and rotating paddle. The bubble size in the reactor was adjustable by changing the power input to the contactor by means of the variable speed motor and Rushton turbine as described previously (Finch and Smith, 1989). There are few sources of cyst loss in this reactor because it is a batch reactor where the liquid is homogeneously mixed. A valved port was used to sample liquid from the middle of the vessel. Mass-transfer and mixing are two of the most important variables in ozone disinfection, and the semi-batch, stirred tank reactor was well suited for conclusions regarding the scale-up of these factors.

4.3 *GIARDIA* METHODS

4.3.1 Parasites

4.3.1.1 *Giardia muris*

The strain of *G. muris* used in this study was originally isolated by Roberts-Thomson (Roberts-Thomson *et al.*, 1976a; 1976b) from a golden hamster and was obtained from B.J. Underdown, McMaster University, Hamilton, Ontario, Canada. The parasite has been maintained in the laboratory since 1981 using bi-weekly passages in CD-1 or C3H/HeN mice. The *G. muris* culture is free of all other contaminating intestinal protozoa including *Hexamita* as shown by fecal and small intestine examinations using light microscopy and histology.

4.3.1.2 *Giardia lamblia*

Trophozoites of WB isolate of *G. lamblia* (ATCC 30957, isolated from a duodenal aspirate of a human male) were used for initial infection. This isolate is routinely maintained *in vitro* using Diamond's TYI-S-33 medium (Belosevic *et al.*, 1982). 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-capped 16 x 125 mm culture tube. The organisms were then sedimented by centrifugation at 200 x *g* for 10 min, washed in phosphate-buffered saline (PBS), and

were inoculated orally with 0.2 mL of PBS containing 1×10^5 *G. lamblia* trophozoites. Subsequently, *G. lamblia* was maintained by cyst passage through gerbils (Belosevic *et al.*, 1983).

4.3.2 Animals

4.3.2.1 Mice

Specific-pathogen-free (SPF) 6- to 8-week-old, male, inbred C3H/HeN mice were used and were obtained from Charles River Breeding Laboratories (St. Constant, Que.) until November 1990. Beginning January 1991, the animals were obtained from Taconic Laboratories Inc. (German Town, N.J.).

Upon arrival, mice were kept under SPF conditions (laminar flow isolator) and were given sterile food and water *ad libitum,* housed 4 to 5 per cage, had sterile bedding changed twice per week and were maintained with a diurnal cycle of 14 h of light and 10 h of dark. The access to the laminar flow isolator was limited to two animal-care technicians. Animals to date have been found to be free of natural *Giardia* infections as shown by fecal examinations for cysts and small intestine examination for trophozoites of randomly chosen animals. Following infection with *G. muris* the mice were individually housed in filter-top (0.22µm filter) cages in a separate SPF isolation room.

4.3.2.2 Gerbils

Six- to 10-week-old male Mongolian gerbils (*Meriones unguiculatus*) were used in all experiments. These animals were purchased from Tumblebrook Farms (West Brookfield, Mass.). Upon arrival at the animal facility, gerbils were placed in filter-top cages (one gerbil per cage) and treated for 3 consecutive days with 20 mg/day/gerbil of Metronidazole (Flagyl®; Rhone-Poulenc, Montreal, Que.), administered by gavage. This treatment ensured that gerbils were free from all previous intestinal infections (Belosevic *et al.*, 1983; Faubert and Belosevic, 1990).

4.3.3 Isolation of Cysts

4.3.3.1 *Giardia muris*

G. muris cysts were isolated from the feces using the modified sucrose gradient centrifugation technique (Roberts-Thomson *et al.*, 1976a; Belosevic and Faubert, 1983a). Feces were collected 7 days after infection from 10 to 15 mice over a period of 2 to 3 h. Feces were soaked for 30 min in Milli-Q® water, emulsified by using applicator sticks, layered on a sucrose solution (spec. grav. 1.12), and centrifuged for 15 min at 400 x *g* in a swinging-bucket centrifuge (GR4.11; Jouan) at 4°C. After centrifugation, cysts trapped at the water-sucrose interface were carefully removed with a pipette and washed in Milli-Q® water by centrifugation at 600 x *g* for 10 min. The pellet containing the cysts was resuspended in a known volume of Milli-Q® water and the number of cysts in the sample determined by counting 4 complete grids of a hemocytometer.

In order to obtain highly purified cysts suitable for use in ozone demand-free experiments, cysts were isolated as above; however, 2 to 4 additional Milli-Q® water washes were required. The absorbance of a cyst suspension of 10^4 cysts/mL at 260 nm was used as a quality control measure. Only cysts with an absorbance less than 0.03/cm were used in experiments (Figure 4.3). The final cyst preparation was resuspended to the desired concentration using ozone demand-free Milli-Q® water. The number of cysts in the sample was determined by counting 4 complete grids of a hemocytometer. Cysts were stored at 4°C for 18 h and were used within 48 h of preparation. It was found that storage of cysts at 4°C for longer than 48 h resulted in decreased viability of the organisms.

4.3.3.2 *Giardia lamblia*

Cysts released in a 4 h fecal collection (9:00 a.m. to 1:00 p.m.) from 15 gerbils were collected in 12 x 75 mm glass tubes from day 7 to day 14 after infection. Cysts were separated from the feces by filtration through

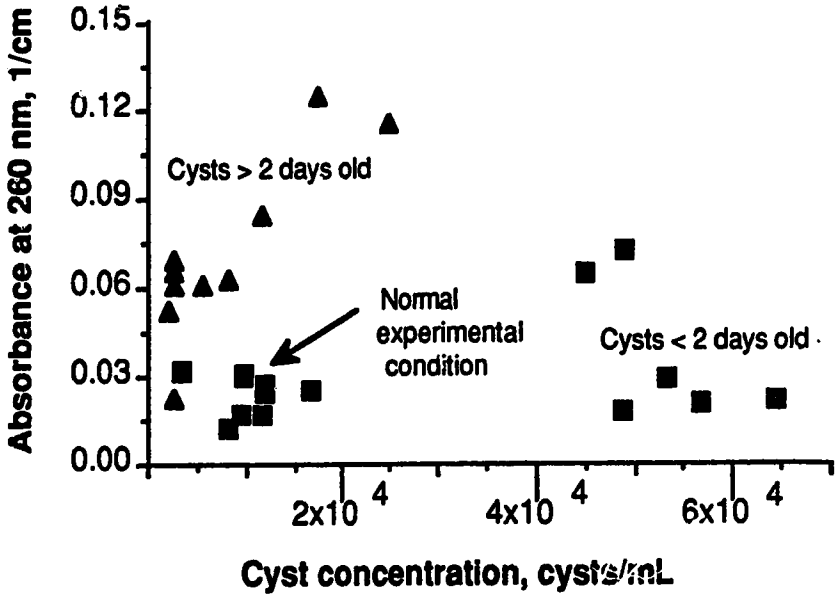

Figure 4.3 Effect of cyst age on absorbance of cyst preparation

three times in Milli-Q® water by centrifugation at 200 x *g* for 10 min (GR4.11; Jouan) so that the absorbance at 260 nm of a cyst suspension of 10^4 cysts/mL did not exceed 0.03/cm. The final cyst preparation was resuspended to 10^6 cysts/mL using ozone demand-free Milli-Q® water. The number of cysts in the sample was determined by counting four complete grids of a hemocytometer.

4.3.4 Isolation of Trophozoites

The upper 25 percent of the small intestine was placed in 3 mL of PBS (Gibco). Four to 10 mucosal scrapings from this segment were placed on glass microscope slides and examined for the presence of trophozoites by using a bright-field microscope.

4.3.5 Fluorogenic Staining

A modified FDA-EB staining technique was used (Jackson *et al.*, 1985). FDA stock solution (5 mg/mL in acetone; ICN Biochemicals Corp.) and EB stock solution (20 µg/mL) in phosphate-buffered saline (Sigma Chemical Corp.) were stored at -30°C prior to use. A working dilution of FDA was made by adding 20 µL of stock FDA to 10 mL of Milli-Q® water. A 100 µL aliquot of *G. muris* cysts was added to a 1:1 solution of diluted FDA (50 µL) and stock EB (50 µL). The mixture was incubated at room temperature for 5 min, and the reaction was then stopped by adding 200 µL of Milli-Q® water. Both sides of the hemocytometer were loaded with a well-mixed stained cyst suspension and the cysts were allowed to settle to the bottom of the hemocytometer chamber for 60 s. The cysts were counted first under incident light and then under u.v. light by using an epifluorescence microscope (Optiphot; Nikon) equipped with an EX330 excitation filter and a BA435 barrier filter. A minimum of four complete grids of the hemocytometer were counted for each sample, and counting was completed within 1 h.

4.3.6 Excystation

A procedure described by Schaefer *et al.* (1984) was used to

cysts contained in 0.5 mL were placed into a 15 mL plastic, screw-cap, graduated, conical centrifuge tube. First, 5 mL of prewarmed (37°C) reducing solution was added, and then 5 mL of prewarmed (37°C) freshly prepared 0.1 M $NaHCO_3$ was added. The tube was vortexed vigorously, incubated in a 37°C water bath for 30 min, and centrifuged for 2 min at 400 x *g* in a swinging-bucket centrifuge, and the supernatant was aspirated down to 0.2 mL. The pellet was resuspended in 10 mL of chilled (4°C) trypsin-Tyrode's solution and vortexed vigorously. The tube was centrifuged for 2 min at 400 x *g* and the supernatant was aspirated down to 0.2 mL. Three-tenths of a mL of prewarmed (37°C) trypsin-Tyrode's solution was added to the tube, and the pellet was resuspended. Three to four drops of the suspension were placed in a chamber slide, the coverslip was sealed with a Vaseline®-paraffin mixture, and the slide incubated in a 37°C air incubator for 30 min. It was then examined with a phase-contrast microscope and a 40X phase objective lens. Empty cyst walls (ECW) and partially excysted trophozoites (PET) were counted as positive excystation; intact cysts (IC) showed no signs of excystation. The total number of ECW, PET, and IC examined was 1,000 or the number present in the entire slide area if it was not possible to find 1,000. The excystation procedure was completed within 3 h.

4.3.7 Animal Models

4.3.7.1 C3H/HeN mouse-*G. muris* model

The C3H/HeN mouse-*G. muris* model (Roberts-Thomson *et al.*, 1976a; Belosevic and Faubert, 1983a; Belosevic *et al.*, 1984) was used to assess *G. muris* cyst infectivity. Data on the effects of different doses of untreated cysts on the duration of the latent period in SPF male C3H/HeN mice are presented in Table 4.2. The data show a significant correlation between the size of the inoculum (cysts/mouse) and the time (days) when all mice in a given dose group were passing cysts. The discriminating variable is not the beginning of the latent period but rather the time when all mice in a group were releasing cysts in the feces.

Table 4.2 C3H/HeN mice infected by *G. muris* as a function of days after infection and inoculum size

Inoculum Size (cysts per mouse)	No. of Mice in Group	Days After Infection* (No. of mice positive for cysts)									
		1	2	3	4	5	6	7	8	9	10
10^5	9	0	0	9	9	9	9	-	-	-	-
10^4	10	0	0	0	10	10	10	10	-	-	-
10^3	10	0	0	0	3	10	10	10	10	-	-
10^2	9	0	0	0	0	5	9	9	9	9	-
10	9	0	0	0	0	3	6	6	9	9	9
1	12	0	0	0	0	0	0	2	3	5	5†

*Data collected until day 10 or for 3 days after all mice in group became positive.

†Trophozoites detected in 9 of the 12 mice in this group.

results. Only 5 of 12 mice inoculated with 1 cyst were releasing cysts on day 10 after inoculation. However, the examination of the small intestine of these mice showed that 9 out of 12 mice (75 percent) were infected with trophozoites of *G. muris*, indicating that C3H/HeN mice are highly susceptible to infection.

4.3.7.2 Mongolian gerbil-*G. lamblia* model

The Mongolian gerbil-*G. lamblia* model was proposed by Belosevic *et al.* (1983). Characteristics of the 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 (Belosevic *et al.*, 1983). 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 (Belosevic *et al.*, 1983). The latent period varied from 4 to 8 days depending on the inoculum size, and the duration of infection was 13 to 86 days; however, most cysts were observed during the second and third weeks. Maximal cyst output occured when the number of trophozoites present in the small intestine was highest (Belosevic *et al.*, 1983). It was concluded that the intermittent nature of cyst release is a feature of the parasite, rather than a host characteristic (Belosevic *et al.*, 1983; Faubert *et al.*, 1983). Mongolian gerbils acquire complete resistance when challenged by the same species of *Giardia*, but only partial resistance when challenged with a different species of *Giardia* and can therefore be used for the identification of the etiological agent as well as determining infectivity (Faubert *et al.*, 1983).

The intermittent nature of cyst release prevents a latent period approach to the determination of inactivation. A most probable number approach has been used (Haas and Heller, 1990; Hibler *et al.*, 1987); however, was beyond the scope of this study due to budget and time constraints. Rather a semiquantitative determination of infection was

4.3.8 Infectivity Testing Procedures

Infectivity testing procedures adhered to the Medical Research Council of Canada and Natural Sciences and Engineering Research Council of Canada guidelines for animal care.

4.3.8.1 *Giardia muris*

All disinfected liquid was centrifuged in 175 mL plastic conical centrifuge tubes at 800 x *g* for 10 min. The supernatant was removed, leaving a 1.5 mL centrifugate, of which 1.0 mL was used for infecting mice. In the viability comparison experiment, the centrifugate was 2.1 mL, of which 1.0 mL was used for infecting mice, 0.5 mL was used for excystation, and 0.100 mL was used for FDA-EB fluorogenic staining; the remaining portion being surplus. A minimum of four hemocytometer counts were made on the 1.0 mL portion of the split sample in order to determine the total number of cysts. Each one of a group of 5 C3H/HeN mice was orally inoculated with 0.200 mL. The size of the inoculum was typically 10^4 cysts/mouse in the screening and ozone/ozone–hydrogen peroxide comparison experiments; and 10^5 cysts/mouse in the viability comparison, specific ozone residual and natural water experiments.

