

Comparison of *Giardia lamblia* and *Giardia muris* Cyst Inactivation by Ozone

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Inactivation of *Giardia lamblia* and *Giardia muris* cysts was compared by using an ozone demand-free 0.05 M phosphate buffer in bench-scale batch reactors at 22°C. Ozone was added to each trial from a concentrated stock solution for contact times of 2 and 5 min. The viability of the control and treated cysts was evaluated by using the C3H/HeN mouse and Mongolian gerbil models for *G. muris* and *G. lamblia*, respectively. The resistance of *G. lamblia* to ozone was not significantly different from that of *G. muris* under the study conditions, contrary to previously reported data that suggested *G. lamblia* was significantly more sensitive to ozone than *G. muris* was. The simple Ct value for 3 log unit inactivation of *G. lamblia* was 2.4 times higher than the Ct value recommended by the Surface Water Treatment Rule.

Giardia muris has been used extensively in ozone disinfection studies as a surrogate for *Giardia lamblia* (10, 21-23, 29, 32, 34, 37-39). *G. muris* is preferred because it is nonpathogenic to humans and has a high rate of in vitro excystation and because the pattern of infection in the mouse model is reproducible (1, 8, 30). In contrast, *G. lamblia* is a human pathogen, has a low rate of in vitro excystation, and has an intermittent release of cysts during the course of infection in the Mongolian gerbil (4, 8, 30). It has been reported that *G. lamblia* cysts are 3.5 times more sensitive to ozone than *G. muris* at 5°C and 1.5 times more sensitive at 25°C (38). The basis for these ratios was the comparison of Ct values for 99% inactivation determined by in vitro excystation (38). In contrast, others have reported that there was little difference between *G. muris* and *G. lamblia* Ct values for 3 log unit inactivation from in vitro excystation (34).

The objective of this work was to compare ozone inactivation of *G. muris* and *G. lamblia* cysts in 0.05 M ozone demand-free phosphate buffer at the bench scale by using the appropriate animal model to determine the viability of the cysts before and after ozonation.

MATERIALS AND METHODS

Phosphate buffer and ozone apparatus. Deionized water was obtained from a Milli-Q system (model OM-140; Millipore Corp.) operated at a resistivity of at least 18 MΩ/cm. The 0.05 M phosphate buffer was prepared by using potassium dihydrogen P_i and disodium hydrogen P_i mixed to provide the required pH (AnalaR grade; BDH Inc.). The resulting solution was made ozone demand free by using procedures described previously (12).

A concentrated stock solution of ozone was prepared in Milli-Q water by a procedure described in an earlier study (23). The concentration of ozone in aqueous solution was determined by using UV spectrophotometry at 260 nm and a molar absorption coefficient of 3,300 M⁻¹ cm⁻¹ (16). Residual ozone was neutralized with 1.0 M sodium formate. The amount of formate that was added was just sufficient to

reduce the ozone A₂₆₀ to zero. The mass of sodium formate added to the test solution did not have an interfering A₂₆₀ and was found to have no adverse effect on the cysts.

The reactor vessel was a 250-ml Erlenmeyer flask reactor containing approximately 200 ml of test liquid. The contents were mixed by using a Teflon-coated magnetic stir bar. All reactors were made ozone demand free prior to use by methods described elsewhere (11, 12).

***G. muris* methods.** The strain of *G. muris* used in this study was originally isolated by Roberts-Thomson et al. (27, 28) from a golden hamster and was obtained from B. J. Underdown, McMaster University. The parasite has been maintained in the laboratory since 1981 by using biweekly passages in CD-1 or C3H/HeN mice. The procedures used to maintain the parasite in the mouse host, to maintain and care for the mice, and to collect and process cysts from the mice were described in detail previously (23).

The dose-response of *G. muris* in C3H/HeN mice was used to estimate the number of viable cysts in the controls and in the ozone-treated samples. The estimation of inactivation by using the C3H/HeN model was described in detail in a previous report (23).

