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Inactivation of *Naegleria Fowleri* by chlorine and ultraviolet light

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Naegleria fowleri is a free-living protozoan that causes the fatal disease primary amoebic meningoencephalitis. The only cases associated with drinking water have occurred in Australia and Arizona. One study found *N. fowleri* in 8% (n = 143) of all municipal untreated drinking water wells tested. The $C \times T$ values (concentration × contact time) for chlorine inactivation of *N. fowleri* trophozoites and cysts at an average disinfectant concentration of 1 mg/L were determined using the efficiency factor Hom kinetic model. The estimated $C \times T$ values for *N. fowleri* cysts are comparable to the published values for *Giardia lamblia* cysts, but are lower than those for *Cryptosporidium parvum* oocysts. In this study, the ultraviolet light dosage required to inactivate the cyst stage of *N. fowleri* was determined to be greater than that of *Cryptosporidium* oocysts but less than that of *Acanthamoeba* cysts.

KEYWORDS: *chlorine, disinfection, Naegleria fowleri, ultraviolet light*

Naegleria fowleri is a water-based protozoan that has been well-documented as a causative agent of primary amoebic meningoencephalitis, a rapidly progressing disease with a fatality rate of 90–98% (Marciano-Cabral et al, 2003; Marshall et al, 1997). The disease can be contracted by water-related recreational activities such as swimming, diving, and jet skiing and by visits to water parks and interactive fountains (Craun et al, 2005; Gyori, 2003). *N. fowleri* is an amoeboflagellate that has three lifecycle stages: trophozoite, cyst, and flagellate (Ma et al, 1990). Infection occurs primarily after flagellated forms of the amoebae come in contact with the nasal passages, but the disease may also be contracted by inhaling cyst-laden dust particles or during face-washing and bath-related activities; this may suggest a possible aerosolization route of exposure (John, 1982; Lawande et al, 1979). Recognized cases of illness associated with this organism are rare, with slightly more than 100 cases reported in the United States (CDC, 2008). Most cases occurred during the summer months, indicating that warm water is a reservoir for this organism. Although the incidence is rare, the highest number of cases in a single year in the United States was in 2007 when six fatalities occurred from primary amoebic meningoencephalitis caused by *N. fowleri* (Bond, 2008).

In October 2002, two children in central Arizona died of meningoencephalitis caused by *N. fowleri*. Both children had been exposed to the same water supply provided by a utility serving more than 2,300 households in the area. *N. fowleri* was later found in the water supply, which made these the first cases of infection in the United States associated with a drinking water supply that had groundwater as its source (Marciano-Cabral et al, 2003). The only other documented case of this parasite being associated with drinking water occurred in Australia (Marciano-Cabral et al, 2003; Dorsch et al, 1983).

Recent research in the authors' laboratory showed that 8% (n = 143) of the wells supplying municipal drinking water tested in central and southern Arizona contained *N. fowleri* (Blair et al, 2008). Data suggest that both the trophozoite and cyst forms of *N. fowleri* are fairly resistant to chlorine (Cassells et al, 1995; Cursons et al, 1980; Chang, 1978; De Jonckheere & Van de Voorde, 1976). Because it is difficult to maintain the infective flagellate form in the laboratory, there are no disinfection studies available for this form of *Naegleria* spp. (De Jonckheere et al, 2001). Chlorination is the most widely used disinfectant in the United States (MWH, 2005); however, none of these studies is useful for establishing $C \times T$ (free chlorine concentration × contact time) values for practical application to drinking water treatment. Chang (1978) showed that 10 min of exposure to a free chlorine concentration of 4 mg/L was required to achieve a 3-log reduction of *N. fowleri* cysts, and Cursons et al (1980) showed that *N. fowleri* trophozoites were inactivated by 30 min of exposure to a 0.74-mg/L concentration of free chlorine—although studies conducted by Rubin et al (1983) of *Naegleria gruberi* cysts reported that a $C \times T$ of 1.29 min-mg/L at 25°C and pH 7 can achieve a 2-log reduction.