Pilot-scale experiments used the above procedure with the exception that an additional concentration step was required because the 1 L aliquot was centrifuged using six 175 mL plastic conical centrifuge tubes. The expected size of the inoculum was 10^4 cysts/mouse.

During experiments, mice were individually housed in filter-top cages (0.22 µm filter) in a SPF isolation room. The feces were checked daily for *G. muris* cysts from day 3 or 4 until day 8 or when they became positive. On day 8 necropsy was performed on all mice which were negative for *G. muris* cysts and the small intestine was examined for the presence of trophozoites.

A different procedure was used in some preliminary work (exps. 18 and 24). Disinfected liquid was centrifuged in a 50 mL plastic conical

leaving a 0.5 mL centrifugate, which was split into a 0.100 mL portion for FDA-EB fluorogenic staining and a 0.4 mL portion for infecting mice. A minimum of 4 hemocytometer counts were made on the 0.4 mL portion in order to determine the total number of cysts. This portion was made up to 0.6 mL with Milli-Q® water. Each of 3 specific pathogen-free, male C3H/HeN mice were inoculated orally with 0.200 mL. The size of the inoculum was typically 10^4 cysts/mouse. All mice were caged separately and the feces checked for *G. muris* cysts every 2 d until day 20 or when they became positive.

4.3.8.2 *Giardia lamblia*

All disinfected liquid from the reactor was centrifuged in 175 mL plastic, conical centrifuge tubes at 800 x *g* for 10 min. The supernatant was removed, leaving a 1.5 mL centrifugate, of which 1.0 mL was used for infecting gerbils. Each one of a group of 5 Mongolian gerbils was inoculated orally with known numbers of *G. lamblia* cysts suspended in 0.2 mL of Milli-Q® water using techniques described previously (Belosevic and Faubert, 1983a; Belosevic *et al.*, 1983; Belosevic *et al.*, 1984). During experiments gerbils were individually housed in filter-top cages (0.22 µm filter) in a SPF 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 using a hemocytometer. On day 14 postinfection necropsy was performed on all gerbils, and the small intestine was examined for the presence of trophozoites (Belosevic *et al.*, 1983).

4.4 DISINFECTION PROCEDURES

4.4.1 Bench-Scale Experiments

Experiments with laboratory and natural waters used the same procedure with the exception that natural waters, with concomitant ozone demand, had a more concentrated stock ozone solution (up to

of test liquid was added to a 250 mL Erlenmeyer flask reactor. The test liquid was seeded with *Giardia* cysts to provide a concentration that was typically 10^4 cysts/mL for *G. muris* and slightly less (5×10^3 cysts/mL) for *G. lamblia*. The concentration of stock ozone solution was measured twice immediately before a calculated volume of ozone solution was added to the liquid using a mass-calibrated pipette. This was the applied ozone dose. Over the duration of the experiment, the test solution was pumped through a thermostatically controlled 35 µL, 1 cm light path length flow cell at approximately 8 mL/min. Residual ozone was neutralized using a slight excess of 1.0 M sodium formate. Sodium formate was also added to the controls. Temperature of the test liquid was maintained by means of an ice-water bath. Figure 4.2 depicts the experimental apparatus (p. 71).

In the screening experiment bentonite (technical grade; BDH) was added to the phosphate buffer to produce turbidity. In the viability comparison, specific ozone residual, natural water and *G. lamblia* experiments, Tween 20 (Sigma Chemical Co., St. Louis, Mo.) was added to the ozone reactor (0.01 percent vol/vol) after the ozone was neutralized to enhance cyst recovery. Following ozonation, but prior to concentration for the infectivity testing procedure, four replicate hemocytometer counts of cyst density were made. This was used to test the homogeneity of the cyst suspension and also permitted an accurate cyst "mass" balance as shown in Figure 4.4.

For the comparison of ozone and ozone–hydrogen peroxide, the procedure was similar to that described in the first paragraph of this section except that hydrogen peroxide (Perhydrol®; E. Merck, Germany) was added to the reactor to provide a final concentration of about 17 mg/L prior to adding cysts. The ozone dose was approximately 1.7 mg/L so that the theoretical hydrogen peroxide to ozone weight ratio was about 10 to 1. Residual hydrogen peroxide was neutralized using a slight excess of catalase. Hydrogen peroxide, sodium formate and catalase were used in the controls.

A different procedure was used in some preliminary work (exps

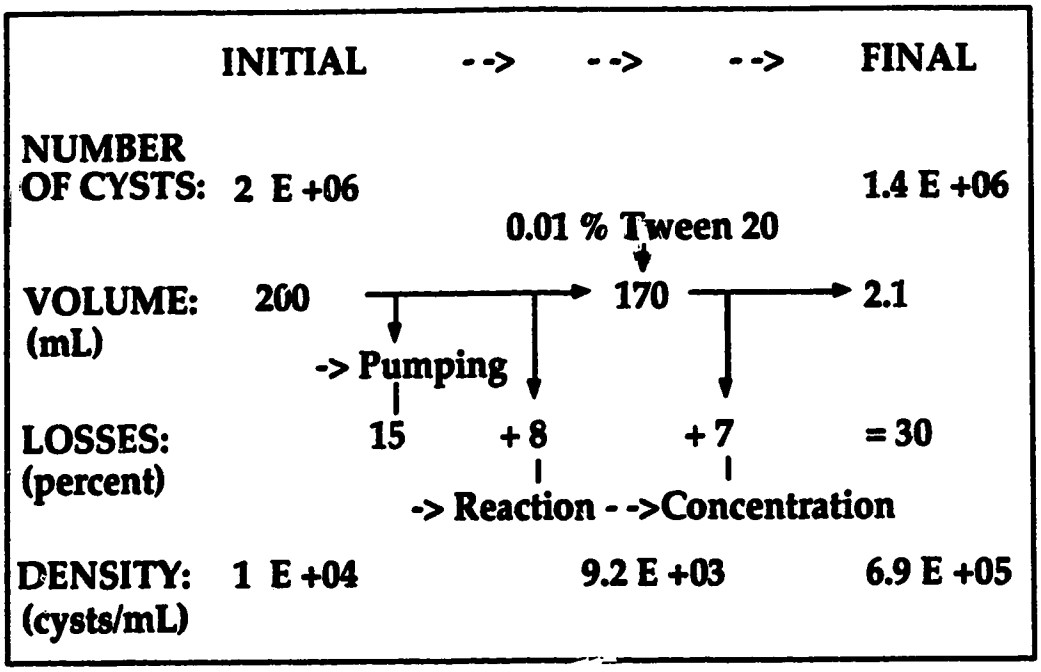

Figure 4.4 Cyst mass balance in control experiments

demand-free Milli-Q® water, *G. muris* cysts were added and then concentrated stock ozone solution was added to provide a total volume of 41 mL. The reactor was mixed rapidly (300 r.p.m.) using a magnetic stirrer (220T; Fischer Scientific) and Teflon®-coated stir bar during cyst addition, moderately during ozone addition (144 r.p.m.), and as slowly as possible (72 r.p.m.) for the 5 min contact time to minimize volatilization of the ozone. Ozone demand-free borosilicate glass pipettes or an Oxford pipette with ozone demand-free disposable tips were used for all liquid or ozone additions and for sample removals. The background u.v. absorbance (260 nm) of the cyst suspension was subtracted from the absorbance at the end of the contact time in order to determine ozone residual. Ozone was purged using 90 s of air-stripping and a final u.v. absorbance measurement was made.

4.4.2 Pilot-Scale Experiments

Pilot-scale experiments were performed using seeded *G. muris* cysts and MS2 coliphage concurrently. HPC bacteria that were native to the test water (NSR water) were enumerated as well. A total of 10^7 cysts were added to the 100 L reactor, providing an initial cyst density of approximately 10^2 cysts/mL. At predetermined times corresponding to the desired applied ozone doses, aliquots were extracted from the sampling port into 1 L Erlenmeyer flasks. These flasks contained 200 µL of 1.0 M sodium formate which neutralized the oxidant. Duplicate samples for ozone residual determination by the indigo method were taken immediately after the 1 L aliquots. As with bench-scale experiments, Tween 20 was added to enhance cyst recovery. Four replicate hemocytometer counts were made prior to concentration for the infectivity testing procedure. The control aliquot was obtained after *G. muris* cyst seeding but when the reactor was operating with oxygen prior to ozonation.

The feed-gas flow-rate was calibrated for standard temperature and pressure using a wet test meter (Precision Scientific, Inc.). Ozone concentration in the off-gas was continuously recorded on a strip chart

operated at 230 r.p.m. which corresponded to a gassed power input of 390 W/m^3 or a velocity gradient of 624/s. The applied ozone dose was calculated from (Finch and Smith, 1989):

$$C = \frac{C_g Q_g t_g}{V} \qquad [4.1]$$

where: C is the applied ozone dose;

C_g concentration of ozone in the carrier gas;

Q_g is the gas flow-rate;

t_g is the ozonation time; and

V is the volume being treated (100 L).

4.5 EXPERIMENTAL DESIGN

Factorial experimental design permits the independent analysis of factors and their interactions using a minimum number of trials (Box *et al.*, 1978; Davies, 1979). Fractional factorial designs can be even more efficient, because negligible higher order interactions can be confounded with additional factors, resulting in fewer trials. A factorial design with two levels and n factors is designated as 2^n. A fractional factorial design is designated as 2^{n-m}, where m is the fraction, $1/2^m$, of the factorial. Factorial or fractional factorial designs were used wherever possible. The main effects and interactions were calculated from the regression coefficients (ß) by the relation:

$$\text{effect} = 2 \times ß \qquad [4.2]$$

A method of analysis of variance (ANOVA) described by Davies (1979) was used to evaluate the factors and interactions. The mean square (MS) of each term was calculated as follows:

$$MS = (TE)^2/(2^{n-m}) \qquad [4.3]$$

$$TE = ß \times 2 \times 2^{n-m-1} \qquad [4.4]$$

where: TE is the total effect

An estimate of the experimental error permits discrimination of real from null effects. The mean square error (MS_d) is an estimate of the error variance and can be calculated from the mean square of insignificant effects and interactions. Half-normal probability plots provide a graphical technique for separating real effects from null effects (Daniel, 1959). The statistic MS/MS_d for each term may be compared with the *F*-statistic, $F\alpha(\lambda_1,\lambda_2)$, where α is the probability (*P*)-level, and λ_1 and λ_2 are the degrees of freedom for MS and MS_d, respectively. Those factors and interactions exceeding the *F*-statistic at the appropriate *P*-level are statistically significant. For most of the results reported here, the *P*-level selected was 0.05.

4.5.1 Comparison of Viability Methods

A single control trial and two or more ozonated trials were performed on each of four cyst preparations (A to D). Each control and ozonated trial had cysts concentrated from them with the resulting concentrate split three ways to provide cysts for the different viability determination methods. This design permitted a comparison of differences in the viability methods while eliminating run-to-run variations in the test water and ozonation conditions. One-way ANOVA was used to compare the three viability methods. The Duncan Multiple Range Test (Duncan, 1955) was used to determine if there was a significant difference between the viability methods.

4.5.2 Comparison of Ozone and Ozone–Hydrogen Peroxide

This experiment was designed as a series of paired trials using two different ozone demand-free laboratory buffer systems with the same cyst preparation. The first water was the 0.05 M phosphate buffer with hydrogen peroxide added in excess. A hydrogen peroxide to ozone weight ratio of 10 to 1 was used because it was found that this ratio completely decomposed ozone in 60 s or less, ensuring a large supply of decomposition radical species and little residual ozone (Yuen, 1990). Lower ratios were found to leave significant concentrations of residual ozone which would have confused the interpretation of the results. The

second water was the 0.05 M phosphate-0.01 M bicarbonate buffer designed to quickly scavenge radical species from ozone decomposition.

The hypothesis being tested was that if the ozone–hydrogen peroxide process was more effective than ozone alone, the large quantity of radical intermediates produced in the peroxide system would result in more inactivation than ozone alone. All trials were conducted in triplicate at pH 5.7 and 22°C and with a 2 min contact time. Differences between mean inactivation in the two buffer systems was evaluated using a t-test.

4.5.3 Factors Affecting Ozone Inactivation

4.5.3.1 Screening experiment

This experiment was conducted to evaluate the significance of various water quality and process control parameters which have been reported to influence the ozone inactivation of *Giardia*. A 2^{5-1} fractional factorial experimental design was chosen to assess 5 factors in 16 runs (Box *et al.*, 1978). The factors and settings used were: applied ozone dose (1.5 and 3.0 mg/L); temperature (5 and 22°C); contact time (2 and 7 min); turbidity (0 and 5 NTU); and pH (5.7 and 7.6). In this type of design, no main effect or two-factor interaction is confounded with any other main effect or two-factor interaction (Box *et al.*, 1978). The experiment was designed in four blocks of four ozonated and two control trials each. In the design the CD and CE (contact time x turbidity and contact time x pH) interactions were confounded with block effects because these interactions were not expected to be significant. This permitted the detection of batch-to-batch differences in the cysts. Blocks and trials within blocks were conducted in random order.

It was assumed that the contact times and temperatures used in this experiment did not affect *G. muris* cyst viability permitting all control runs to be done at 22°C and for a contact time of 5 min. The effect of turbidity and pH on control cysts and, subsequently, the animal model, was not known, so these factors were varied in the controls.

4.5.3.2 Specific ozone residual experiment

This experiment was designed to further examine the effects of temperature and pH and to better define the kinetics of *Giardia* inactivation. Decomposition tests without cysts were used to calculate the applied ozone doses necessary to achieve a specific ozone residual level of approximately 0.4 mg/L in all trials (refer to Section 5.1.1). This is in contrast to the screening experiment where ozone dose was specified and the ozone residual was happenstance. Initially a full 2^2 factorial design with 2 centre-point replications was performed. Subsequently, additional trials were done in which contact time, temperature and pH were varied.