***G. lamblia* maintenance.** Trophozoites of *G. lamblia* WB (ATCC 30957; isolated from a duodenal aspirate taken from a human male) were used for initial infection. This isolate is routinely maintained in vitro in Diamond's TYI S-33 medium (3). Actively growing *G. lamblia* trophozoites (48- to 96-h-old culture) were incubated on ice for 15 min to detach the parasite from the walls of the screw-cap 16- by 125-mm culture tube. The organisms were then sedimented by centrifugation at 500 × g for 10 min, washed in phosphate-buffered saline (PBS), and resuspended in 0.2 ml of PBS to contain 10⁵ trophozoites. Gerbils were inoculated orally with 0.2 ml of PBS containing 10⁵ *G. lamblia* trophozoites. Subsequently, *G. lamblia* was maintained by cyst passage through gerbils (4).

Gerbils. Male Mongolian gerbils (*Meriones unguiculatus*), 6 to 10 weeks old, were used in these experiments. The animals were purchased from Tumblebrook Farms, West Brookfield, Mass. On arrival at the animal facility, the gerbils were placed in filter-top cages (one gerbil per cage), and each gerbil was treated for three consecutive days with 20 mg of metronidazole (Flagyl; Rhone-Poulenc, Montreal,

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Quebec, Canada) per day administered by gavage. This treatment ensured that gerbils were free from all previous infections of the small intestine; this was confirmed by the use of sentinel animals (2, 8).

***G. lamblia* cyst collection.** Cysts released in a 4-h fecal collection from 15 gerbils were collected in 12- by 75-mm glass tubes from days 7 to 14 after infection. The cysts were separated from the feces by filtration through 180- and 45- μ m stainless-steel sieves and concentrated by using the sucrose gradient centrifugation technique described above. They were washed three or more times in Milli-Q water by centrifugation at $500 \times g$ for 10 min (Jouan model GR4.11 centrifuge). The final cyst preparation was resuspended to 10^6 cysts per ml with ozone demand-free Milli-Q water. The number of cysts in the sample was determined by counting four complete grids of a hemocytometer.

We examined the animals for the presence of trophozoites by placing the upper half of the small intestine in 3 ml of PBS (GIBCO). We placed 4 to 10 mucosal scrapings from this segment onto glass microscope slides and examined them for the presence of trophozoites by using a bright-field microscope.

Mongolian gerbil-*G. lamblia* model. Characteristics of the Mongolian gerbil-*G. lamblia* model include a susceptibility to infection with *G. lamblia* cysts that is much higher than that for either rats or dogs, a pattern of cyst release and duration of infection similar to that for the human infection, and the nontransient nature of the infection as demonstrated by an increase in trophozoite colonization and concomitant cyst formation at higher levels than the original inoculum (4). Cyst release is intermittent, with cysts first detected on day 8 for Mongolian gerbils infected with *G. lamblia* cysts, day 9 for gerbils orally inoculated with *in vitro* trophozoites, and day 12 for gerbils duodenally inoculated with *in vitro* trophozoites (4). The latent period varies from 4 to 8 days depending on the inoculum size, and the infection lasts from 13 to 86 days; however, most cysts are observed during weeks 2 and 3. Maximum cyst output appears to occur when the number of trophozoites present in the small intestine is largest (4). It has been concluded that the intermittent nature of cyst release is a feature of the parasite rather than a host characteristic (4, 9). Mongolian gerbils acquire complete resistance when challenged by the same *Giardia* species but only partial resistance when challenged with a different *Giardia* species; they can therefore be used for the identification of the etiological agent as well as for determination of infectivity (9).

Disinfection procedure. A 200-ml volume of the phosphate buffer was added to a 250-ml Erlenmeyer flask reactor. The test solution was then seeded with cysts. Experimental trials 1 to 3 involved a *G. lamblia* cyst concentration of about 3×10^3 cysts per ml. The rest of the experimental trials involved a *G. lamblia* cyst concentration of about 3×10^4 cysts per ml to improve the range of inactivation which could be determined. The phosphate buffer was seeded with *G. muris* cysts for a final cyst concentration of between 10^4 and 10^5 cysts per ml. The initial cyst concentration of the test water was checked by hemocytometer counts.

The concentration of stock ozone solution was measured twice immediately before a calculated volume of ozone solution was added to the buffer via a calibrated pipette. The reactor was continuously sampled by using a peristaltic pump with a flow rate of 8 ml/min in a closed loop. The sample was carried through a short piece of small-diameter Teflon tubing to a 35- μ l flow cell with a light path of 1 cm. A diode array spectrophotometer (model 8452A; Hewlett

Packard) was used to continuously monitor the A_{260} . Experiments were conducted at 22°C. At the end of the predetermined contact time, the residual absorbance was removed with sodium formate. Residual ozone at the end of the contact time could be calculated from the change in absorbance after the addition of formate.