Factors such as concentration of the organism, concentration and type of disinfectant, temperature, pH, and the presence of interfering substances must be taken into consideration (Rice & Gomez-Taylor, 1986). The disinfection efficacy of chlorine in this study was modeled using the efficiency factor Hom model (EHM) to account for dynamic disinfectant concentrations caused by the protozoa and the effect of pH and temperature on disinfection (John et al, 2005). Through kinetic modeling, microbial reductions can be determined at comparable disinfectant doses. Kinetic modeling also takes into account the nonlinearity of disinfection kinetics (Thurston-Enriquez et al, 2003a, 2003b). Kinetic models

have been previously used in the formulation of disinfection design criteria for water treatment (Thurston-Enriquez et al, 2003a, 2003b; Gyurek & Finch, 1998). To determine the $C \times T$ values of chlorination for *N. fowleri* trophozoites and cysts, the EHM was applied to the observed bench-scale values to generate $C \times T$ values for 2-, 3-, and 4-log inactivation. Because chlorination can cause formation of harmful disinfection by-products in the presence of organic matter and humic acid, there has been an increased interest in evaluating alternative disinfectants such as ultraviolet (UV) light, which does not produce any such harmful by-products (Hanzon & Vigilia, 1999). UV-C radiation inactivates microorganisms by causing dimerization of thymine bases, which in turn blocks nucleic acid replication (Bitton, 2005). Therefore, UV light doses for the inactivation of *N. fowleri* cysts and trophozoites were determined.

MATERIALS AND METHODS

Culture propagation and assay procedure. A pure culture of *N. fowleri*[1] was maintained axenically on Nelson's medium. Culture propagation and assay methods have been described by Cassells et al (1995). Stock cultures were renewed by transferring them to fresh media every seven days. Trophozoites were harvested for disinfection experiments after five days of growth before cyst formation by washing them off the flasks with 1 *M* buffered demand-free (BDF) water, which is a phosphate-buffered saline made in chlorine-demand-free (CDF) water, followed by centrifugation at 1,090 × *g* for 10 min. The supernatant was decanted and the pellet was resuspended in BDF. This step was repeated three times, and the final pellet was resuspended in BDF and immediately checked for chlorine demand. The trophozoites transform into a flagellate stage when the ionic concentration of the milieu changes and can be observed microscopically (Visvesvara et al, 2007). Cyst suspensions were obtained by using the methods described by Marciano-Cabral et al (2003). Cyst and trophozoite suspensions were counted using a hemocytometer to attain appropriate concentrations. The most probable number (MPN) method with 95% confidence limits was used for the quantification of viable *N. fowleri* (Cassells et al, 1995; Hurley & Roscoe, 1983). The concentration of amoebas/cysts per millilitre was calculated by counting the number of positive-growth wells for each dilution in triplicate aliquots using the MPN computational program devised by Hurley and Roscoe (1983) that is an adaptation of the MPN technique from *Standard Methods* (2005).

Reagents and glassware. All reagents and glassware used for disinfection experiments were prepared and treated to eliminate chlorine demand according to methods described by Thurston-Enriquez et al (2003a). Glassware was soaked overnight in a solution of at least 100 mg of free chlorine/litre to make them CDF. The glassware was then rinsed in this water, followed by baking for 2 h at 200°C. Free chlorine stock solution (125 mg/L) was prepared using reagent-grade 5.0% sodium hypochlorite[2] diluted in CDF water. The required free chlorine concentration was achieved by diluting the free chlorine stock solution in CDF water. All disinfection experiments were conducted in either BDF or groundwater. BDF water (phosphate-buffered saline) was prepared by dissolving 0.54 g of disodium phosphate (anhydrous) and 0.88 g of monopotassium phosphate (KH_2PO_4 anhydrous) per litre of deionized CDF water[3]. The pH required for each experimental condition was adjusted with 1 *M* sodium hydroxide or 1 *M* KH_2PO_4 to attain the required pH range of 7.5–9. BDF water was stored in CDF bottles at 4°C until use. Groundwater samples were obtained from wells that supply the city of Tucson, Ariz. The city is supplied by more than 300 wells located throughout the region. The groundwater used in these studies had a pH of 7.3–8.2, a turbidity of 0.04 ntu, and total organic carbon concentration of 0.7–1.0 mg/L.

Experimental design. Immediately before each experiment, the free chlorine concentration in stock solution was measured by the DPD method, and the volume necessary to achieve the initial free-chlorine dose in each experimental beaker was calculated

TABLE 1 Calculated $C \times T$ values for inactivation of *Naegleria fowleri* trophozoites with free chlorine in BDF water and groundwater

Conditions	Log_{10} Inactivation	$C \times T$ Values
BDF at pH 7.5 and 25°C	2	6
	3	9
	4	12
BDF at pH 9.0 and 25°C	2	18
	3	23
	4	27
Groundwater at pH 8.21 and 31°C	2	10
	3	14
	4	19

BDF—buffered demand free, $C \times T$—concentration × contact time

TABLE 2 Calculated $C \times T$ values for inactivation of *Naegleria fowleri* cysts in BDF water and groundwater