4.5.3.3 Natural waters experiment

This experiment was designed to verify the laboratory water results for two natural waters that were significantly different in water quality parameters affecting ozone decomposition. A 2^{3-1} fractional factorial design was performed using both NSR and GVRD waters. The factors and settings were: ozone residual (0.2 and 0.8 mg/L); temperature (5 and 22°C) and contact time (2 and 5 min). In addition, a 2^2 factorial design at 5°C was performed using GVRD water with factors and settings of: ozone residual (0.4 and 0.8 mg/L) and contact time (4 and 10 min).

4.5.4 Comparison of *G. muris* and *G. lamblia*

Four *G. lamblia* trials were performed: a control trial with a contact time of 5 min and ozonated trials with residuals of 0.2, 0.5 and 0.8 mg/L, and contact times of 2 and 5 min. A semiquantitative determination of *G. lamblia* inactivation was made. These results were compared qualitatively with *G. muris* inactivation results from other experimental trials made under similar ozonation conditions.

4.6 ANALYTICAL

The statistical application Data Desk Professional® v.3.0 (Odesta Corporation, Northbrook, Ill.) was used to perform least squares regression and routine statistical analyses. Systat® v.5.1 (Systat, Inc., Evanston, Ill.) was used to perform nonlinear analyses.

4.6.1 Fluorogenic Staining

The relation used for determining inactivation (N/N_0) from FDA-EB data was:

$$\frac{N}{N_0} = \frac{\text{\% of stained cysts that are FDA-positive ozonated}}{\text{\% of stained cysts that are FDA-positive control}} \quad [4.5]$$

where: FDA-positive cysts are viable and have a green perimeter when viewed under u.v. light by using an epifluorescence microscope; and

EB-positive cysts are non-viable and fluoresce red by using epifluorescence microscopy.

4.6.2 Excystation

The equation used for determining percent excystation from *G. muris* excystation data was (Schaefer, 1990; USEPA, 1991):

$$\text{\% excystation} = \frac{ECW + PET}{ECW + PET + IC} \times 100 \quad [4.6]$$

where: ECW is the number of empty cyst walls;

PET is the number of partially excysted trophozoites; and

IC is the number of intact cysts.

Inactivation (N/N_0) was calculated from:

$$\frac{N}{N_0} = \frac{\text{\% excystation ozonated}}{\text{\% excystation control}} \quad [4.7]$$

4.6.3 C3H/HeN Mouse Model

The relation used for determining inactivation (N/N_0) from mouse model data was:

$$\frac{N}{N_0} = \frac{n \times I}{n_0 \times I_0} \qquad [4.8]$$

where: n is the number of positive mice in an ozonated trial;

I is the number of infectious cysts per positive mouse after ozonation, determined using the latent period (if the inoculum had 10^5, 10^4, 10^3, 10^2, or 10 infectious cysts, mice became positive on days 3, 4, 5, 6 or 8, respectively);

n_0 is the number of positive mice in the control; and

I_0 is the inoculum size in the control, determined by hemocytometer count.

In equation [4.8], the latent period definition is the time when all mice became positive. The term n was required because, on occasion, all mice in ozonated trials did not become positive. If all the mice in an ozonated trial did not become positive, the last day which showed an increase in the number of positive mice was used as the latent period. The term n_0 was required in equation [4.8] because an occasional mouse in the ozonated cohort died before day 8 and it was necessary to use the same number of animals in the control cohort to estimate the initial number of viable cysts. An example of this calculation is provided in Appendix B.

4.7 QUALITY ASSURANCE AND QUALITY CONTROL

4.7.1 Biological

The most important component of QA/QC in *Giardia* work deals with maintaining SPF colonies of mice and gerbils. It is well established that prior exposure of animals to either *G. muris* or *G. lamblia* can lead

to resistance to reinfection (Roberts-Thomson *et al.*, 1976b; Belosevic and Faubert, 1983b; Belosevic *et al.*, 1983; Lewis *et al.*, 1987). Thus it is imperative that all animals be maintained in a SPF facility before experimental use. Mice and gerbils were purchased from SPF facilities and were kept under SPF conditions (laminar flow isolator) upon arrival. Access to the laminar flow isolator was limited to two animal-care technicians. Animals entering the laboratory were treated for three consecutive days with Metronidazole (Flagyl®; Rhone-Poulenc) to ensure that they were free from all possible protozoan infections (Belosevic and Faubert, 1983a; 1983b; Belosevic *et al.*, 1983). Fecal examinations as well as small intestine examinations for trophozoites of animals chosen at random were performed one week after treatment.

Giardia determinations were performed using groups of five mice for each experimental trial including controls. Invariably C3H/HeN mouse control groups became positive on day 3 or day 4 depending on the size of the inoculum, indicating that *G. muris* cyst preparations were of high quality and the experimental protocol was satisfactory. QA/QC measures for *G. muris* demand-free work included:

1. the absorbance of a cyst suspension of 10^4 cysts/mL at 260 nm was never greater than 0.03/cm;
2. cysts were stored at 4°C for 18 h and were used within 48 h of preparation.

Only the absorbance QA/QC measure was applicable to *G. lamblia* demand-free work.

The underlying probability distribution of replicate hemocytometer cyst count type data is the Poisson distribution (Fisher *et al.*, 1922). The Fisher D^2 statistic and four replicate hemocytometer counts of the cyst density following the ozonation reaction were used to test the homogeneity of the cyst suspension. If the variation among replicates exceeded that from chance alone ($P \leq 0.05$) the data were discarded. An example of this test is provided in Appendix C. It was not necessary to discard any data due to this criterion.

A critical aspect of QA/QC was liaison between the environmental engineering laboratory where ozonation was done and the zoology laboratory where infectivity testing was performed. A written plan for each experiment was prepared in advance. Forms were developed to facilitate the recording of data and communication between laboratories.

4.7.2 Chemical

Reagents which came into contact with ozone were prepared using high-purity chemicals (AnalaR grade; BDH). The quality of reagents had an extremely important effect on ozone decomposition.

Aqueous residual ozone was determined using u.v. absorbance. The indigo trisulfonate method (APHA *et al.*, 1989) was periodically used as a check. The two methods agreed within 5 percent, however the indigo method was consistently higher due to the different molar absorption coefficient (2,950 M^{-1} cm^{-1}) used to initially calibrate the procedure (Bader and Hoigné, 1981). Typical results are given in Appendix D.

4.7.3 Instrumentation

Although iodometric methods for ozone determination may proceed through a variety of pathways resulting in non-constant stoichiometry, they are useful as an independent check of gas phase u.v. monitors (Gordon *et al.*, 1989). The gas phase u.v. monitor was checked on a monthly basis using an iodometric method recommended by the manufacturer (Birdsall *et al.*, 1952). Spectrophotometers were checked monthly with solutions of known absorbances in the visible and u.v. wavelength regions. Other instruments and equipment were maintained and checked in accordance with manufacturers' instructions.

5. RESULTS AND DISCUSSION

5.1 PRELIMINARY RESULTS

5.1.1 Ozone Decomposition

Decomposition kinetics of ozone in the laboratory waters were determined using the u.v.-visible diode-array spectrophotometer and the bench-scale disinfection procedure without cyst addition. Trials were carried out in a darkened room and reactors were mixed at 72 r.p.m. using a Teflon®-coated stir bar and a magnetic stirrer (220T; Fischer Scientific). Experimental conditions for Data Set A trials were 22°C and pH 6.9. Conditions for Data Set B trials were as noted in Appendix E.

Data Set A was developed to study the combinations of ozone reactor, applied ozone dose and contact time that would provide ozone residuals in the range necessary to achieve 3 log inactivation of *Giardia*. A replicated complete factorial design was used to examine 3 factors in 24 trials (Table E.1, Appendix E). The factors and settings used were: size of ozone reactor (50, 250 and 2,000 mL); applied ozone dose (0.5 and 1.0 mg/L); and type of laboratory water (ozone demand-free phosphate buffer and ozone demand-free deionized water). The ozone half-lives that could be achieved in the 250 mL Erlynmeyer reactor were 15–20 min for phosphate buffer and 30–60 min for deionized water. A 15–20 min ozone half-life is significant in terms of the contact times which would normally be considered in engineering applications for disinfection (10 min or less). An advantage of the 250 mL Erlynmeyer reactor compared with the 2 L vessel is that six to eight trials can be done with the same quantity of cysts. A disadvantage of the 250 mL Erlynmeyer reactor is potential volatilization, however, with a test liquid volume of 200 mL, the surface area exposed to the atmosphere is minimal. An ANOVA using mean half-lives from Data Set A is given in Table E.2 in Appendix E. The significant factors were type of laboratory water and size of ozone reactor ($P \leq 0.05$). Interestingly, ozone decomposition was independent of the applied ozone dose, which indicated that ozone decomposition

generally appeared to be a first-order reaction. This conclusion was verified by plotting decomposition values as log C/C_0 versus time and as $[(1/C) - (1/C_0)]$ versus time. The first-order plot (log C/C_0 vs time) was linear in 21 trials while the second-order plot appeared linear in 3 trials.

Data Set B was used to predict the applied ozone doses necessary to obtain the desired residuals in the specific ozone residual experiment. Data Set B was a 2^4 factorial designed to assess 4 factors in 16 runs. The factors and settings used were: applied ozone dose (1.5 and 3.0 mg/L); temperature (5 and 22°C); turbidity (0 and 5 NTU); and pH (5.7 and 7.6). This was similar to the screening experiment design. Work was done at the same time for quality control. Figures 5.1–5.4 show the behavior of ozone in the phosphate buffer and 250 mL Erlynmeyer reactor (Data Set B, Appendix E). In Figures 5.1–5.4 ozone decomposition appears independent of the applied ozone dose and the linearity of the log C/C_0 versus time plots also indicates first-order decomposition. Decomposition results at each pH value were repeatable. The regression equation for the first-order decomposition coefficient was:

$$k = [\, 6.54 + 1.80(pH) + 0.34(Temp) - 0.31(pH)(Temp) \,]\, 10^{-4} \qquad [5.1]$$

where: (pH) assumes a value of -1 for pH 5.7 and +1 for pH 7.6;

(Temp) assumes a value of -1 for 5°C and +1 for 22°C; and

k is the first-order decomposition coefficient in s^{-1}.

C_0 was calculated using k from equation [5.1] and the relation:

$$C_0 = C_r / 10^{-kt} \qquad [5.2]$$

where: t is the contact time in seconds;

C_r is the ozone residual in mg/L; and

C_0 is the applied ozone dose in mg/L.

Tables E.3 and E.4 in Appendix E summarize the regression and indicate the significance of coefficients in equation [5.1]. Turbidity did not significantly affect ozone decomposition in Data Set B.

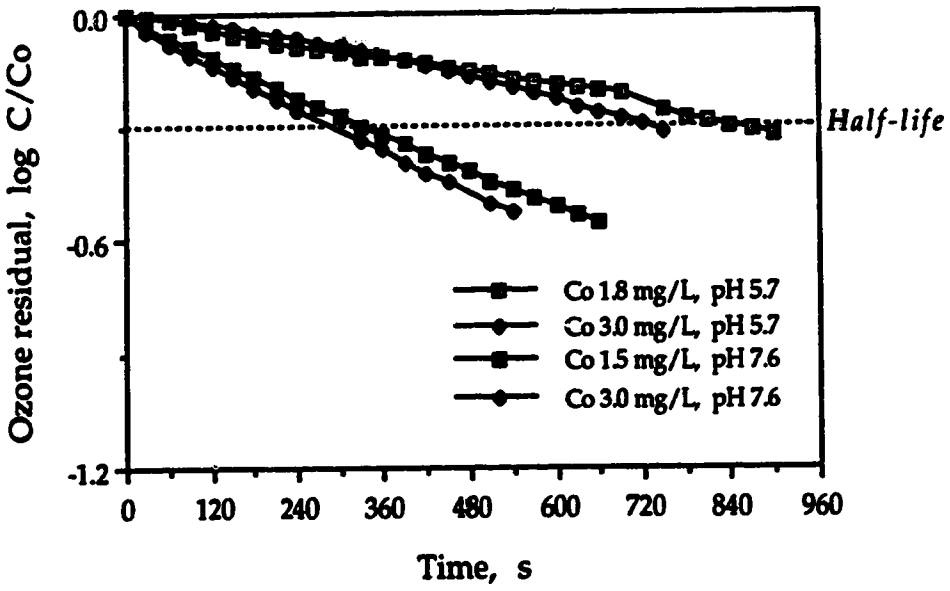

Figure 5.1 Ozone decomposition in 0.05 M phosphate buffer and 250 mL Erlynmeyer reactor (5°C, 0 NTU)

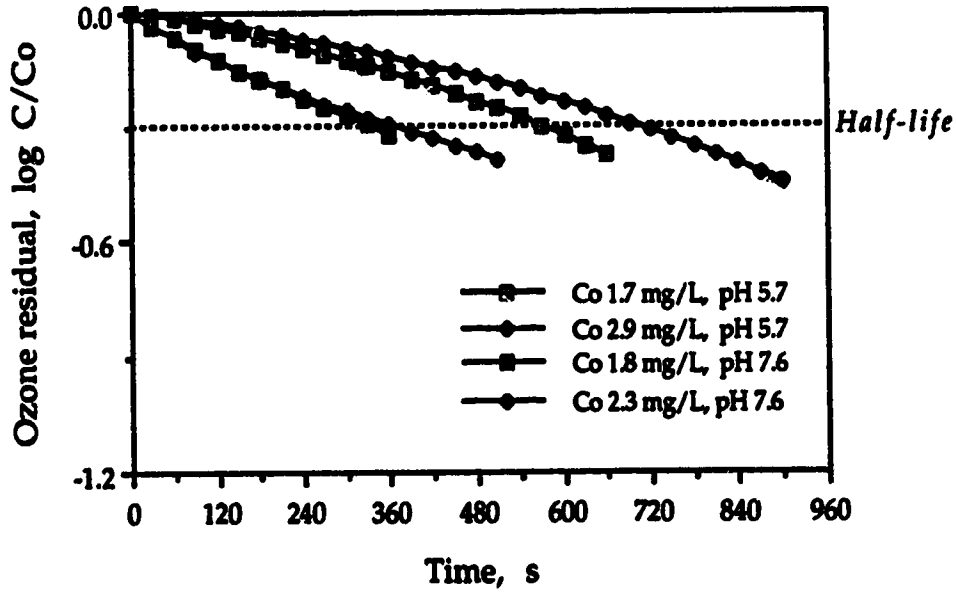

Figure 5.2 Ozone decomposition in 0.05 M phosphate buffer and 250 mL Erlynmeyer reactor (22°C, 0 NTU)