Cyst concentration and animal infection procedure. Each test vessel had Tween 20 (Sigma Chemical Co.) added to a final concentration of 0.01% (vol/vol). The liquid was centrifuged in 175-ml conical plastic centrifuge tubes at $800 \times g$ for 10 min. The supernatant was removed, leaving a 1.5-ml centrifugate, of which 1.0 ml was used for infecting animals. A minimum of four hemocytometer counts were made on the sample to determine the number of cysts.

Each one of a group of five Mongolian gerbils was inoculated orally with known numbers of *G. lamblia* cysts suspended in 0.2 ml of Milli-Q water by previously described techniques (1, 4, 5). Gerbils were individually housed in filter top cages (0.22- μ m-pore-size filter) in a specific-pathogen-free isolation room. The total number of cysts released in a 4-h fecal collection (9:00 a.m. to 1:00 p.m.) by individual gerbils was determined for four consecutive days starting on day 10 postinfection. Cysts were isolated by sucrose gradient centrifugation and counted with a hemocytometer. On day 14 postinfection, necropsy was performed on all gerbils, and the small intestine was examined for the presence of trophozoites (4).

Each one of a group of five C3H/HeN mice was inoculated orally with an equal proportion of the centrifugate. Mice were individually housed in filter top cages (0.22- μ m-pore-size filter) in a specific-pathogen-free isolation room. The feces were checked daily for *G. muris* cysts from days 3 to 8 or until they became positive. On day 8 necropsy was performed on all mice that were negative for *G. muris* cysts, and the small intestine was examined for the presence of trophozoites.

Calculation of inactivation. The definition of inactivation by using the *G. muris*-mouse model is (23)

$$\frac{N}{N_0} = \frac{nI}{n_0I_0} \quad (1)$$

where N is the surviving number of viable cysts, N_0 is the initial number of viable cysts, I is the infective dose in treated animals, I_0 is the infective dose in control animals, n is the number of positive mice in a trial with ozone, and n_0 is the number of positive mice in the control trial.

A logistic dose-response model for *G. lamblia* in Mongolian gerbils was developed by using data from this study and from two earlier reports (31, 33). These data are tabulated in Table 1. Logit response as a function of *G. lamblia* dose is plotted in Fig. 1 with 95% confidence limits. The parameters for the logit model were estimated by using the method of least squares. The resulting model is

$$\text{logit} = -3.688 + 1.542 \log_{10}(\text{inoculum}) \quad (2)$$

where logit is $\ln[P/(1 - P)]$; P is the proportion of animals infected for the specified inoculum. Equation 2 was used to estimate the number of viable *G. lamblia* cysts after ozone treatment.

RESULTS AND DISCUSSION

Kinetics of disinfection. The Chick-Watson (7, 15, 35) pseudo-first-order rate law states that

TABLE 1. Summary of dose-response data reported in the literature and from this study for the *G. lamblia*-Mongolian gerbil model

Inoculum (no. of cysts/gerbil)	No. of gerbils inoculated	No. of gerbils infected	Proportion infected	Reference
4	8	0	0	This study
20	10	1	0.10	This study
40	10	3	0.30	This study
50	5	3	0.60	This study
100	10	3	0.30	This study
200	11	7	0.64	This study
300	3	3	1	This study
1,000	5	5	1	This study
10	10	1	0.10	31
20	10	0	0	31
40	10	1	0.10	31
80	10	4	0.40	31
100	10	4	0.40	31
160	10	4	0.40	31
320	10	5	0.50	31
640	10	6	0.60	31
1,000	10	6	0.60	31
10,000	10	10	1	31
10	24	0	0	33
15	4	0	0	33
45	26	2	0.08	33
100	26	15	0.58	33
1,000	2	2	1	33

$$dN/dt = -kNC^n \quad (3)$$

where dN/dt is the rate of inactivation, k is a pseudo-first-order rate constant found experimentally, N is the number of survivors, C is the concentration of the disinfectant, and n is a dilution factor or empirical constant. Integration of equation 3 yields