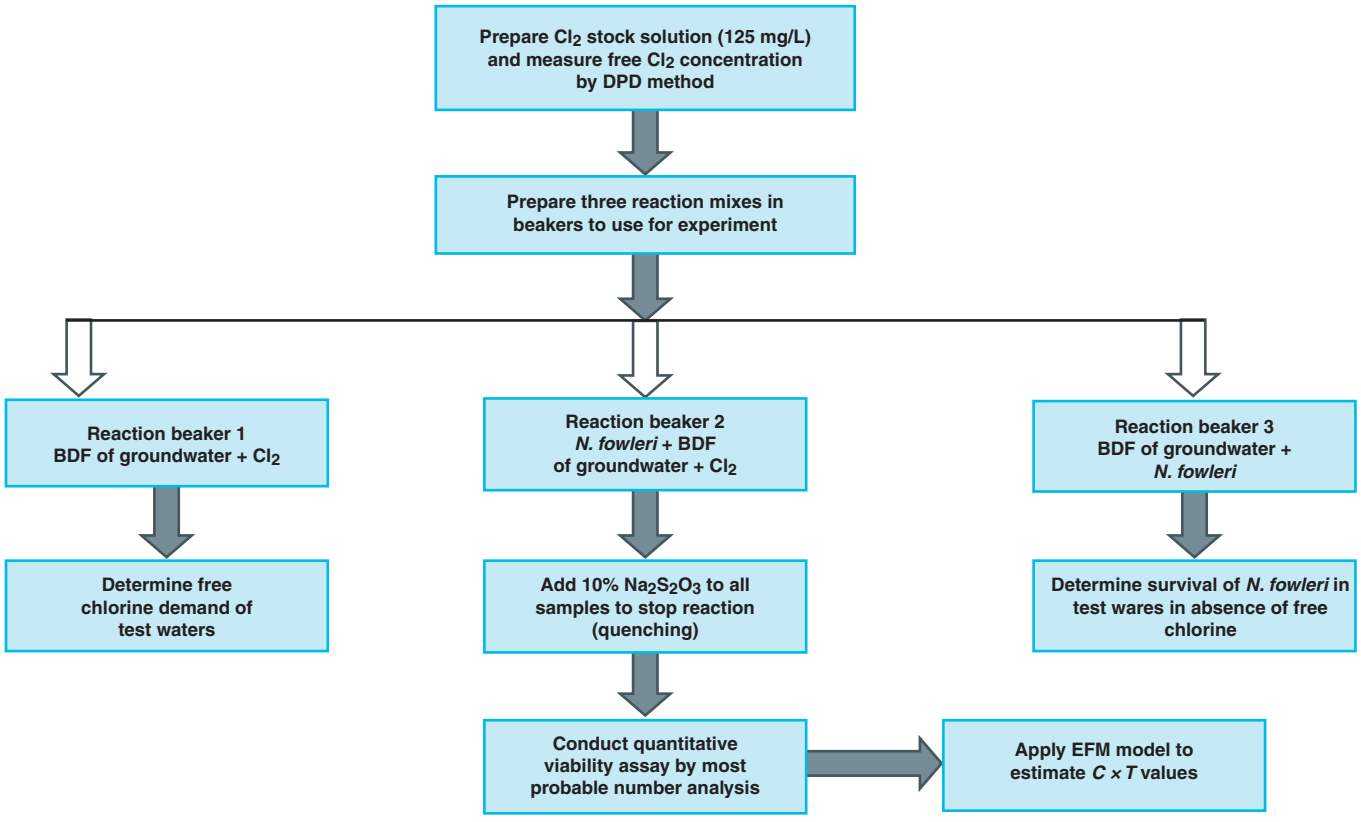
Conditions	Log_{10} Inactivation	$C \times T$ Values
BDF at pH 7.5 at 25°C	2	31
	3	42
	4	53
BDF at pH 9.0 at 25°C	2	37
	3	50
	4	62
Groundwater at pH 8.01 at 38°C	2	40
	3	48
	4	56
Groundwater at pH 7.38 at 28°C	2	25
	3	35
	4	44

