillate was analyzed by infrared spectroscopy and by conventional av. Cl analysis. Calculations of HOCl concentrations in the original solutions took into account the appropriate enrichment factors.

Disinfection Data – Disinfection data (99% kill time) of Andersen (Andersen 1965) for *Streptococcus faecalis* at 20°C and pH 7 and 9 for various cyanuric acid and av. Cl concentrations are listed in Table 4 along with the calculated concentration of hypochlorous acid (at 25°C). The HOCl concentrations were calculated at 25°C because the complete set of equilibrium constants for the isocyanurates are known only at this temperature. The data show that the 99% kill

pH	CA ppm	Av. Cl ppm	99% Kill Time (min) at 20°C	HOCl (ppm as av. Cl) at 25°C
7	0	0.23	0.3	0.178
7	25	0.25	7.2	0.00525
7	50	0.24	11.5	0.00254
7	100	0.24	21.7	0.00127
7	25	0.48	3.2	0.0102
7	50	0.48	4.7	0.00511
7	100	0.48	10.2	0.00255
7	25	0.98	1.6	0.0214
7	50	0.97	2.4	0.0104
7	100	0.97	4.1	0.00520
9	0	0.23	3.5	0.00780
9	25	0.24	15.5	0.00209
9	50	0.24	29.5	0.0012
9	100	0.24	55.3	0.000644
9	0	0.47	1.6	0.0160
9	25	0.48	7.7	0.00422
9	50	0.48	12.1	0.0241
9	100	0.47	20.4	0.00127
9	0	0.96	0.9	0.0326
9	25	0.98	3.3	0.00876
9	50	0.97	5.5	0.00492
9	100	0.95	9.4	0.00258

Table 4 – Disinfection Data of Andersen and Calculated Hypochlorous Acid Concentrations (Organism: *S. Faecalis*)

time: a) increased with increasing cyanuric acid concentration at constant av. Cl, b) decreased with increasing av. Cl concentration at constant cyanuric acid concentration, c) increased with increasing pH at constant cyanuric acid and av. Cl concentrations, and d) increased with increasing pH whether cyanuric acid was present or not. The relative importance of HOCl and the chloroisocyanurates in disinfection can be evaluated statistically.

Statistical Analysis – A plot of 99% kill time at 20°C against the calculated HOCl concentration at 25°C is shown in Figure 4. The plot shows very good correlation of kill times with calculated HOCl concentrations. The fact that data for cyanurate and cyanurate-free media fall on the same line is probably fortuitous. They should, in fact, most likely fall on separate lines as discussed below. However, this will not affect statistical analysis since the cyanurate data can be analyzed separately.

Extrapolation of the calculated HOCl concentrations to 20°C should cause the two sets of data to diverge and fall on separate parallel lines because the temperature dependence of the HOCl concentration is different for stabilized and unstabilized chlorine. The only parameter that changes in transforming the calculated HOCl concentrations from 25 to 20°C is the intercept; the slope should remain the same. For unstabilized chlorine, the HOCl concentration will increase from 25 to 20°C due to decreased ionization of HOCl. By contrast, the HOCl concentration in stabilized chlorine solutions decreases with decreasing temperature due to decreased hydrolysis of chloroisocyanurates. The extent of separation of the two lines will depend on the temperature dependence of ionization of HOCl and hydrolysis of chloroisocyanurates. Thus, for the lines to be coincident at 20°C, the calculated HOCl concentrations at 25°C for the isocyanurate data would have to be a little higher.

Regression analysis (Table 5) shows that the kill time correlates excellently with the free HOCl concentration ($r^2 = 0.98$). By contrast, correlations against the concentrations of the various chloroisocyanurates were poor. The lone exception was dichloroisocyanurate ion (Cl_2Cy^-) which showed a high correlation ($r^2 = 0.95$). However, this is to be expected since the concentration of Cl_2Cy^- correlates with the concentration of HOCl (see Figure 3). Indeed, any significant bactericidal activity for $\text{Cl}_2\text{Cy}^{2-}$ is very unlikely given the poor statistical correlations found for the four other chloroisocyanurates. The following equation was obtained which shows that kill time at 20°C varies inversely with the calculated HOCl concentration at 25°C.

$$t_{0.99} = (0.0268q)[\text{HOCl}]^{-1.014}$$

Where: q is the relative disinfection time at 25°C versus that at 20°C. This equation conforms to the Chick–Watson Law: $C^n t = k$, where C is the disinfectant concentration, t the kill time, and n and k are constants. The fact that $n = 1$ means that the concentration of HOCl and time are equally important in determining the inactivation rate of bacteria.

Effect of Temperature on Kill Time – The rate of disinfection increases with increasing temperature. Disinfection data can be extrapolated to other temperatures using the following equation:

$$t_2/t_1 = q = \exp[-(\Delta E/R)(1/T_2 - 1/T_1)]$$

Where: t_1 and t_2 are the 99% kill times at temperatures T_1 and T_2 (in kelvins), R is the gas constant (8.314 J/deg/mol; 1.987 cal/deg/mol), and ΔE is the disinfection activation energy. For enteric bacteria (such as *E. coli*) in a cyanuric acid–free system, $\Delta E = \sim 30.5$ kJ/mol

Species	Coefficient of Determination r^{2*}
HOCl	0.98
ClO^-	0.07
H_2ClCy	0.37
HClCy^-	0.59
ClCy^{2-}	0.08
HCl_2Cy	0.60
Cl_2Cy^-	0.95

* r^2 is the fraction of the variability in the data explained by the regression equation.