$$\log N/N_0 = -kC^n t \quad (4)$$

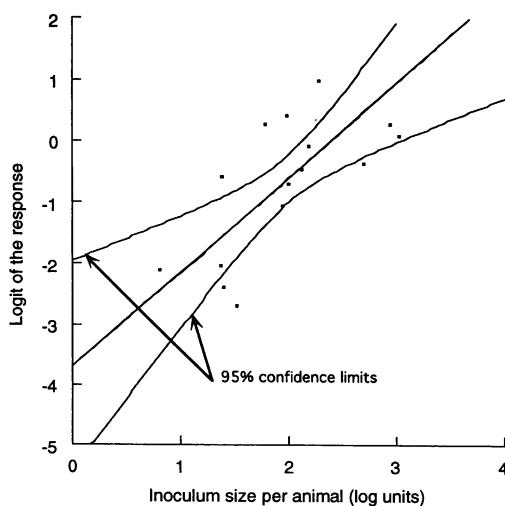


FIG. 1. Logit response as a function of *G. lamblia* cyst inoculum size per gerbil from data from this study and the literature by using a quantal response defined by the presence or absence of infection (31, 33).

Hom (19) proposed an alternate model to account for deviations from equation 4 encountered in practice, expressed by the differential equation

$$dN/dt = -kNC^n t^m$$

Letting $C^n t = k^0$,

$$dN/dt = -kk^0 N(t^m/t) \quad (5)$$

where m is an empirical constant and t is the contact time. Assuming, as in equation 3, that the concentration of the disinfectant remains approximately constant during the course of the contact time, equation (5) is integrated as follows:

$$\int_{N_0}^N \frac{dN}{N} = -kk^0 \int_{t_0}^t t^{m-1} dt$$

$$\ln N/N_0 = -kC^n t(t^m/m)$$

$$\log N/N_0 = -2.303(k/m)C^n t^{m+1}$$

$$\log N/N_0 = -k^* C^n t^{m^*} \quad (6)$$

In ozone studies, assuming that $C^n t$ is constant is misleading; ozone is reactive and continuously disappears during the contact time. Also, there are several methods for defining the concentration of ozone depending on the method of adding ozone to the liquid and the type of reactor (24). In the present study, in which ozone is added from a concentrated stock solution into a batch reactor, the reactor vessel can be considered a reactive flow segment with ideal plug flow (24). Consequently, the concentration of ozone in the flow segment decreases with time as the segment passes through the length of the reactor. Assuming first-order ozone decomposition, the ozone residual in the reactive flow segment at each contact time is

$$C = C_0 e^{-k't} \quad (7)$$

where C is the final ozone residual at the end of the contact time, C_0 is the initial ozone residual at time zero, and k' is the ozone decay constant calculated from the initial and final ozone residuals over the contact time.

Table 2 summarizes the ozone concentrations for each experimental trial. The applied ozone dose is the mass of ozone obtained from the stock solution divided by the final volume of the test liquid in the batch reactor. The initial ozone residual is the dissolved ozone observed in the vessel at time zero. The initial ozone residual is analogous to the transferred ozone dose in a system in which ozone was added as a gas. The degree of immediate ozone demand can be estimated from the ratio of the initial ozone residual to the calculated applied ozone dose. Utilized ozone is the difference between the calculated applied ozone dose and the final ozone residual at the end of the contact time. The ozone decay constant can be estimated by solving equation 7 for k' for each trial.

To account for the decreasing ozone concentration in the flow segment, equation 7 can be substituted into the Hom differential model (13a):

$$dN/dt = -kNC_0^n (e^{-k't})^n t^m \quad (8)$$

Integration of equation 8 yields a Hom-type model that

TABLE 2. Experimental conditions and estimated inactivation of *G. muris* and *G. lamblia* cysts in 0.05 M phosphate buffer at 22°C