BDF—buffered demand free, $C \times T$—concentration × contact time

(*Standard Methods*, 2005). Three reaction beakers were analyzed for every experimental condition. The first reaction beaker, containing only BDF or groundwater, was tested after 15 s to determine the initial and final free-chlorine dose (in the absence of any chlorine demand that may occur with groundwater or protozoa addition). The second reaction beaker was inoculated with the cysts or trophozoites of *N. fowleri* at a concentration that would allow detection of a 2- to 4-log inactivation in either BDF or treated groundwater. The same initial concentration was used for both trophozoites and cysts (~ 10^6 organisms/mL). This beaker was then inoculated with the free-chlorine stock solution and immediately stirred, followed by continuous stirring throughout the course of experiment. The second beaker was sampled to determine the free chlorine concentration at the beginning and end of each disinfection reaction to determine the free-chlorine decay during the experiment. To determine protozoan inactivation by free chlorine, 1-mL samples were also taken from the second beaker at predetermined times throughout the experiment. Residual free chlorine was immediately quenched by placing the 1-mL samples into collection tubes containing 10 µL of sterile 10% sodium thiosulfate solution. The third reaction beaker contained only protozoa and test water and was considered to be representative of protozoa concentrations in beakers 2 and 3. This beaker was used as the control beaker to determine the initial protozoa concentration in the reaction beaker and to evaluate whether protozoa inactivation occurred under the tested pH and temperature conditions (in the absence of chlorine). All experiments were conducted in triplicate. The disinfection experiment protocol is depicted in Figure 1. Because *N. fowleri* grows best at warm temperatures in the range of 25–42°C (Marciano-Cabral et al, 2003), the disinfection experiments under laboratory conditions were conducted at 25°C, which approximates the median temperatures of groundwater in central and southern Arizona. The groundwater sample was tested at a range of 25–38°C, which was the temperature of the water in the wells from which the samples were collected. In this study, laboratory test waters and groundwater samples at a pH range of 7.5–9 along with a temperature range of 25–38°C were used to evaluate $C \times T$ values at a free chlorine concentration of 1 mg/L.

Data analyses. Log_{10} reduction of resulting number of amoebas/cysts after disinfection was calculated as $\log_{10} N_t/N_0$ in which N_t and N_0 are final and initial concentration of viable organisms, respectively. The average $\log_{10} N_t/N_0$ values were then incorporated into the EHM, and the kinetic modeling of all experiments was performed using the equation solver function in a spread-

FIGURE 1 Flow chart for *Naegleria fowleri* disinfection protocol



BDF—buffered demand free water, Cl_2—chlorine, $C \times T$—concentration × contact time, DPD—N,N-diethyl-p-phenylenediamine, EFH—efficiency factor Hom, $Na_2S_2O_3$—sodium thiosulfate

sheet software program[4] (Thurston-Enriquez et al, 2003a; Gyurek & Finch, 1988). Chlorine first-order decay constants (k') were obtained in the same manner to regress the first-order kinetic equation using the least squares method.

$$C = C_0 \exp(-k't) \quad (1)$$

in which C, C_0 = disinfectant residual (milligrams per litre) at time t and time zero, respectively. The MPN values for each experiment were organized by the life stage of the organism, time of inactivation, and pH level. The values were then fit into both the Chick-Watson disinfection model (Eq 2; Thurston et al, 2003b) and the EHM (Eq 3).

$$e^{-k/k'n}(C_0^n - C_t^n) = N_t/N_0 \quad (2)$$

$$Ln\ N_t/N_0 = -kC_0\, t^m \times [1 - \exp(-nk't/m)(nk't/m)] \quad (3)$$

in which k is the inactivation rate constant for the organism, k' is the first-order free chlorine decay constant for disinfectant (min^{-1}), t is the time required to achieve a given level of inactivation, n is the coefficient of dilution that represents the average number of molecules to have combined with the organism necessary to cause inactivation, and m is the microbial inactivation constant for the inactivation rate law that describes deviation from ideal Chick-Watson kinetics (Gyurek & Finch, 1998). C_0 and $C \times T$ represent the concentration of disinfectant at time zero and after the exposure time indicated, respectively. $Ln(N_t/N_0)$ is the natural log of survival ratio (number of cysts/trophozoites remaining at time t divided by the number at time zero). The sum of squares of the difference between the observed and calculated $Ln\ N_t/N_0$ was minimized using the solver function of the spreadsheet software. The minimized sums of squares of differences were subsequently used to determine the values of coefficient of each model. The microbial inactivation curves were plotted using the software to compare observed and calculated log-inactivation values wherein the observed curve depicts the average log-inactivation value of replicate bench-scale experiments versus sampling time.