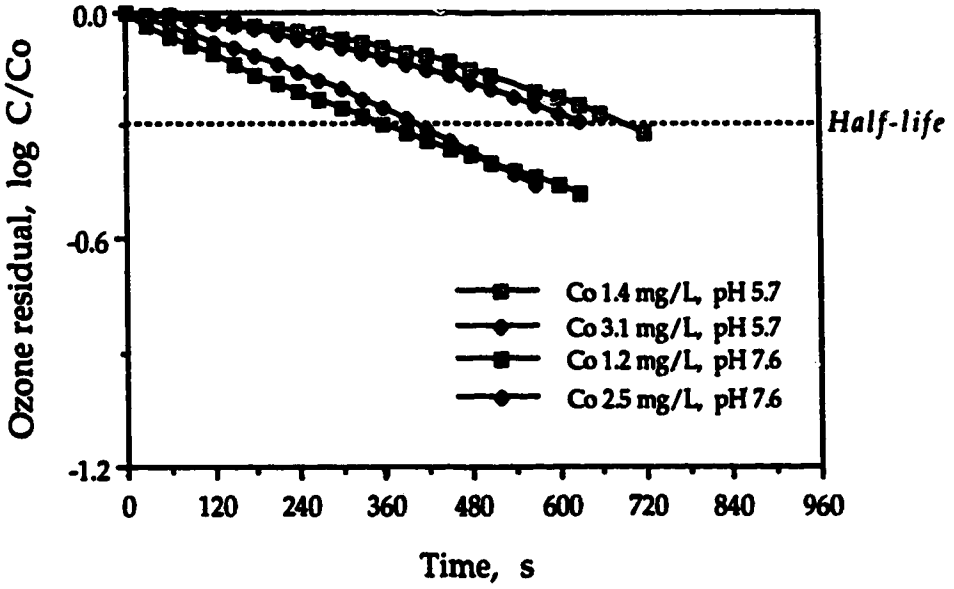

Figure 5.3 Ozone decomposition in 0.05 M phosphate buffer and 250 mL Erlynmeyer reactor (5°C, 5 NTU)

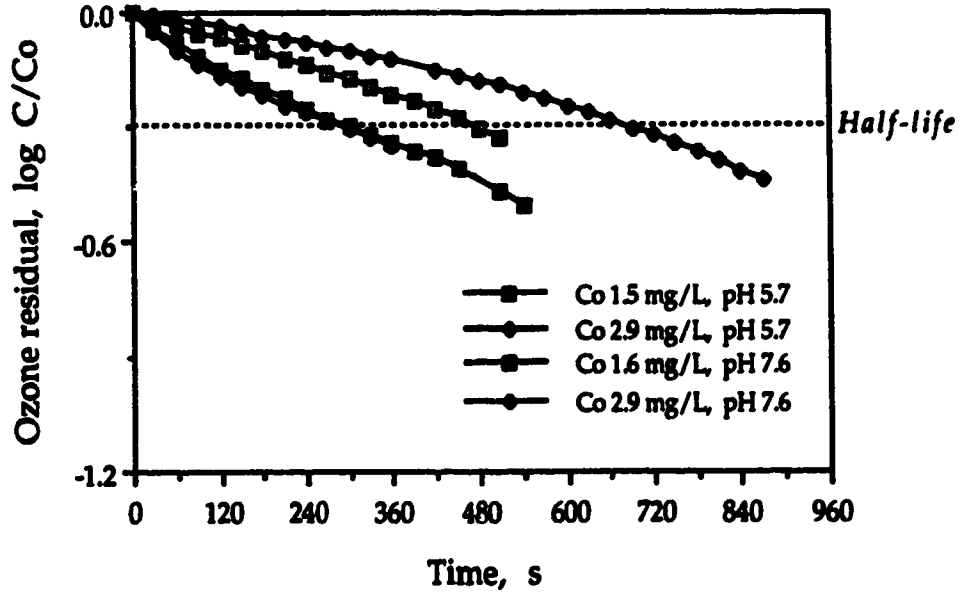

Figure 5.4 Ozone decomposition in 0.05 M phosphate buffer and 250 mL Erlynmeyer reactor (22°C, 5 NTU)

5.1.2 Verification of Mouse Model

The mouse model was originally described by Iwanczuk (1968) and Roberts-Thomson *et al.* (1976a) and the correlation between oral dosage and course of infection was further defined by Belosevic and Faubert (1983a). The results reported in 1983 were for CD-1 Swiss mice; therefore, it was necessary to verify the results for male C3H/HeN mice. Data verifying the effect of different cyst doses on the latent period in SPF male C3H/HeN mice are presented in Table 4.2 (p. 80). Further replications of the effect of different doses of cysts on the time until all mice in a group released cysts in the feces are found in the control trial animal responses in Appendices G and H. Refer to trial C1 in Table H.2 for the 10^3 cyst dose; control trials in Tables H.1 and H.2 for the 10^4 cyst dose (11 replications); and control trials in Table G.3, H.1 and H.3 for the 10^5 cyst dose (10 replications). In each of these control trials, all mice in the group were positive on day 3 for the 10^5 cyst dose; day 4 for the 10^4 cyst dose; and day 5 for the 10^3 cyst dose. Not only were all control mice groups positive, but groups invariably were positive with a latent period that depended on the inoculum size.

An additional experiment was designed to replicate animal responses to 10^2, 10 and 1 cyst doses. All mice in a group of 9 became positive on day 6 for the 10^2 cyst dose. Five mice in a group of 5 became positive on day 8 for the 10 cyst dose. These latent periods agree with those presented in Table 4.2. However, none of the mice in the 1 cyst dose group were positive for cysts on day 10 postinfection, nor were trophozoites detected on day 12. This result was not obtained previously. Consequently the C3H/HeN mouse-*G. muris* model was not used in the range of 10 or less cysts. Latent periods of 3 days for a 5×10^4 cyst dose and 5 days for a 10^3 cyst dose were reported by Schupp and Erlandsen (1987b) for non-Swiss CF-1 mice and are similar to those reported here for C3H/HeN mice (Table 4.2).

Equation [4.8] (p. 92) is an empirical relation based on observed experimental results rather than a theoretical dose-response relation. In equation [4.8], the latent period is used to assess infectivity. The effect of

different doses of *G. muris* cysts on the latent period is shown in Figure 5.5. Although a quantal (infected or not infected) response was observed in mice exposed to ozone treated cysts, the classical ID_{50} technique (Klaassen *et al.*, 1986) was not appropriate for assessing infectivity since as few as one *G. muris* cyst was an infectious dose to 9 of a group of 12 inbred male C3H/HeN mice. Advantages of the latent period approach include the need for just a single cohort of mice and the avoidance of an assumption regarding the probability of infection as in most probable number based methods (Haas and Heller, 1990).

Tween 20 increased cyst recovery in control trials from 6.2 ± 1.6 percent to 69 ± 2.8 percent (mean ± SD). Consequently, the inoculum size was large and it was necessary to collect data on day 3 to properly use the C3H/HeN mouse model. Tween 20 was used in bench- and pilot-scale experiments as noted in Sections 4.4.1 and 4.4.2. Tween 20 also increased recovery in the ozonated trials, however interpretation was complicated by variable experimental conditions and ozonation levels as well as the suspected lysing of cysts. For a variety of reasons including cyst clumping, declining ozone residual during the experiment, possible nonuniformity in the action of ozone, or simply high inactivation, not all mice in an ozonated trial group may become infected. When this occurs, it can be concluded that more cyst inactivation was obtained than if all mice in the group had been infected. The relation for determining N/N_0 from infectivity data [equation 4.8] was formulated on this basis.

In-depth analysis of sources of variance in the mouse model was beyond the scope of this work, however, a number of observations can be made. Sampling was not a major source of error in the mouse model since typically 60 percent of the initial cyst population was being assessed. However, because mice were only checked daily, the C3H/HeN mouse model must be considered a discontinuous rather than a continuous function. Many inactivations were separated by a whole number (i.e. 1.2, 2.2 etc.). This was because a 1, 2 or 3 day lag in the latent period of the ozonated trial relative to the control was indicative of 1, 2 or 3 log inactivation. The impact on the accuracy of the mouse model is a potential overestimation of inactivation to approximately 3 log-units.

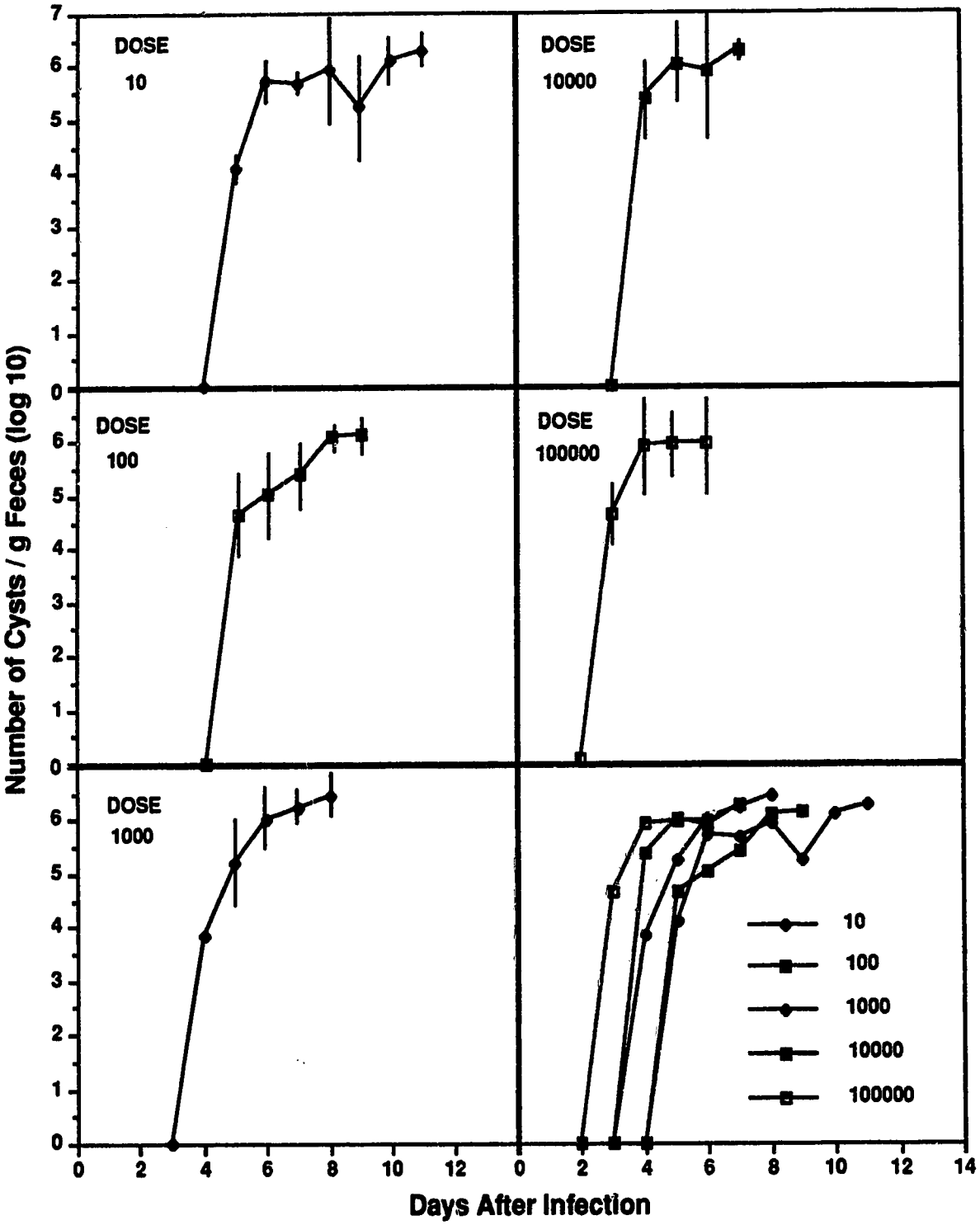

Figure 5.5 Pattern of cyst release in C3H/HeN mice infected with different doses of *Giardia muris* cysts. Each point represents the mean number of cysts released for 10 animals ± SD.

5.2 COMPARISON OF VIABILITY METHODS

5.2.1 Objective

The objective of this experiment was to compare *G. muris* cyst inactivation in laboratory water at bench-scale following ozonation using three viability methods on split samples. The methods used were a FDA-EB staining technique, the C3H/HeN mouse-*G. muris* model, and *in vitro* excystation.

5.2.2 General Discussion

Experimental conditions and inactivation results for the three viability methods are summarized in Table 5.1. Details on experimental conditions, hemocytometer count data and animal responses are presented in Appendices F and G (Tables F.1, G.1, G.2 and G.3).

The final centrifugate in controls contained approximately 1.4×10^6 *G. muris* cysts in 2.1 mL. Mean recovery of control cysts was 68 ± 9 percent (mean ± SD). Cyst losses totalled approximately 30 percent with the greatest loss (15 percent) accounted for by pumping during the reaction. Excystation values in controls were 96.3, 99.1, 95.8 and 93.0 percent for preparations A, B, C and D, respectively, as indicated in Figure 5.6. The mean control excystation was 96.1 ± 2.5 percent, indicating that cyst preparations were of high quality. Following fluorogenic staining three populations of cysts were observed: green (FDA-positive), red (EB-positive), and black (unstained). It was easier to observe black cysts under bright-field than with epifluorescence microscopy. The fraction of control cysts that were nonviable or EB-positive ranged from 9 to 12 percent (Figure 5.6). Forty to 60 percent of *G. muris* cysts in controls were unstained. However this fraction decreased to 6.9 ± 5.5 percent following ozonation, with a range of 1.9 to 16.1 percent. Figure 5.7 summarizes the effect of ozone on fluorogenic staining results.