Organism	Trial	pH	Contact time (min)	Applied ozone dose (mg/liter)	Initial ozone residual (mg/liter)	Initial residual/applied dose ratio	Final ozone residual (mg/liter)	Ozone decay constant (k') (min^{-1}) ^a	Survival ratio ($\log N/N_0$)	% Inactivation (N/N_0)
<i>G. lamblia</i>	4	6.85	13	2.52	1.76	0.70	0.32	0.13	<-4.4 ^b	>99.996 ^b
<i>G. lamblia</i>	5	6.85	2	1.69	0.93	0.55	0.56	0.25	-3.1	>99.92
<i>G. lamblia</i>	6	6.85	5	2.16	1.44	0.67	0.49	0.22	-3.5	99.97
<i>G. lamblia</i>	7	6.85	5	1.88	1.17	0.62	0.54	0.16	<-4.3 ^b	>99.995 ^b
<i>G. lamblia</i>	8	6.85	2	1.10	0.79	0.72	0.43	0.30	-2.3	99.5
<i>G. muris</i>	9	6.70	5	0.62	0.47	0.76	0.26	0.12	-4.2	99.994
<i>G. muris</i>	10	6.70	5	0.64	0.52	0.81	0.37	0.07	-4.2	99.994
<i>G. muris</i>	11	6.70	5	0.26	0.18	0.69	0.08	0.16	-3.1	99.92
<i>G. muris</i>	12	6.70	5	0.30	0.22	0.73	0.11	0.14	-3.1	99.92
<i>G. muris</i>	13	7.60	5	0.82	0.70	0.85	0.36	0.13	<-5.1 ^c	>99.9992 ^c

^a Based on initial and final ozone residuals over the contact time.

^b 0/5 gerbils responded.

^c Exceeded detection limit of method.

accounts for the first-order ozone decay in the reactive flow segment and describes the inactivation of microorganisms by ozone as a function of the ozone residual at the end of the contact time:

$$\log N/N_0 = -kC_0^n \int_{t_0}^t (e^{-k't})^n t^m dt \quad (9)$$

Equation 9 must be modified to a closed form, however, to estimate the kinetic parameters and to evaluate the resistance of *G. lamblia* to ozone relative to *G. muris*. It is possible to approximate the integral with a Riemann sum based on appropriately small Δt partitions:

$$\log N/N_0 = -kC_0^n \sum_{p=1}^z (e^{-k't_p})^n t_p^m \Delta t_p \quad (10)$$

where z is the number of partitions, which is dependent on the contact time of each experimental trial.

Ozone disinfection parameters. To estimate the kinetic parameters k , n , and m of the Hom-type inactivation model (equation 10), two statistical techniques must be used: the methods of least squares and maximum likelihood. Both approaches used Δt intervals of 0.10 min. The least-squares technique can be performed provided that all experimental trials with survival ratios exceeding the detection limit are excluded from the analysis. Assuming that below-detection-limit data were at the detection limit is inappropriate for the purpose of a kinetic comparison of the two *Giardia* species. Trials 4, 7, and 13 had survival ratio observations exceeding animal infectivity detection limits and were therefore omitted from the least-squares approach.

The least-squares approach estimates the kinetic parameters by minimizing the least-squares residual (S):

$$S = \sum_{i=1}^{n_0} (y_i - \mu_i)^2 \quad (11)$$

$$\mu_i = -kC_0^n \sum_{p=1}^z (e^{-k't_p})^n t_p^m \Delta t_p$$

where y_i is the observed survival ratio for each trial, μ_i is the

predicted survival ratio for each trial, and n_0 is the number of trials with observed survival ratios above the detection limit. The solver routine of Microsoft Excel 4.0 (Microsoft Corp.) was used to minimize S for an n_0 of seven trials given equal weights, omitting trials 4, 7, and 13. Parameter estimates of k , m , and n based on the least-squares approach are summarized in Table 3.

It is known that when data exceed the detection limits of the analysis method, ignoring the data may leave out valuable information (17). Omitting trials with survival ratios below the detection limit may lead to reduced precision in the parameter estimates and to possible systematic bias in the resulting model. The use of maximum-likelihood estimation has been found to yield estimates with less bias and greater precision for such data, provided the residuals are normally and independently distributed with the same standard deviation (14).