Generation of *C × T* values. The $C \times T$ value is the concentration (milligrams per litre) of free chlorine multiplied by the time (minutes) when a specific log inactivation—2, 3, or 4 logs—occurred. $C \times T$ values were used to assess the sensitivity of *N. fowleri* trophozoites and cysts to chlorine. EHM parameters were applied to observed bench-scale free chlorine concentration values in order to generate $C \times T$ values for each cellular stage of *N. fowleri* under a given set of conditions (Tables 1 and 2). R^2 values were calculated using Excel to determine the fit of calculated EHM inactivation curves to observed bench-scale inactivation curves. Thus, the observed (experimental) data were fit by least-squares fitting to Eq 2 and 3 to obtain the model parameters.

UV irradiation. The UV irradiation experimental setup consisted of an 8-W, low-pressure mercury UV lamp[5] that was suspended horizontally in a wood box (Thurston-Enriquez et al, 2003b). A black polyvinyl chloride pipe measuring 53.3 cm long with a diameter of 7.62 cm was attached to the UV lamp box that acts as a collimating tube. The UV box and the colli-

FIGURE 2 Predicted and observed inactivation curves of *Naegleria fowleri* trophozoites with free chlorine in BDF water at pH 7.5 and pH 9.0 at 25°C

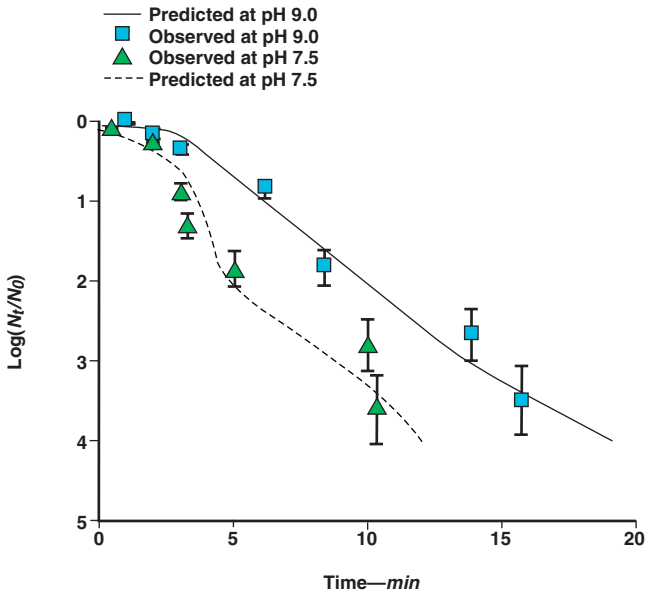
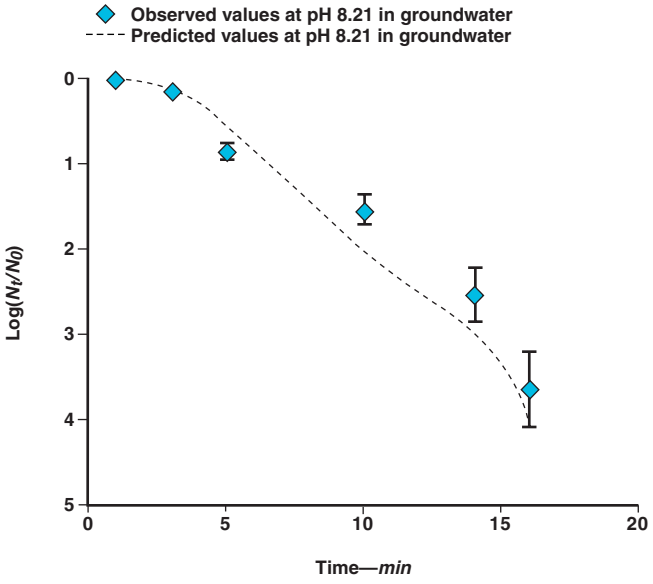


FIGURE 3 Predicted and observed inactivation curves of *Naegleria fowleri* trophozoites with free chlorine at pH 8.21 in ground water at 31°C



mating apparatus were painted black to minimize light diffraction. A stir plate was placed directly below the collimating tube. The UV intensity of each experiment was measured by placing a calibrated radiometer at approximately the same level and location of the irradiated samples.

UV irradiation of samples. Samples were irradiated in sterile 60 × 15-mm glass dishes containing 10 × 2-mm stir bars stirring at low speed. The cyst suspension was diluted in phosphate-buffered solution such that the initial concentration of cysts in the sample was approximately 10^6 cysts/mL. The total volume and depth of cyst suspension within the dish was 21 mL and 1 cm, respectively. Before the experiment, the absorbance of the cyst suspension was measured at 254 nm on a spectrophotometer[6]. Cyst suspensions were placed under the collimated beam and irradiated for predetermined time intervals. One-millilitre samples were withdrawn for the cyst assay. Control samples that were not subjected to UV irradiation were collected at the same time. Each experiment was conducted in triplicate.