Table 5.1 Experimental conditions and bench-scale ozone inactivations estimated by three viability methods for *G. muris* in ozone demand-free phosphate buffer (pH 6.7; 22°C).

Trial	Contact time (min)	Residual ozone (mg/L)	Inactivation (log N/N_O)†		
			FDA-EB staining	*In vitro* excystation	C3H/HeN mouse model
A1	5	0.3	>2.1	>2.8	4.2
A2	5	0.4	>2.2	>2.7	4.2
B1	5	0.1	2.3	3.0	3.1
B2	5	0.1	2.7	>3.0	3.1
C1	5	1.3	>2.8	>2.9	4.4
C2	0.58	0.04	0.2	0.5	1.2
C3	0.25	0.2	0.006	0.7	ND
C4	0.33	0.1	0.004	0.2	ND
D1	0.58	0.2	0.5	1.8	2.2
D2	1	0.1	0.7	2.1	ND
D3	2	0.1	2.4	2.0	ND
D4	1.53	0.02	0.5	2.4	ND

† >, detection limit;
FDA, fluorescein diacetate;
EB, ethidium bromide;
ND, not determined.

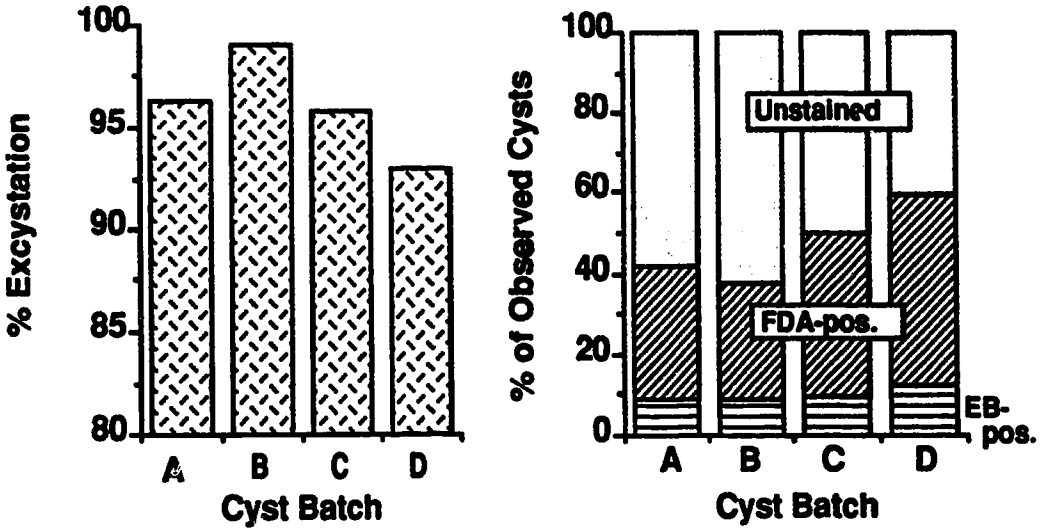

Figure 5.6 Excystation and fluorogenic staining in control *G. muris* cysts in the viability comparison experiment

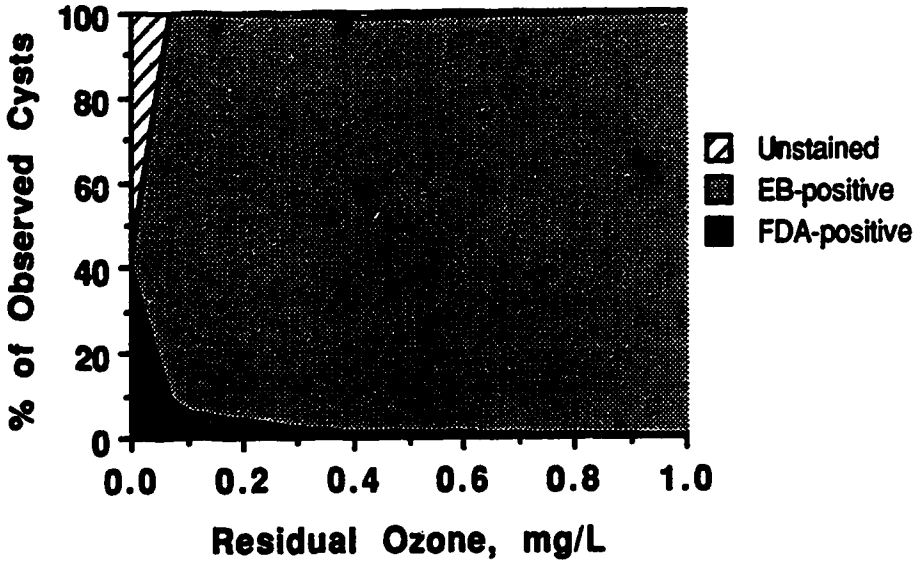

Figure 5.7 Effect of ozone on *G. muris* cyst staining (22°C, pH 6.9, 300 s contact time)

Trials A1, A2 and C1 had ozone residuals of 0.3 mg/L or higher. Although the entire slide area was examined in the excystation method for these trials, it was only possible to count 669, 546 and 782 cysts, respectively, with all cysts being intact cysts. The corresponding counts in the fluorogenic dye method were 143, 169 and 675, with all cysts being either EB-positive or unstained. These trials were at the detection limit since there was neither positive excystation, nor were FDA-positive cysts observed. None of the mouse model results were at the detection limit since at least one mouse became positive in each of the trials.

The three viability methods were compared using one-way ANOVA. Results are summarized in Table 5.2. In the analysis, trials A1, A2 and C1 were excluded because they were beyond the detection limit of all methods except infectivity. Fluorogenic staining was used as a reference method because the difference between all of the other methods could be calculated. The ANOVA was performed by using differences from the reference method. The underlying assumptions of ANOVA were tested and found to be satisfied. The Duncan multiple range test was used to determine if there was a significant difference between the viability methods (Duncan, 1955). Fluorogenic staining significantly underestimated inactivations determined by using infectivity ($P \leq 0.05$). This would suggest that not all FDA-positive cysts were capable of producing an infection. In contrast, the difference between infectivity and excystation, and between fluorogenic staining and excystation was not statistically significant ($P \leq 0.05$).

Table 5.1 indicates that fluorogenic staining gave the highest viabilities, or smallest inactivations, and infectivity provided the lowest viabilities. Excystation provided results intermediate to the other two methods. There was a greater difference between fluorogenic staining and the other methods at lower inactivations than at higher ones, as can be seen by comparing those trials which had an ozone residual of 0.1 to 0.2 mg/L. However, the animal model is a measure of the potential of the cysts to complete their life cycle in the host. In contrast, fluorogenic staining measures the presence or absence of metabolic activity. It is possible that cysts which show some metabolic potential

Table 5.2 One-way ANOVA for three viability determination methods performed on split-samples following ozone inactivation of *G. muris*.

Source	Sum of squares	Degrees of freedom	Mean square	*F*-ratio	Probability
Between viability methods	1.97	2	0.986	6.005	0.022
Error	1.48	9	0.164		
Total	3.45	11			

will not cause an infection in the animal host, thereby causing an apparent overestimation of viability.

A qualitative comparison of the three viability methods in terms of basis of the method, equipment, time required, and theoretical and observed detection limits following ozonation is provided in Table 5.3. Observed detection limits did not always equal their theoretical values and infectivity required considerably more time than the other methods. The time given in Table 5.3 for the mouse model was that required for a test trial latent period of up to five days greater than that of the control. To demonstrate 3 log inactivation, it would only be necessary to check control mice on day 3 and test mice on day 6. In the numerical comparison of inactivation estimates following ozonation, it should be remembered that the basis of each viability method, as indicated in Table 5.3, is different. The fact that two *in vitro* methods are being compared with one *in vivo* method adds an additional dimension of uncertainty.

Observed fluorogenic staining and *in vitro* excystation detection limits were 2.1 to 3.0 log inactivation depending on the number of cysts counted or found. In three of the twelve excystation trials, there were less than 1,000 cysts available for counting. FDA-EB inactivation estimates were based on stained cysts only, and the number counted ranged from 140 to 966 in the 12 trials. The theoretical detection limit for both of these methods is 3.0 log inactivation based on the microscopic examination of 1,000 cysts. The theoretical detection limit for the C3H/HeN mouse-*G. muris* model is based on the size of the inoculum in the control (10^5 cysts) and is 5 log inactivation. The observed mouse model detection limit was greater than 4.4 log inactivation.

The relation for determining N/N_0 from infectivity data [equation 4.8] was formulated to provide higher inactivations when fewer mice became positive. An example is trials C1 and A1, with 4.4 and 4.2 log inactivation, respectively. In trial C1 there was only one positive mouse on day 8 and the ozone residual was 1.3 mg/L. In trial A1 there were four positive mice on day 8 and the ozone residual was 0.3 mg/L. Trial C1 had one-fourth the number of positive mice compared

Table 5.3 Generic comparison of three viability methods in terms of basis of method, equipment and time required, and theoretical and observed detection limits following ozonation.

Viability method	Basis of method	Detection limit (log N/N_0)		Time required	Specialized equipment
		Theoretical	Observed		
FDA-EB	Biochemical metabolism or exclusion	3.0	2.1 to 2.8	1 h	Epifluorescent microscope
In vitro excystation	Chemical induction	3.0	2.7 to 3.0	3 h	Phase contrast microscope
C3H/HeN mouse model	Measure of infectivity	5.0	> 4.4	8 d	Animal care facilities

FDA, fluorescein diacetate;
EB, ethidium bromide.

with trial A1 at an ozone residual that was about four times as high.

5.2.2.1 Fluorogenic staining

A potential problem with fluorogenic dyes is that they are sometimes affected by the particular disinfectant used (Sauch *et al.*, 1991). This did not appear to be the case with ozone and FDA-EB. Unstained cysts were not used in calculating inactivation because their numbers decreased markedly following ozonation, and assumptions would have been required regarding unstained cyst viability. Schupp and Erlandsen (1987b) found that unstained *G. muris* cysts were infective to mice in a study that did not involve disinfectants. Other researchers found that 21 percent of *G. lamblia* cysts were unstained with FDA-EB after 30 min of exposure to 1 mg/L of chlorine (Hale *et al.*, 1985). Unstained cysts decrease the efficiency of fluorogenic staining procedures in determining inactivation because 1,000 stained cysts rather than 1,000 total cysts must be counted to determine 99.9 percent inactivation.

5.2.2.2 Excystation

In vitro excystation is based on chemical induction. Care must be taken to determine whether the excysted trophozoites are alive or dead in order to avoid counting aborted attempts at trophozoite emergence (Schaefer, 1990; Schupp and Erlandsen, 1987b). Results from excystation replicates B1 and B2 agreed even though one of the values was at the detection limit. In trials A1, A2 and C1, which had ozone residuals of 0.3 mg/L or higher, it was not possible to find 1,000 cysts using the *in vitro* excystation procedure. It was suspected that one of the modes of action of ozone was to lyse cysts, because all factors except ozone residual were the same as in other trials where 1,000 cysts were found.

5.2.2.3 Infectivity

Animal infectivity provided a direct measure of the ability of cysts to cause infection. Infectivity provided information on groups of cysts rather than on individual cysts, and consequently detected high inactivations. No other viability method was capable of detecting

inactivations with ozone residuals of 0.3 mg/L or higher in the demand-free phosphate buffer. C3H/HeN mouse model replicate trials B1 and B2 both had an inactivation of 3.1 log-unit. Using the error variance found in Table 5.2 and the appropriate t-statistic, the 95 percent confidence limit for the mean inactivation in trials B1 and B2 was:

$$\bar{x} \pm \frac{2.26\sqrt{0.164}}{\sqrt{2}} \text{ or } \bar{x} \pm 0.65 \log$$

Alternative infectivity methods to that used in this work include the ID_{50} technique and the most probable number method. Although ID_{50} has superior statistical properties, it was not used because the latent period approach avoided the expense of a cohort of mice at each dilution level, as required with the ID_{50} and most probable number methods.

5.2.2.4 Comparison with other studies

DeRegnier *et al.* (1989) noted that all *Giardia* cysts capable of producing infection would show viability by fluorogenic dyes or excystation, but not *vice versa*. Inspection of Table 5.1 (p. 103) reveals that generally infectivity indicated the highest inactivations, followed by *in vitro* excystation, and inactivation estimates from fluorogenic staining were the lowest. Smith and Smith (1989), working with *G. lamblia* reported that FDA staining consistently overestimated cyst viability compared with *in vitro* excystation. This result is in agreement with the results reported here for *G. muris* where lower FDA-EB inactivations were indicated in Table 5.1 except for one trial where excystation was lower. It is also consistent with the results of Ongerth *et al.* (1989), who reported that fluorogenic staining indicated a slightly higher proportion of viable *G. muris* cysts than did *in vitro* excystation after heat inactivation.

Based on very limited replication, the 95 percent confidence limit was ± 0.65 log-units for *G. muris* inactivations determined by the mouse model. This confidence limit is also applicable to the two other viability methods because it was calculated using a pooled error variance. For comparison, the 95 percent confidence limit for *E. coli* in ozone demand-

free solutions has been reported as ± 0.5 log-unit in the 3 to 7 log inactivation range (Finch *et al.*, 1988). It is expected that sampling may be a major source of error in the fluorogenic staining and *in vitro* excystation methods. An estimate of sampling variance can be made using sampling theory (Cochran, 1977). The sample size n was nominally 10^3 for FDA-EB and excystation. The population size N was 6.9×10^4 for FDA-EB and 3.5×10^5 for excystation in trial B1. The 95 percent confidence limit due to sampling is ± 0.3 log for trial B1 as estimated by FDA-EB and ± 0.6 log for trial B1 as estimated by excystation.