The maximum-likelihood approach estimates the kinetic parameters k , m , and n by maximizing the log-likelihood function ($\ln L$):

$$\ln L = -n_0 \ln \sigma - \frac{1}{2} \sum_{i=1}^{n_0} \left(\frac{y_i - \mu_i}{\sigma} \right)^2 + \sum_{i=n_0+1}^n \ln \phi \left(\frac{y_i \leq - \mu_i}{\sigma} \right) \quad (12)$$

$$\sigma = \sqrt{\frac{\sum_{i=1}^{n_0+n} [(y_i \text{ or } y_i \leq) - \mu_i]^2}{n_0 + n - p}} \quad (13)$$

$$\Phi(z) = \frac{1}{\sqrt{2\pi}} \int_{-\infty}^z \exp \left(-\frac{x^2}{2} \right) dx \quad (14)$$

TABLE 3. Summary of parameter estimates for the Hom-type model for *G. lamblia* and *G. muris* inactivation by ozone at 22°C

Estimation technique	S	Λ_1	k	m	n
Least squares	1.20		1.00	-0.74	0.10
Maximum likelihood		2.91	1.04	-0.84	0.12

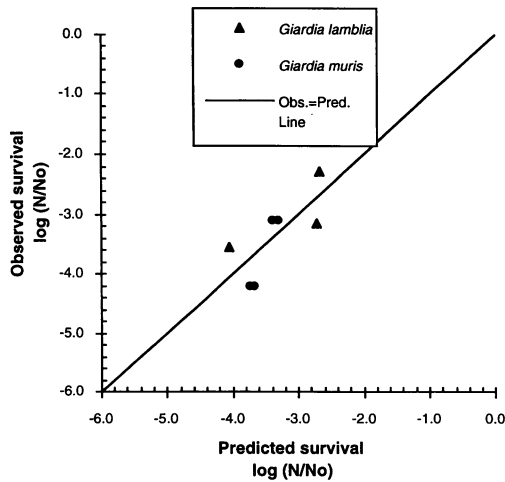


FIG. 2. Observed versus predicted values for *G. muris* and *G. lamblia* inactivation as a function of kinetic parameters k , m , and n estimated by using the least-squares technique.

where n is the number of trials with observed survival ratios below the detection limit, y_i^c is the detection limit (which may differ between trials), σ is the standard deviation of the errors estimated as part of the iterative solution using the detection limit in place of y_i for trials which had survival ratios exceeding the detection limit, p is the number of parameters, and $\Phi(z)$ is the standard normal distribution. The solver routine of Microsoft Excel 4.0 was used to maximize $\ln L$ for an n_0 and n of seven and three trials, respectively; the numerical value of $-\ln L$ at the optimum point is termed Λ_1 . The statistical nature of the maximum-likelihood technique is described elsewhere (14). Parameter estimates based on the maximum-likelihood approach are summarized in Table 3.

On the basis of these parameter estimates, predicted survival ratios can be calculated in log units for the experimental trials. The observed and predicted survival ratios are plotted in Fig. 2 and 3 for the least-squares and maximum-

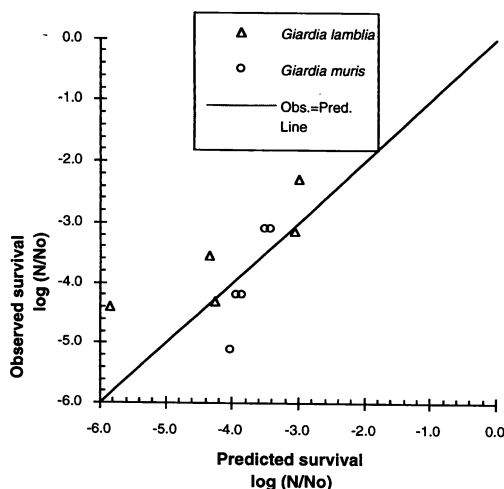


FIG. 3. Observed versus predicted values for *G. muris* and *G. lamblia* inactivation as a function of kinetic parameters k , m , and n estimated by using the maximum-likelihood technique.