Dose determination. UV light dose for the inactivation of *N. fowleri* was determined as previously described (Meng & Gerba, 1996). UV dose is calculated as the product of the average intensity (mW·s/cm^2) multiplied by the time of UV exposure.

$$\text{UV Dose} = I \times t \quad (4)$$

in which I = mW·s/cm^2.

According to Beer's law, the measured intensity was corrected for absorbance of the liquid sample by the following equation:

$$I_{ave} = I_O \times (1 - e^{-aeL})/aeL \quad (5)$$

in which I_{ave} is the intensity in mW/cm^2, I_o is the average measured intensity in mW/cm^2 (using a radiometer), a is absorbance at 254 nm, dose = I_{ave} × exposure time (mW·s/cm^2), e = 2.303 constant, and L = 1.0 cm (optical depth of sample).

Log_{10} survival of cysts was taken for each experiment to plot the inactivation curve against UV dose. Chick's law $(N_t/N_0) = e^{-kit}$ was used to obtain the log_{10} survival of cysts, in which N_t = the number of cysts at time t (time of UV exposure), N_0 = number of cysts at time zero without application of UV light to sample, k = inactivation rate constant or slope of inactivation curve, i = intensity of UV light energy (mW/cm^2), and t = exposure time.

RESULTS

All the free-chlorine disinfection experiments were carried out in triplicate at high- and low-pH values (7.0–9.0) and temperature conditions at an average concentration of 1 mg/L. A 1-mg/L free chlorine concentration was chosen for all disinfection experiments because that is the reported approximate average free chlorine residual used in the US drinking water industry (White, 1999). $C \times T$ values, the concentration of free chlorine multiplied by time of contact with *N. fowleri* trophozoites and cysts, were calculated using the EHM. The calculated $C \times T$ values and inactivation curve of *N. fowleri* trophozoites by free chlorine are shown in Table 1 and in Figures 2 and 3, respectively. The calculated $C \times T$ values and the inactivation curve according to the EHM parameters of the *N. fowleri* cysts are shown in Table 2 and Figures 4 and 5, respectively. Significant differences ($p < 0.05$) in inactivation rates as shown by the analysis of variance test were observed for both *N. fowleri* trophozoites and cysts at

TABLE 3 Summary of parameters for best-fit model for chlorine disinfection of *Naegleria fowleri* trophozoites based on efficiency factor Hom kinetic model for dynamic disinfectant conditions

Conditions	Number of Replicates	k'	n	m	R^{2*}	ssq
BDF, pH 7.5 at 25°C	3	1.03	0.672	0.421	0.97	8.662
BDF, pH 9.0 at 25°C	3	0.88	2.441	1.039	0.98	9.712
Groundwater, pH 8.21 at 31°C	3	0.317	1.2	0.552	0.98	2.68

BDF—buffered demand free, k'—chlorine decay, n—coefficient of dilution, m—constant for the inactivation rate law, ssq—sum of squares value (least squares) regression analysis.

*R^2 values were calculated to determine the fit of predicted efficiency-factor Hom kinetic model inactivation curves to bench-scale curves.

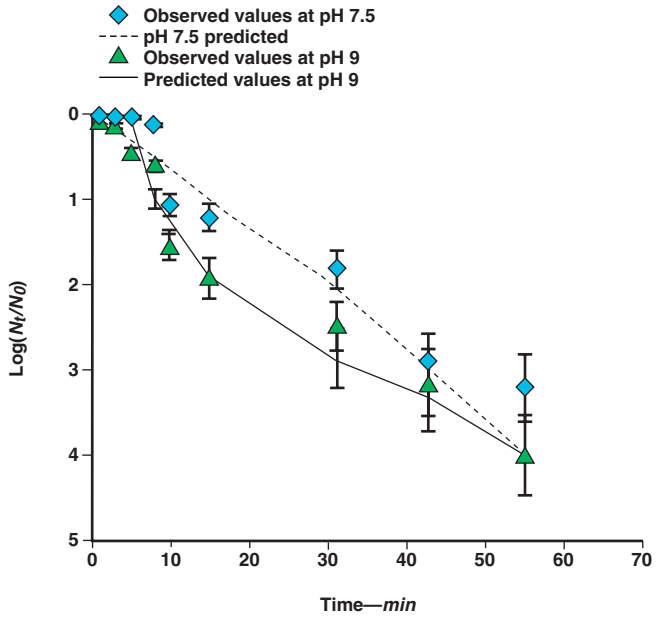
TABLE 4 Summary of parameter values for best-fit model for chlorine disinfection of *Naegleria fowleri* cysts based on Hom's model for dynamic disinfectant conditions