Previous studies comparing excystation and infectivity following chemical disinfection are rare. Wallis *et al.* (1990) reported $Ct_{99.9}$ values for *G. muris* cyst ozonation of 1.6 mg·min/L as estimated by excystation and 1.1 mg·min/L as estimated by animal infectivity. Animal results were interpolated from graphs at points where a mixed infection of five animals occurred. Another study showed little difference between infectivity and excystation up to 99.9 percent inactivation when using *G. muris* cysts and an ID_{50} chlorination technique (Hoff *et al.*, 1985). The results of this work by using a course of infection based animal model and ozonation support the hypothesis that *in vitro* excystation and infectivity provide similar results when using *G. muris* cysts. However, in the present work, only animal infectivity had the sensitivity to detect inactivations greater than 99.9 percent. Because of its high sensitivity, animal infectivity was used exclusively in further experimental work in this research.

5.3 COMPARISON OF OZONE AND OZONE–HYDROGEN PEROXIDE

5.3.1 Objective

The objective of this experiment was to distinguish between the disinfection effects of the ozone molecule alone and that of ozone decomposition products when inactivating *Giardia* cysts. The bench-scale disinfection procedure was used in conjunction with the C3H/HeN mouse-*G. muris* model.

5.3.2 Discussion

Figures 5.8 and 5.9 show ozone concentration versus time for the three trials using each laboratory buffer system. Figure 5.9 clearly indicates the beneficial effects of bicarbonate in quenching the ozone decomposition reaction. Inactivation results are presented in Figure 5.10. Further details on experimental conditions and animal responses can be found in Appendices F (Table F.3, exp. 31 data) and H (Table H.1).

The mean *G. muris* cyst inactivation in the phosphate-bicarbonate buffer system was 3.9 ± 0.9 log inactivation (mean ± SD) compared with 2.3 ± 0.2 log inactivation in the hydrogen peroxide system. The phosphate-bicarbonate buffer system, which had little decomposition of ozone over the duration of the experiment (mean residual ozone of 1.5 ± 0.1 mg/L at 2 min which was 92 ± 2 percent of the applied ozone dose), had significantly greater ($P \leq 0.05$) inactivation of *G. muris* cysts than that observed in the phosphate buffer hydrogen peroxide system where the ozone was completely decomposed in less than 60 s.

The hydroxyl radical is extremely reactive and very short-lived in aqueous solutions, so it is most likely to react with molecules of dissolved species, which have greater surface area to volume ratios than the larger microorganisms. By inference, the results reported here indicated that the direct action of the ozone molecule appeared to be the most significant factor for inactivation of *G. muris*. The 2.3 log inactivation in the peroxide system can be attributed to the average ozone residual (0.75 mg/L) that was present over the 60 s it took the ozone to decompose. This amount of ozone is sufficient to inactivate at least 99 percent of the *G. muris* cysts in 60 s and is 150 times greater than the ozone residual required to inactivate 99.9 percent of *E. coli* in 60 s in ozone demand-free solutions (Finch *et al.*, 1988).

Researchers working with *B. cereus* and *G. muris* concluded that ozone–hydrogen peroxide was applicable for disinfection but was less efficient than ozone alone, particularly at higher hydrogen peroxide to ozone weight ratios (Duget *et al.*, 1989; Wolfe *et al.*, 1989b). Observations

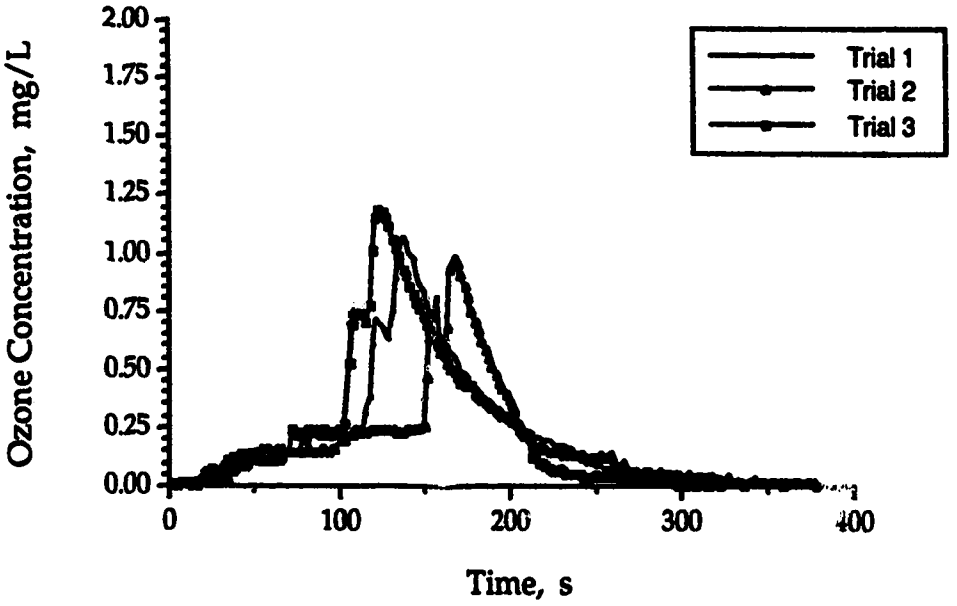

Figure 5.8 Ozone decomposition in 0.05 M phosphate buffer with a 10 to 1 hydrogen peroxide to ozone weight ratio.

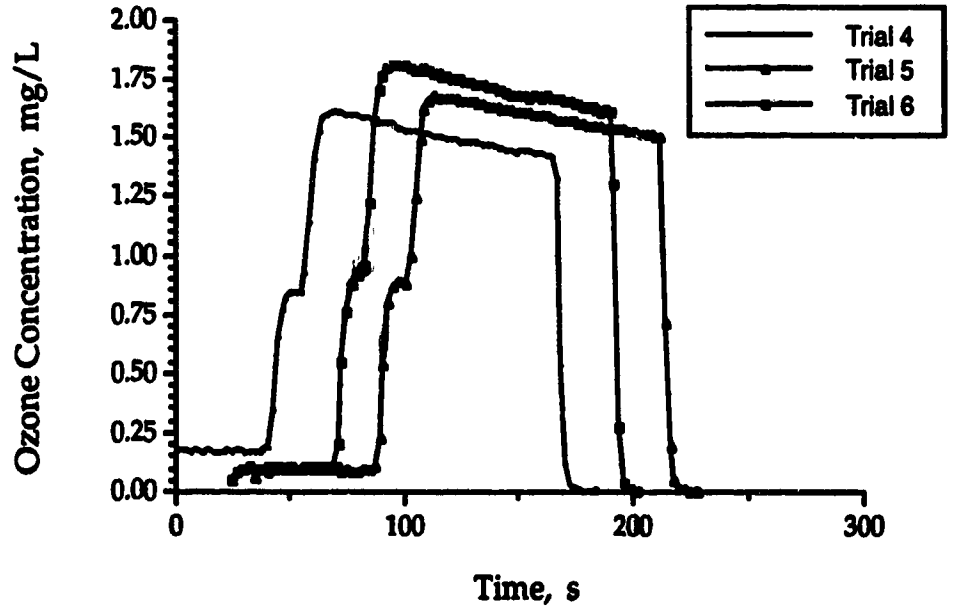

Figure 5.9 Ozone decomposition in 0.05 M phosphate-0.01 M carbonate buffer.

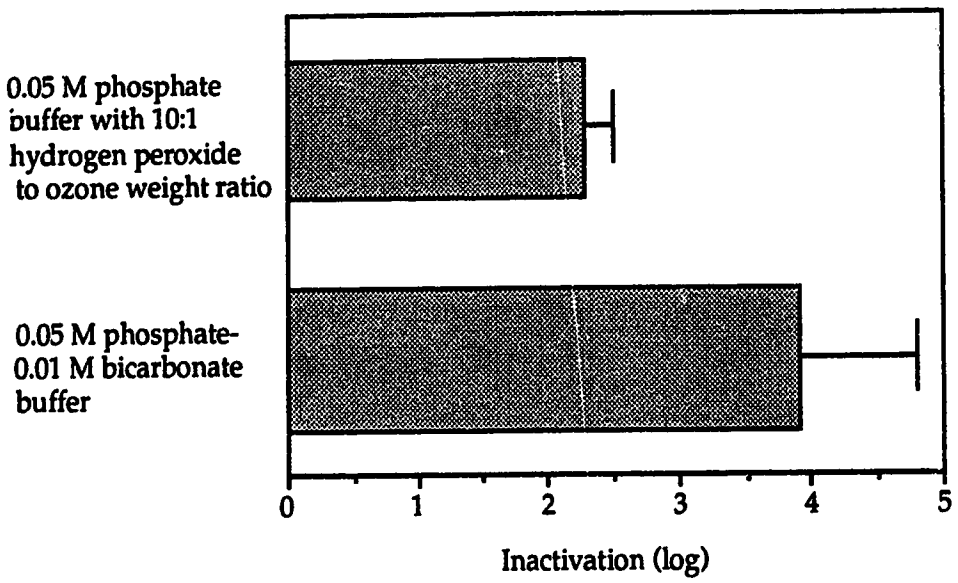

Figure 5.10 Mean *G. muris* cyst inactivation (mean of three trials plus one standard deviation) in two different laboratory buffer systems

made with *E. coli* and two buffer systems similar to those reported here could be explained either in terms of ozone decomposition products or the ozone molecule; however, inactivation continued in the presence of an ozone residual and not in the phosphate buffer hydrogen peroxide system (Yuen, 1990; Finch *et al.*, 1992). These findings in conjunction with the work reported here suggest that ozone alone is a more effective disinfectant. The ozone–hydrogen peroxide process was not researched further because there did not appear to be a significant disinfection effect associated with the hydroxyl radical reaction pathway.

5.4 FACTORS AFFECTING OZONE INACTIVATION

5.4.1 Objective

The objective of this experiment was to systematically evaluate the significance of parameters including temperature, turbidity, pH, ozone concentration and contact time in the ozone inactivation of *G. muris*. The bench-scale disinfection procedure was used in conjunction with the C3H/HeN mouse-*G. muris* model. Phosphate buffer was used in the screening and specific ozone residual experiments while NSR and GVRD waters were used in the natural waters experiment.

5.4.2 General Discussion

Experimental conditions and inactivation results for the screening, specific ozone residual and natural waters experiments are summarized in Table 5.4, 5.5 and 5.6, respectively. Further details on experimental conditions can be found in Appendix F (Table F.2, exp. 30 data for screening; Table F.3, exps. 36 and 41 data for specific ozone residual; Table F.4, exp. 39 data for natural waters). Animal responses are presented in Appendix H (Tables H.2, H.3 and H.4).

5.4.2.1 Screening experiment

Inactivation results, calculated using equation [4.8] (p.92) are shown in the last column of Table 5.4(a). In 4 of the 20 test trials no mice

Table 5.4(a) Screening Experiment: Design and bench-scale ozone inactivation results for *G. muris* in ozone demand-free phosphate buffer.

		Factor					Inactivation, $\log \frac{N}{No}$
Block	Trial	A	B	C	D	E	
1	5	-	-	+	-	-	3.5
	8	+	+	+	-	-	3.2
	10	+	-	-	+	+	4.4
	11	-	+	-	+	+	4.0
	C1*	C	+	0	-	-	0.0
	C4	C	+	0	+	+	0.0
1R†	R5	-	-	+	-	-	3.5
	R8	+	+	+	-	-	3.1
	R10	+	-	-	+	+	3.1
	R11	-	+	-	+	+	>5.0
	RC1	C	+	0	-	-	0.0
	RC4	C	+	0	+	+	0.0
2	1	-	-	-	-	+	4.3
	4	+	+	-	-	+	3.4
	14	+	-	+	+	-	4.1
	15	-	+	+	+	-	2.5
	C6	C	+	0	+	-	0.0
	C7	C	+	0	-	+	0.0
3	6	+	-	+	-	+	3.8
	7	-	+	+	-	+	4.0
	9	-	-	-	+	-	3.7
	12	+	+	-	+	-	>4.3
	C2	C	+	0	+	-	0.0
	C3	C	+	0	-	+	0.0
4	2	+	-	-	-	-	>4.4
	3	-	+	-	-	-	2.4
	13	-	-	+	+	+	3.8
	16	+	+	+	+	+	>4.4
	C5	C	+	0	-	-	0.0
	C8	C	+	0	+	+	0.0

> Exceeded detection limit; ± Factor levels, refer to Table 5.4(b);

†1R - Repeat of Block 1

*C1 - Control 1

Table 5.4(b) Screening Experiment: Factors and Levels

Factors	Levels (-)	Levels (+)
(A) Ozone dose (mg/L)	1.5	3.0
(B) Temperature (°C)	5	22
(C) Contact time (min)	2	7
(D) Turbidity (NTU)	0	5
(E) pH	5.7	7.6

Table 5.5(a) Specific Ozone Residual Experiment: Design and bench-scale ozone inactivation results for *G. muris* in ozone demand-free phosphate buffer.

Trial	Residual Ozone	Utilized Ozone	Factor				Inactivation, $\log \frac{N}{No}$
	(mg/L)	(mg/L)	A	B	C27	C12	
1	0.35	0.24	-	-	0		2.1
2	0.35	0.24	+	-	0		>5.1
3	0.40	0.33	-	+	0		4.5
4	0.36	0.46	+	+	0		>5.1
5	0.37	0.29	0	0	0		3.6
R5*	0.37	0.34	0	0	0		3.6
6	0.30	0.32	+	0	0		4.2
R6*	0.40	0.24	+	0	0		4.2
7	0.29	1.25	+	+	+		4.0
8	0.42	0.35	0	0	+		3.5
9	0.38	0.09	-	+	-	+	3.8
10	0.41	0.07	0	0	-	+	3.1
11	0.41	0.12	+	-	-	+	3.2
12	0.39	0.03	-	-		-	3.1
13	0.40	0.03	-	+		-	3.1
14	0.20	0.08	+	0		-†	2.2

> Exceeded detection limit; ± Factor levels, refer to Table 5.5(b);
*R5, R6, repeat of trial 5 and 6
†Contact time was 0.5 min

Table 5.5(b) Specific Ozone Residual Experiment: Factors and Levels

Factors	Levels		
	(-)	(0)	(+)
(A) Temperature (°C)	5	14	22
(B) pH	5.7	6.7	7.6
(C27) Time (min)	2	5	7
(C12) Time (min)	1	1.5	2

Table 5.6(a) Natural Water Experiment: Design and bench-scale *G. muris* ozone inactivation results.