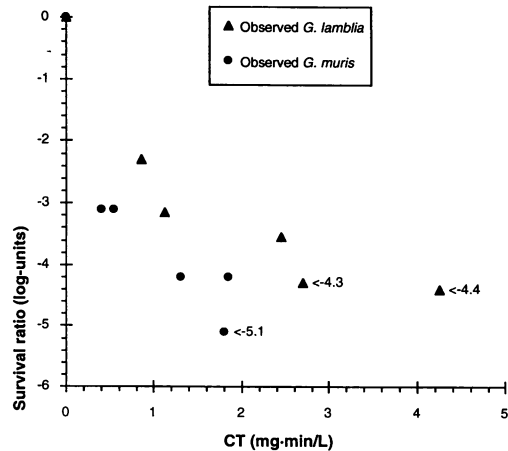


FIG. 4. *G. muris* and *G. lamblia* inactivation as a function of the final ozone residual \times contact time product.

likelihood techniques, respectively. The vertical distance between the data points and the observed equals predicted line are the residuals as measured by the observed survival axis. If *G. lamblia* were more resistant than *G. muris* to ozone, the *G. lamblia* data would have plotted above the observed equals predicted line, with the *G. muris* data plotted below the line. In general, the residuals are evidence of no significant distinction between the resistance of *G. lamblia* to ozone and that of *G. muris* under the study conditions. The kinetic analysis suggests that the ozonation effort required to inactivate *G. lamblia* is not significantly different from the effort required to effect greater than 3 log units of inactivation of *G. muris*.

Ct comparison. Even though the kinetic analysis suggests that the resistance of *G. lamblia* to ozone was not significantly different from that of *G. muris*, the experimental data were also evaluated in terms of the simple Ct product for comparison with previously published studies. Typically chemical disinfection results are expressed in terms of a concentration-time product (Ct) for different levels of inactivation (18). For this purpose, all the experimental trials were considered. The Ct concept attempts to define the survival ratio as a linear function of ozone concentration and contact time. Consequently, the comparison of *G. lamblia* with *G. muris* by using the simple Ct product will differ from the kinetic analysis based on the Hom-type model, which accounted for the nonlinear behavior with the parameters n and m .

Several approaches have been suggested for estimating the Ct product (26). The discussion here will focus on the reactive-flow segment, which is conceptually similar to the reactor used in this study. If there are no means of measuring the ozone residual continuously in a reactive-flow segment, it has been recommended that the ozone residual at the end of the contact time (outlet to the reactor) be used for Ct calculations (24, 26). In a flowing system, a tracer study would have to be performed to determine the characteristic t for the reactor (25). When using a batch reactor such as used in this study, the actual contact time in each experimental trial can be used as the characteristic t .

Inactivation of *Giardia* species. The experimental conditions and inactivation results for *G. lamblia* and *G. muris* are summarized in Table 2. Figure 4 illustrates the survival of *G. muris* and *G. lamblia* as a function of Ct based on the final

ozone residual and the contact time of the experimental trial. Figure 4 can be used to determine the simple Ct product for different levels of inactivation. The simple Ct for 2, 3, and 4 log units of *G. muris* inactivation is about 0.24, 0.45, and 0.86 mg · min/liter, respectively. *G. lamblia* Ct data were more difficult to interpret. The approximate Ct for consistently achieving 2 log units of inactivation of *G. lamblia* was about 0.65 mg · min/liter. This was more than twice the requirement for a similar inactivation of *G. muris*. The approximate Ct value for 3 log units was 1.23 mg · min/liter, i.e., more than 2.5 times greater than that for *G. muris*. The approximate Ct value for 4 log units was 2.57, i.e., nearly 3 times greater than that for *G. muris*. Five log units of inactivation of *G. lamblia* was beyond the detection limit of the model system used.

Several issues arise from the data in Table 2. First, two inactivation data points exceeded the detection limits of the *G. lamblia* animal model, making quantitative analysis difficult. Consequently, the approximate detection limits were used for analysis by assuming that about 30 viable *G. lamblia* cysts could cause an infection in the gerbil model. This value was the average of the two lowest cyst doses (20 and 40 cysts per animal) that produced an infection in a significant proportion of animals (Table 1). The *G. muris* data had one observation which exceeded the detection limit. In all cases, the detection limit was used in the numerical analysis of the data. Second, the inactivation data shown in Fig. 4 do not form a linear plot as would be predicted by the pseudo first-order Chick-Watson model (equation 3) with $n = 1$. This is a result of the tailing effect reported for microorganisms chemically inactivated with ozone (6, 11, 13, 20). Therefore, extrapolation of these data to higher levels of inactivation by using the Chick-Watson model could result in serious underestimation of the required ozone for higher levels of inactivation.