Conditions	Number of Replicates	k (min^{-1})*	n	m	R^2	ssq
BDF, pH 7.5 at 25°C	3	0.084	0.15	0.513	0.97	7.642
BDF at pH 9.0, 25°C	3	0.049	0.64	0.48	0.95	7.338
Groundwater at pH 8.01 at 38°C	3	0.114	0.009	1.144	0.97	9.455
Groundwater at pH 7.38 at 28°C	3	0.241	0.7	0.41	0.95	8.21

BDF—buffered demand free, k'—chlorine decay, n—coefficient of dilution, m—constant for the inactivation rate law, ssq—sum of squares value (least squares) regression analysis.

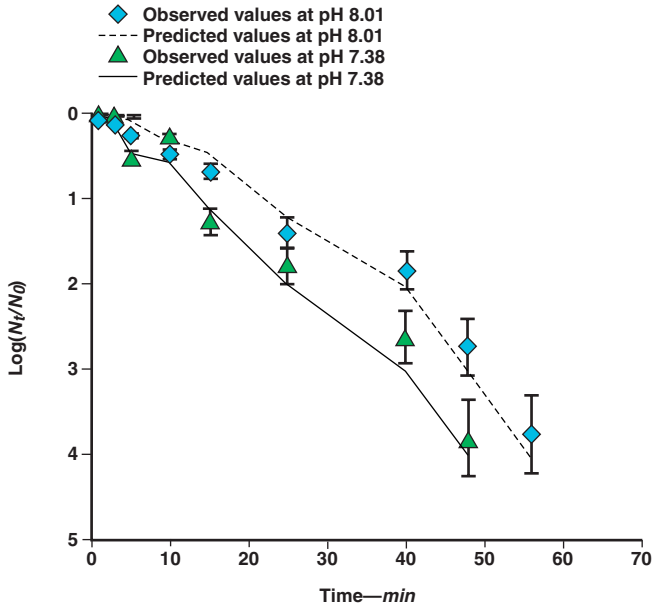
*Average k value for replicate experiments.

FIGURE 4 Predicted and observed inactivation curves of *Naegleri fowleri* cysts with free chlorine at pH 7.5 and pH 9 in BDF water at 25°C



BDF—buffered demand free

FIGURE 5 Predicted and observed inactivation curves of *Naegleria fowleri* cysts with free chlorine at 38°C, pH 8.01 and at 28°C, pH 7.38 in ground water



different temperatures, pH levels, and water types. The $C \times T$ values for trophozoites and cysts at a higher pH were greater than those observed at a lower pH. In all the experiments, the EHM produced the best fit to the observed bench-scale inactivation curves. Tables 3 and 4 show the EHM parameters and the R^2 values for observed and calculated $C \times T$ values for trophozoites and cysts as shown in Tables 1 and 2, respectively. The highest inactivation rate constants (k) were observed in experiments conducted with *N. fowleri* trophozoites at pH 7.5. A high $k9$ indicates that the concentration of free chlorine decreased rapidly throughout the experiment. Experiments were also conducted in groundwater samples in which *N. fowleri* was detected, and the $C \times T$ values were found to be in a similar range as those observed for the studies using demand-free buffer (Tables 1–4). UV doses of 63 and 13 mW·s/cm^2 were found to be effective to attain a 2-log inactivation of *N. fowleri* cysts and trophozoites, respectively, as shown in Figure 6 and Tables 5 and 6.

CONCLUSIONS

Because both the free-living form and the cysts are potentially important in the transmission of *N. fowleri*, both were included in this study. A 1-mg/L free chlorine initial concentration was chosen for all disinfection experiments because that is the reported approximate average free chlorine residual used in the US drinking water industry (White, 1999). The results of this study suggest that both the trophozoite and the cyst forms of *N. fowleri* are fairly resistant to free chlorine at this concentration in comparison to that of *Giardia* ($C \times T$ is in the range of 31–45 at 25°C). Although the authors' work cannot be directly compared with previous disinfection studies with *N. fowleri*, it does confirm that it is one of the more resistant water transmitted organisms to chlorine and UV light inactivation (Gerba et al, 2002).