Water (Date)	Trial	Residual Ozone (mg/L)	Utilized Ozone (mg/L)	Factor A	Factor B	Factor C25	Factor C410	Inactivation, $\log \frac{N}{N_o}$
NSR	1	0.25	0.71	-	-	-		2.7
(14-01-91)	2	0.70	1.88	+	+	-		3.7
	3	0.83	1.87	+	-	+		>5.0
	4	0.24	2.79	-	+	+		2.0
GVRD	5	0.65	1.45	+	-	+		4.1
(22-02-91)	6	0.15	0.94	-	-	-		2.1
	7	0.35	2.27	-	+	+		>5.1
	8	0.83	1.54	+	+	-		3.1
GVRD	9	0.37	0.91	-	-		-	3.1
(13-03-91)	10	0.76	0.90	+	-		-	3.1
	11	0.77	1.28	+	-		+	3.0
	12	0.34	1.37	-	-		+	3.3

> Exceeded detection limit; ± Factor levels, refer to Table 5.6(b);
NSR = North Saskatchewan River water;
GVRD = Greater Vancouver Regional District water.

Table 5.6(b) Natural Water Experiment: Factors and Levels

Factors	Levels (-)	Levels (+)
(A) Ozone residual (mg/L)	0.30	0.80
(B) Temperature (°C)	5	22
(C25) Time (min)	2	5
(C410) Time (min)	4	10

were positive, and inactivation is shown as being greater than the detection limit. Significant factors and interactions affecting inactivation were determined by ANOVA (Table 5.7). The error variance in Table 5.7 was estimated from the smallest interactions with the aid of a half-normal probability plot (Daniel, 1959). The error variance was 0.0746 with 7 d.f. In Table 5.7 the CD and CE interactions were not significant, indicating there were no batch-to-batch differences in cyst preparations.

The ANOVA results indicated that main effects applied ozone dose, temperature and pH were significant ($P \leq 0.05$). The two-factor interactions AE (ozone dose x pH) and BE (temperature x pH) and the main effect pH were significant ($P \leq 0.01$). Contact time was not a significant factor over the range examined (2–7 min) nor was it involved in any interactions that influenced the inactivation of *Giardia* cysts.

Temperature and pH are known to be two of the more important factors influencing ozone decomposition (Gurol and Singer, 1982; Bablon *et al.*, 1991a). On the basis of the screening experiment it was not possible to tell if the significance of temperature and pH was due to effects on ozone residual, or due to a direct influence on the *G. muris* cyst itself.

5.4.2.2 Specific ozone residual experiment

Inactivation results for the specific ozone residual experiment are shown in the last column of Table 5.5(a). The significant factors and interactions were determined by ANOVA for contact times less than or equal to 2 min [Table 5.8(a)] and greater than 2 min [Table 5.8(b)]. A pooled estimate of error variance was obtained from the mean square of the insignificant interactions in Tables 5.8(a) and 5.8(b) as determined by regression analysis and half-normal probability plots. Contact time for periods less than or equal to 2 min and temperature at all contact times were significant ($P \leq 0.05$). However, for contact times greater than 2 min, pH and all interactions including pH were also significant ($P \leq 0.05$). Apparent Ct products were calculated for each combination of ozone residual and contact time for each set of temperatures, and were plotted in Figure 5.11. Figure 5.12 presents the *G. muris* inactivations in terms of

Table 5.7 Screening Experiment ANOVA

Factor	MS	MS/MS_d[†]
(A) O_3 Dose	0.423	5.66*
(B) Temperature	0.456	6.10*
(C) Time	0.141	1.88
(D) Turbidity	0.276	3.69
(E) pH	0.951	12.74**
AB	0.076	1.01
AC	0.031	0.41
AD	0.141	1.88
AE	1.626	21.78**
BC	0.010	0.13
BD	0.723	9.68*
BE	1.000	13.40**
CD	0.123	1.64
CE	0.160	2.14
DE	0.003	0.03

MS = mean square of the total effect

[†]MS_d = 0.0746 with 7 degrees of freedom

*$P \leq 0.05$; **$P \leq 0.01$.

Table 5.8(a) ANOVA for a contact time less than or equal to two minutes in the specific ozone residual experiment

Factor	MS	MS/MS_d†
(A) Temperature	0.743	10.7*
(B) pH	0.004	0.1
(C) Time12	0.605	8.7*

MS = mean square of the total effect
†MS_d = 0.0696 with 6 degrees of freedom. Based on pooled estimate of error variance in laboratory water specific ozone residual experiment (Table 5.5).
*$P \leq 0.05$.

Table 5.8(b) ANOVA for a contact time greater than two minutes in the specific ozone residual experiment

Factor	MS	MS/MS_d†
(A) Temperature	4.50	64.7**
(B) pH	2.88	41.4**
(C) Time27	0.32	4.6
AB	2.88	41.4**
AC	0.32	4.6
BC	0.50	7.2*
ABC	3.38	48.6**

MS = mean square of the total effect
†MS_d = 0.0696 with 6 degrees of freedom. Based on pooled estimate of error variance in laboratory water specific ozone residual experiment (Table 5.5).
*$P \leq 0.05$; **$P \leq 0.01$.

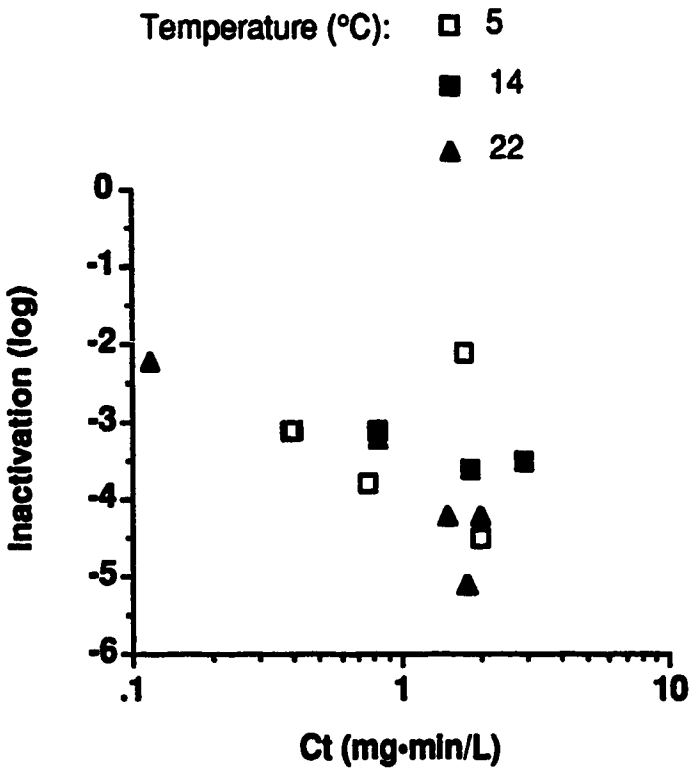

Figure 5.11 Effect of the apparent Ct on *G. muris* inactivation in specific ozone residual experiment.

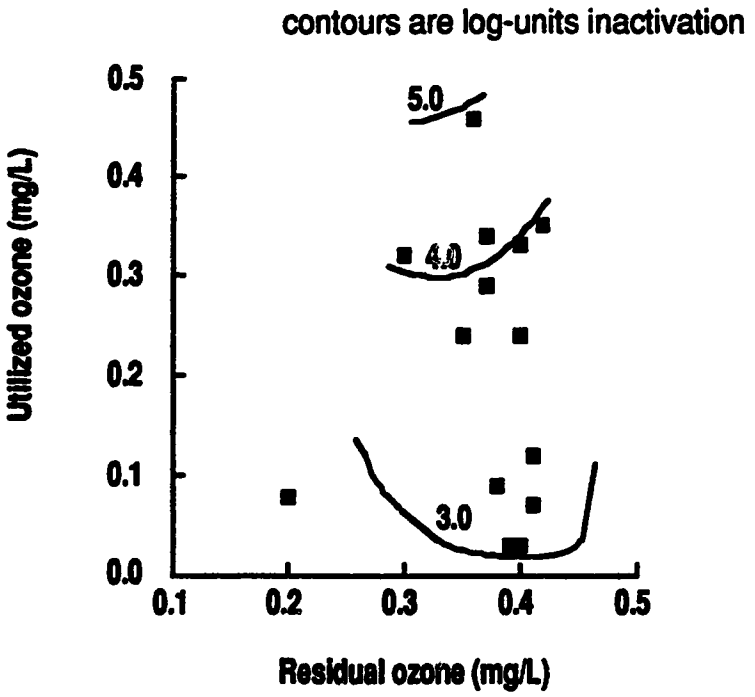

Figure 5.12 *Giardia muris* inactivation contours as a function of residual and utilized ozone in the specific ozone residual experiment.

utilized ozone and residual ozone (contours fitted quadratically). The effects of temperature and pH are accounted for by the utilized term which is directly affected by these factors. The sum of ozone residual and utilized ozone for each coordinate is the applied ozone dose. The inactivation data at about 0.4 mg/L of ozone residual in Figure 5.12 ranges from about 3 log inactivation for essentially zero ozone utilized (applied ozone equals residual ozone) to 5 log inactivation for utilized ozone of 0.45 mg/L (applied ozone is 0.45 + 0.40 or 0.85 mg/L). The higher inactivations occurred when the ozone utilized was more than ten times that for the lower inactivations.

5.4.2.3 Natural waters experiment

Inactivation results for the natural waters experiments are shown in the last column of Table 5.6(a). Inspection of Table 5.6(a) indicates that residual ozone was the most important factor and contact time the least important factor in the ozone inactivation of *G. muris* in NSR water. Unexpectedly, contact time between 2 and 5 min was found to be more significant than either temperature or residual ozone for *G. muris* inactivation in GVRD water, as can be seen from trials 5–8 in Table 5.6(a). However, contact time between 4 and 10 min was not significant, as indicated by the similar inactivations for trials 9–12 in Table 5.6(a). Apparent Ct products were calculated for each natural surface water for each set of temperatures and were plotted in Figure 5.13. Figure 5.14 presents the natural water inactivation response surface in terms of residual and utilized ozone (contours fitted quadratically).

5.4.3 Experimental Factors in Laboratory Water

In the screening and constant residual experiment it was observed that ozone rapidly inactivated *G. muris* and that contact time beyond 2 min resulted in little additional inactivation. The screening experiment demonstrated that applied ozone dose (and resulting residual) was significant ($P \leq 0.05$), with greater inactivation associated with the higher applied ozone dose. This is consistent with the ozone inactivation of other microorganisms where ozone concentration has been the most

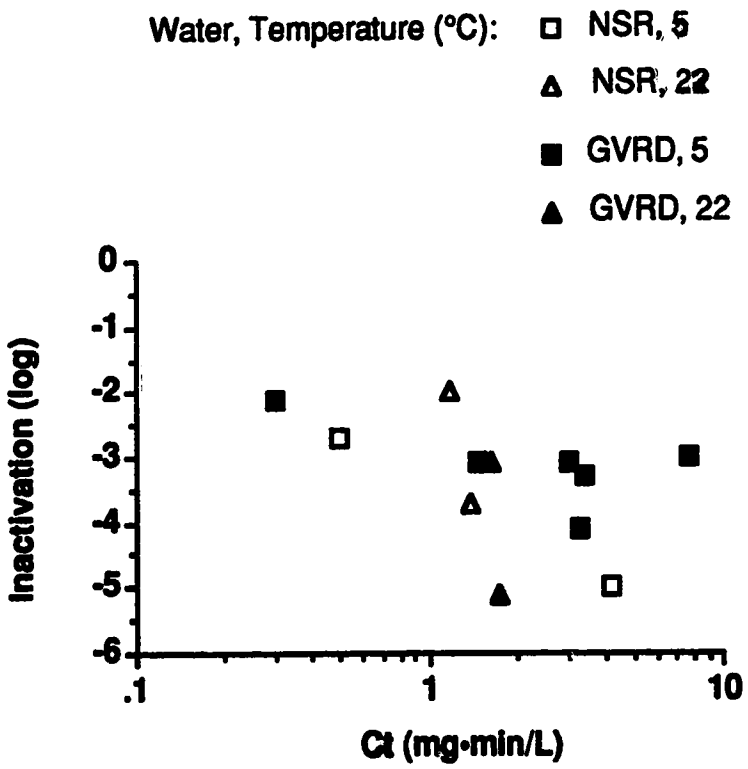

Figure 5.13 Effect of the apparent Ct on *G. muris* inactivation in North Saskatchewan River (NSR) water and Greater Vancouver Regional District (GVRD) water.