A third observation of importance is that the amount of ozone required to effect 2 log units of inactivation of *G. lamblia* is significantly greater than that previously reported. Previously published data indicated that the simple Ct value for 2 log units of inactivation of *G. lamblia* was about 0.17 mg · min/liter at 25°C (36), whereas the data from the present study suggest that the Ct value could be about 3.8 times higher. There are two critical differences between this study and the earlier work by Wickramanayake et al. (36–38). First, the earlier study used *in vitro* excystation to determine the viability of the *G. lamblia* cysts, whereas this study used animal infectivity. Animal infectivity is a better indicator of the infection-blocking potential of a disinfectant. Also, *in vitro* excystation rarely exceeds 50% in controls and is limited to little more than 2 log units of inactivation (30).

A second significant difference between the current study and the earlier work is the ozonation apparatus. Wickramanayake et al. (36) used a semibatch, ozone gas-fed, bench-scale system to which ozone was continuously added to maintain a constant ozone residual in the test batch of water. The ozone dose was not reported. In the study reported here, ozone was added from a concentrated stock solution to a batch reactor and was continuously monitored throughout the experimental trial. The significance of the differences in experimental protocol between this study and earlier work is that the ozone dose was probably higher than the reported steady-state ozone residual concentration. The importance of initial ozone residual measurement is illustrated in Fig. 5. A higher initial ozone residual would result in a higher apparent Ct product.

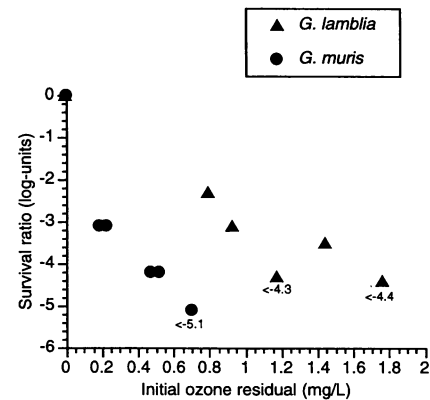


FIG. 5. *G. muris* and *G. lamblia* inactivation as a function of the initial ozone residual.

In another study, Wallis et al. (34) reported a Ct of 1.7 mg · min/liter to achieve 3 log units of *G. lamblia* inactivation in natural water at 8 to 9°C. The *Giardia* strain in their study was derived from a muskrat. The suggested Ct value for water temperatures similar to those reported by Wallis et al. (34) was 1.5 mg · min/liter, including the safety factor (26). The ozonation apparatus of Wallis et al. (34) was conceptually similar to that of Wickramanayake et al. (36) in that a constant ozone residual was maintained after ozone demand was satisfied.

Implications of results. The U.S. Environmental Protection Agency used the data of Wickramanayake et al. to formulate the published Ct values for *G. lamblia* (26). The published values for 2 log units of inactivation of *G. lamblia* were those reported by Wickramanayake et al. with a safety factor of 2. The published values for 3 log units of inactivation were obtained by extrapolating the data of Wickramanayake et al. from 2 log units to 3 log units by using equation 3 (26). The published value for 3 log units of inactivation was 0.52 mg · min/liter at 22°C, including the safety factor. The value obtained from the present study was about 2.4 times greater. The reasons for the apparently higher ozone requirements for 3 log units of inactivation of *G. lamblia* in this study may derive from the different ozonation protocol that was used, the different viability assay procedure, and differences in the strains of *G. lamblia* used. The results of this study suggest that the human-derived strain of *G. lamblia* used in this study is more resistant to ozone than was *G. muris*, commonly used in mice as a surrogate for *G. lamblia*.

It is evident that although *Giardia* species have been studied extensively, much remains uncertain regarding their inactivation by chemical disinfectants. Therefore, the use of a chemical disinfectant as the only barrier to the transmission of *Giardia* cysts in potable water supplies cannot be recommended. The data reported in this study suggest that although *G. muris* may be a reasonable surrogate for *G. lamblia*, the ozonation effort required to inactivate *G. lamblia* is not significantly different from the effort required to effect greater than 3 log units of inactivation of *G. muris*. More data are needed on the inactivation of *G. lamblia* at different water temperatures, in water of different qualities, and with different strains of *G. lamblia* to adequately judge the reliability of chemical oxidation alone as a barrier to the transmission of giardiasis by the water route.

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