In this study, trophozoites required $C \times T$ values in the range of 6–27 compared with cysts that required $C \times T$ values in the

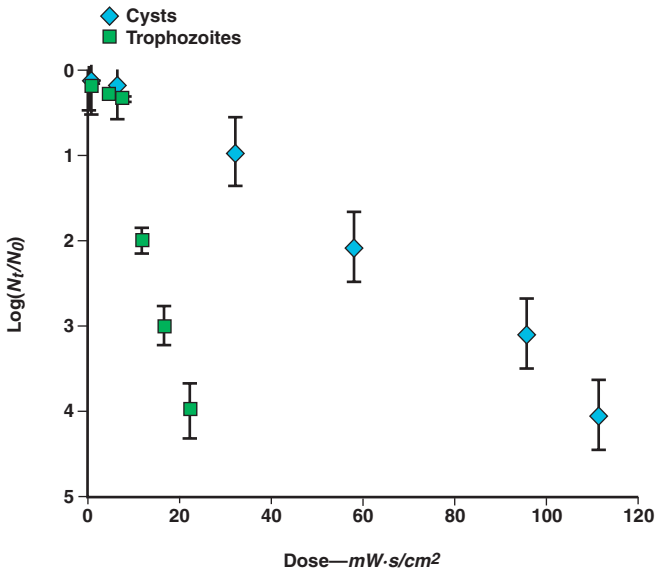
TABLE 5 Calculated ultraviolet doses required for inactivation of *Naegleria fowleri* cysts and trophozoites in buffered demand-free water at room temperature

Cellular Stage of *N. fowleri*	Dose Required to Inactivate 99% mW·s/cm^2	Dose Required to Inactivate 99.9% mW·s/cm^2	Dose Required to Inactivate 99.99% mW·s/cm^2
Trophozoite	13	18	24
Cyst	63	104	121

TABLE 6 Inactivation constants and R^2 values for triplicate experiments by regression analysis for ultraviolet disinfection of *Naegleria fowleri* cysts and trophozoites at pH 7.5 in buffered demand-free water at room temperature

N. fowleri Stage	*K* (Coefficient)	R^2
Trophozoite	0.0126	0.98
Cyst	0.0344	0.95

FIGURE 6 UV light inactivation curve of *Naegleria fowleri* trophozoites and cysts at pH 7.5 in BDF



range of 31–62 for chlorine to achieve a 4-log inactivation at 25°C. The reason for the greater resistance of cysts to chlorine disinfection is because of the hardier structure (thick wall) of the cysts compared with that of the trophozoites (Visvesvara et al, 2007). The current studies were conducted at 24°C because the organism usually grows in natural waters at temperatures higher than 20°C. In Arizona the organism was detected in wells with temperatures ranging from 20 to 38°C (Blair et al, 2008).

The $C \times T$ values for chlorine disinfection of *N. fowleri* cysts as estimated by applying the EHM parameters are comparable to published C × T values of *Giardia lamblia* cysts ($C \times T$ = 30–45) but are considerably lower than those of *Cryptosporidium parvum* oocysts, which have a high $C \times T$ value of 7,200 at a chlorine residual of 80 mg/L in order to achieve a 2-log inactivation at pH 7 and 25°C (Rose et al, 1997; Sobsey, 1989). *N. fowleri* cysts were more resistant to UV light than were *C. parvum* oocysts (estimated dose ~ 10 mW·s/cm^2; Craik et al, 2001; Clancy et al, 1998). This may occur because unlike *Cryptosporidium* spp., *Naegleria* spp. is a free-living organism commonly found in surface waters where it is always exposed to UV light; therefore, its greater resistance may be the result of the presence of DNA repair enzymes, which would make it more resistant to UV light damage (Hillebrandt & Muller, 1991). The UV dose for a 2-log *N. fowleri* cyst inactivation is less than that of cysts of *Acanthamoeba* spp., another free-living amoeba found in surface waters (Hijnen et al, 2006). As demonstrated by this study, chlorination or UV disinfection can be used as effective methods of control of *N. fowleri* transmission via drinking water with the demonstrated dosages and contact times under the conditions described in this study.

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FOOTNOTES

[1]Lee strain, *Naegleria fowleri* Carter, ATCC, Manassas, Va.
[2]Baker Co., Phillipsburg, N.J.
[3]Nanopure RO purifier, Barnstead, Dubuque, Iowa
[4]Solver function, Microsoft Excel, Microsoft Corp., Seattle, Wash.
[5]G8T5.2N, Sankyo Denki, Kanagawa, Japan
[6]Spectrotronic Genesis 5 spectrophotometer, Milton Roy Co., Rochester, N.Y.

PEER REVIEW

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