Table 5. Regression Analysis of Disinfection Data

(~ 7.3 kcal/mol) for disinfection by hypochlorous acid (Fair *et al* 1948). This value represents the combined temperature dependence of HOCl ionization and inactivation of bacteria. Although the concentration of HOCl decreases with increasing temperature at constant pH due to increased ionization, the disinfection rate in cyanuric acid–free media actually increases due to greater disinfection efficiency. By contrast, in a cyanuric acid–available chlorine system, increased temperature can lead to an increase in the HOCl concentration due to increased hydrolysis of chloroisocyanurates, resulting in an additional increase in the disinfection rate.

Although the activation energy for disinfection in a cyanurate system has not been determined, it can be estimated. The value of 30.5 kJ/mol (7.3 kcal/mol) for a cyanurate–free system can be adjusted to that in a cyanurate system by combining it with the appropriate energy term applicable to a cyanurate system. The principle chloroisocyanurate in pool water is monochloroisocyanurate ion. It hydrolyzes to a small extent at pool pH, providing increased HOCl concentrations as temperature increases. A value of 99.2 kJ/mol (23.7 kcal/mol) has been reported for the energy (i.e., heat of hydrolysis) that determines the temperature dependence of its hydrolysis (Wojtowicz to be published). Therefore, the overall estimated activation energy for disinfection in a cyanurate system is: $30.5 + 99.2 = \sim 129.7$ kJ/mol (31.0 kcal/mol). At 25°C, this will result in a decrease in kill time by a factor (q) of 2.44 compared to that at 20°C.

Extrapolation of Andersen’s Data to Swimming Pools – Since hypochlorous acid and chloroisocyanurates cannot be distinguished by test kit analysis (which is typically based on diethyl–p–phenylenediamine), the total free av. Cl concentration is the only indicator of adequate disinfection. However, because disinfection is affected by the presence of cyanuric acid, a better indicator is the ratio of total cyanurate to av. Cl since it correlates perfectly with the HOCl concentration at pH 7 and 20°C in Andersen’s study:

$$\text{HOCl (pH 7 \& 20°C)} = 0.576(\text{C}_{\text{T}}/\text{Cl}_{\text{T}})^{-1.008}, r^2 = 1.00$$

This equation shows that the HOCl concentration varies inversely with the ratio of total cyanurate to total free av. Cl.

Regression analysis shows that Andersen’s data for 99% kill time ($t_{0.99}$) at pH 7 and 20°C varies linearly with the ratio of total cyanurate (C_{T}) to total free available chlorine (Cl_{T}) as shown by the following equation:

$$t_{0.99} \text{ (pH 7 and 20°C)} = 0.119 + 0.0516\text{C}_{\text{T}}/\text{Cl}_{\text{T}}, r^2 = 0.98$$

The Chemistry and Treatment of Swimming Pool and Spa Water

In order to apply Andersen's data to swimming pool conditions, the data must be adjusted for the effect not only of temperature but pH as well. Since kill time varies inversely with pH (which influences the HOCl concentration), the 99% kill time at pH 7 and 20°C was first adjusted to pH 7.5 (typical in swimming pools) by multiplying by the factor 1.40, which represents the ratio of HOCl at pH 7 to that at pH 7.5 in stabilized water. Cyanuric acid moderates the effect of pH on the HOCl concentration since the factor in unstabilized water is 1.48.

$$t_{0.99} (\text{pH } 7.5 \text{ and } 20^\circ\text{C}) = [0.167 + 0.0722C_{Y_T}/Cl_T]/q$$

The factor q allows extrapolation of kill times from 20°C to other temperatures. The higher the temperature, the higher the value of q and the lower the kill time. At a swimming pool temperature of 80°F (26.7°C), $q = 3.29$ which corresponds to 99% kill times of 0.6–0.2 minutes over the recommended free av. Cl range of 1–3 ppm at 25 ppm cyanuric for enteric bacteria such as *S. faecalis*. Higher cyanuric acid concentrations will cause the 99% kill times to increase. By contrast, higher temperatures, especially in spas, will decrease kill times.

Effect of Swimming Pool Contaminants on Disinfection Rate—Longer kill times were observed in pool water, compared to distilled water, whether cyanuric acid was present or not (Swatek *et al* 1967). This was attributed to unknown variables, which in reality are swimming pool contaminants. In swimming pools, nitrogenous compounds such as ammonia, amino acids, and creatinine, introduced by bathers, can react with free av. Cl. This forms bactericidally ineffective combined chlorine, resulting in increased kill times. Combined chlorine compounds do not hydrolyze to a significant extent to HOCl. Thus, it is important to maintain a low combined available chlorine level by frequent superchlorination or shock treatment in order to oxidize chlorinated compounds such as monochloramine, N-chloroamino acids, and N-chlorocreatinine, which are much poorer disinfectants than HOCl.

Addition of ammonia to stabilized aqueous av. Cl results in increased kill times proportional to the amount added (Fitzgerald and DerVartanian 1967). By contrast to ammonia, urea, a major swimming pool contaminant, does not affect disinfection in short term experiments (Fitzgerald and DerVartanian 1967). Although urea is a potential source of ammonia, its hydrolysis is very slow under swimming pool conditions. However, since urea serves as a nutrient for bacteria and algae, it is necessary to oxidize it by periodic shock treatment.

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