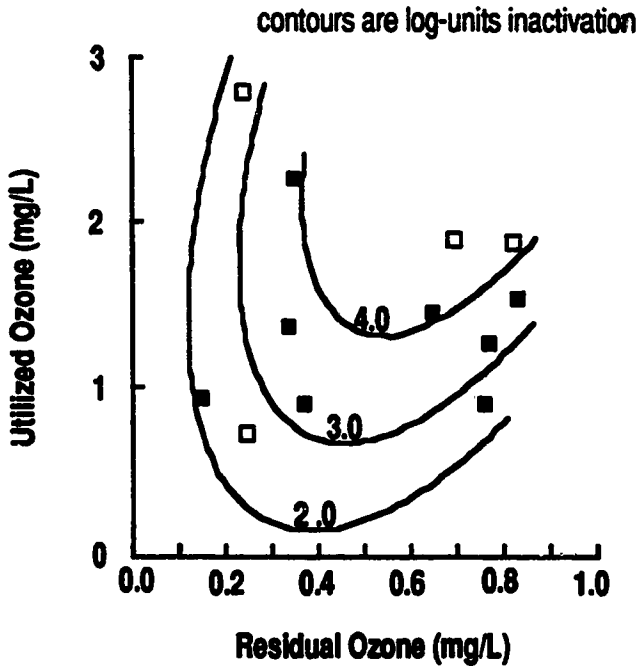

Figure 5.14 *Giardia muris* inactivation contours as a function of residual and utilized ozone for the natural waters experiment (open symbols, North Saskatchewan River water; solid symbols, Greater Vancouver Regional District water).

important design factor and ozone inactivation is relatively independent of contact time after an initial stage of about 60 s in clean water (Coin *et al.*, 1964; Farooq *et al.*, 1977; Finch *et al.*, 1988; Katzenelson *et al.*, 1974; Roy *et al.*, 1982).

pH was a significant factor ($P \leq 0.05$) in the screening experiment, which examined contact times from 2 to 7 min, as well as in the specific ozone residual experiment for contact times greater than 2 min. pH was not a significant factor in the specific ozone residual experiment for contact times less than or equal to 2 min. Other researchers have reported that pH had a minimal effect on ozone disinfection of water (Farooq *et al.*, 1977; Venosa, 1972).

Temperature was a significant factor ($P \leq 0.05$) in all experiments performed [Tables 5.7, 5.8(a) and 5.8(b), p. 122 and 123]. However, the sign of the total effect was not consistently positive or negative, indicating that the effect of increased temperature on *G. muris* cyst inactivation was adverse in some cases, and beneficial in others. One hypothesis which could account for the importance of temperature is that the *G. muris* organism itself is more susceptible to ozone inactivation at the higher temperatures. It is known that the viability of *G. muris* cysts is prolonged when the organism is stored at 5°C (deRegnier *et al.*, 1989; Wickramanayake *et al.*, 1985). Another factor is that chemical reaction rates double for each 10°C rise in temperature. Thus, at a temperature of 22°C, one could expect that the reaction of molecular ozone and *G. muris* cysts would be almost four times faster than at 5°C. Yet another factor is that cold temperatures increase ozone stability.

The turbidity caused by adding bentonite to the laboratory water did not have a significant effect on ozone inactivation; however, it was observed to have a beneficial effect on the physical removal of *Giardia* through microflocculation enhancement by the use of ozone. This phenomenon following the ozonation of turbid waters containing *Giardia* has been previously reported (Wallis *et al.*, 1990). Other researchers reported that turbidity adversely affected ozone inactivation of *E. coli*, human enteric viruses, porcine picornavirus and coliphage f_2

(Sproul *et al.*, 1979). However the sizes of these organisms are smaller than that of *Giardia*, and thus entrapment in suspended particles may not be as significant an effect with *Giardia* (Hoff, 1978).

Utilized ozone is computed as applied ozone less residual ozone, and combines the effects of contact time, temperature and pH. Parameters influencing ozone utilization in water include: pH, which influences ozone residual; temperature, which influences kinetic rate constants; the presence or absence of initiators or promotors, which influences ozone decomposition; and organic and inorganic impurities, which react selectively with ozone as defined by the competing reaction rate kinetics. The importance of utilized ozone in *G. muris* inactivation is demonstrated graphically in Figure 5.12 (p. 125) for the specific ozone residual data. In the screening experiment it was postulated that the significant interactions (ozone dose x pH and temperature x pH) also indicated the importance of utilized ozone. Trial 1 (the uppermost open square) in Figure 5.11 (p. 124) may be an outlier while trial 3 appears to be more appropriate relative to the other data. However, trial 1 was conducted at pH 5.7, and consequently had a utilized ozone value that was 0.73 times that of trial 3, which was conducted at pH 7.6.

In Figure 5.11 the variance in *G. muris* inactivation increases as the Ct increases. This may be due to the confounding of pH and temperature with time in achieving a target ozone residual of 0.4 mg/L at the end of the specified contact time. Longer contact times required more applied ozone at higher temperatures and pH than at lower temperatures and pH. Consequently, ozone doses fluctuated markedly accounting for the significant variation in the response of the *G. muris* cysts to ozone as Ct (or time) increased. The effects of pH are confused with time effects because pH also alters the rate of ozone decomposition. The Ct product in Figure 5.11 does not account for the decomposition of ozone, while the utilized ozone term in Figure 5.12 does.

5.4.4 Experimental Factors in Natural Waters

NSR water was hard and of high alkalinity and pH while GVRD water was extremely soft and low in alkalinity and pH (Table 4.1, p. 68).

There was a much higher level of organic matter, turbidity and absorbance in the GVRD water than in the NSR water. Thus parameters which affected ozone decomposition and consumption were significantly different for these two waters. Increased alkalinity inhibits the decomposition process though possibly to a limited extent given the relatively low concentrations found even in hard waters. The effect of pH was very apparent in these two waters and NSR water decomposed more readily than GVRD water. The higher organic carbon content of the GVRD water would be expected to result in more competing ozone reactions than in the NSR water. This is illustrated by the values of w (equation [2.33], p. 43) and Ω_M (Section 2.3.3) in these waters. The specific ozone utilization rate, w, is 11.1 h^{-1} for NSR water (13-02-91 sample), resulting in a 5 min ozone utilization of 0.93 mg/L. For GVRD water (22-02-91 sample) w is 18.2 h^{-1}, however the water quality parameters for GVRD water are outside the range for which the regression coefficients in equation [2.33] were determined. Ω_M can be estimated using a value of 0.25 mg/L per mg/L DOC, resulting in 0.4 mg/L for NSR water (13-02-91 sample) and 2.3 mg/L for GVRD water (22-02-91 sample). It is not surprising that higher ozone doses were required in the GVRD water to achieve the same inactivations as in the NSR water.

In all natural water trials except one, a higher value of utilized ozone resulted in greater inactivation. In NSR water (pH 8.3) utilized ozone correlated directly with residual ozone, and the inactivation values (mean ± SD) were 2.4 ± 0.5 log-units at an ozone residual of approximately 0.3 mg/L and 4.4 ± 0.9 log-units at the 0.8 mg/L residual. In GVRD water (pH 6.3) utilized ozone appeared to correlate with contact time rather than residual ozone.

Figure 5.14 indicated that *G. muris* inactivation in the natural waters was dependent on residual and utilized ozone as was the case in the laboratory waters. When residual ozone was less than or equal to 0.3 mg/L, N/N_0 was less than 3 log inactivation regardless of the value of utilized ozone. Figure 5.14 also indicated that for residual ozone values greater than 0.4 mg/L, more inactivation occurred in NSR water than in GVRD water. Several factors likely contributed to this: the

higher utilized ozone levels in the NSR water, and the greater organic levels and concomitant competing reactions in the GVRD water. Contact time beyond 2 min resulted in additional inactivation in GVRD water, but not in NSR water. This may also be an effect of the higher organic levels in GVRD water. It has been shown using bacteria and wastewater that the presence of suspended particles required substantially longer ozone contact times to achieve inactivations equivalent to those obtained when suspended matter was absent (Kirk *et al.*, 1972).

Figure 5.13 (p. 127) presents data indicating that it was more difficult to achieve 2 or 3 log inactivation of *G. muris* cysts in the natural waters at 22°C than at 5°C. Temperature has opposing influences on ozone stability and disinfection efficiency. It should be noted that 4 log inactivation of *G. muris* occurs more readily at 22°C than at 5°C. While it has been observed by other researchers that at higher temperatures the increased reaction rate effect exceeds that of ozone instability (Farooq *et al.*, 1977; Roy *et al.*, 1982), this was not the case for *G. muris* cyst inactivation in the natural waters used in this study, although it appeared to be so in the laboratory water. This can be explained by the increased contact time required for inactivation of *G. muris*, particularly in natural waters, compared with that required for other organisms which can often be inactivated in less than 60 s. It appears that the positive effect of colder temperatures in preserving ozone residual exceeds negative kinetic influences in the ozone inactivation of *G. muris* cysts.

5.5 COMPARISON OF *G. MURIS* AND *G. LAMBLIA*

5.5.1 Objective

The objective of this experiment was to compare *G. muris* and *G. lamblia* infectivity following ozonation using the C3H/HeN mouse-*G. muris* and Mongolian gerbil-*G. lamblia* models. A semi-quantitative determination of *G. lamblia* inactivation was made. The bench-scale disinfection procedure and phosphate buffer were used.

5.5.2 Discussion

Experimental conditions are summarized in Table 5.9(a) and inactivation results for *G. lamblia* and *G. muris* are presented in Table 5.9(b). Further details on experimental conditions and animal responses for the *G. lamblia* trials can be found in Appendix F (Table F.3, exp. 43 data). *G. muris* inactivation results from other experimental trials made under similar ozonation conditions were used for comparison. For example, trial 30-13 in Table 5.9(a) designates trial 13 of experiment 30. Details on these *G. muris* trials can also be found in Appendix F. From the data available it was not possible to choose three *G. muris* trials to match the *G. lamblia* trials precisely; however, applied ozone doses and apparent Ct values are reasonably close. All trials including control trials had sodium formate added to neutralize ozone. The amount used, 50 µL of 1 M solution, was not toxic to either *G. lamblia* or *G. muris* cysts. All animals in control trials were positive in both the gerbil and mouse models.

The semiquantitative *G. lamblia* cyst inactivation determination method is presented in Table 5.10. In Table 5.10, N was the total number of viable cysts in the experimental cohort, estimated using a literature value for the number of *G. lamblia* cysts that would cause infection to a given proportion of Mongolian gerbils (Figure 1 in Schaefer *et al.*, 1991). For consistency, the N_0 definition used for the mouse model was also used for the gerbil model. This was defining N_0 as the total inoculum in the control cohort, determined by four replicate hemocytometer counts.

G. muris cysts were typically collected on day 7 and cyst preparations were of high quality as shown in Figure 5.6 (p. 104). *G. lamblia* cysts were collected from day 7 to day 14 and cyst preparations required more effort in purification. Fluorogenic staining of the *G. lamblia* cyst preparation resulted in: 15 percent FDA-positive, 4 percent EB-positive and 81 percent unstained. It is not known if the unstained population is viable or nonviable. If the *G. lamblia* cyst preparation was just 50 percent viable, the difference in inactivation estimates would be only 0.3 log-units less than if the preparation was 100 percent viable.

Table 5.9(a) Experimental conditions for *G. muris* and *G. lamblia* trials in ozone demand-free phosphate buffer (pH 6.8; 22°C).

Trial	Contact Time (min)	Residual Ozone (mg/L)	Utilized Ozone (mg/L)	Applied Ozone (mg/L)	Apparent Ct (mg•min/L)
G. lamblia					
1	5	0.8	0.6	1.4	4.0
2	5	0.5	0.4	0.9	2.5
3	2	0.2	0.3	0.5	0.4
C	5	-	-	-	-
G. muris					
30-13	7	0.6	1.0	1.6	4.2
33-A2	5	0.4	0.2	0.6	2.0
41-6	2	0.4	0.1	0.5	0.8

C - control.

Table 5.9(b) Bench-scale ozone inactivations as estimated by the C3H/HeN mouse-*G. muris* and Mongolian gerbil-*G. lamblia* models.

Trial	Animals in Cohort	Positive Animals in Cohort	Inactivation, $\log \frac{N}{N_O}$
G. lamblia			
1	5	0 on day 14	≥3.8
2	5	0 on day 14	≥3.8
3	4	1 on day 14	3.0
C	5	5 on day 11	0.0
G. muris			
30-13	5	3 on day 8*	3.8
33-A2	5	4 on day 8	4.2
41-6	5	4 on day 6	3.2

*Three of 5 mice were positive for cysts on day 8; all 5 mice had trophozoites detected on day 8;
C - control.

Table 5.10 Semiquantitative determination of *G. lamblia* inactivation

Trial	Percent of Mongolian gerbils infected in experimental cohort			
	0%	25%	50%	75%
G. lamblia inoculum (cysts/gerbil)†	11	60	282	1,320
Viable cysts in experimental cohort, N				
1	≤55	-	-	-
2	≤55	-	-	-
3	-	240	-	-
Total number of cysts in control cohort, N_0				
C*	3.1×10^5	3.1×10^5	3.1×10^5	3.1×10^5
Inactivation, $\log \frac{N}{N_0}$				
1	≥3.8	-	-	-
2	≥3.8	-	-	-
3	-	3.0	-	-

† From Figure 1 of Schaeffer *et al.*, 1991;

* Value is 2.5×10^5 for trial 3 because there were only 4 gerbils.

The gerbil and mouse models are very different, as discussed in Sections 2.1.5, 4.3.7 and 4.3.8. The mouse model uses the latent period to provide a quantitative estimate of inactivation while the gerbil model provides a semiquantitative inactivation estimate. Therefore, this is not a strict mathematical comparison. On the basis of number of animals infected, trial 1 does not provide any additional information over trial 2. In trial 2, 0 out of 5 gerbils became infected, while 4 out of 5 mice became infected by day 8. On this basis, it appears that *G. lamblia* is less resistant to ozone than *G. muris*. However, it should be noted that the *G. muris* inocula were about twice as large as those for *G. lamblia*, mainly due to the greater difficulty in obtaining *G. lamblia* cysts. In trial 3 the contact time and ozone residual were decreased in an effort to have at least part of the gerbil cohort become positive. Under these conditions 1 out of 4 gerbils became infected whereas 4 out of 5 mice became infected on day 6. The *G. muris* inactivation in trial 3 was 3.2 log-units compared with a *G. lamblia* inactivation of 3.0. The result of trial 3 indicates that *G. lamblia* cysts may be of similar resistance to ozone as *G. muris* cysts at 22°C. Although the apparent Ct values in trial 3 are not the same, the applied ozone doses are. Additional observations and replicates to determine assay variability are required to make firm conclusions. A large amount of the uncertainty in *G. lamblia* inactivation estimates can be explained by the inherent variability of the biological assay and differences in host susceptibility. The *G. lamblia* ID_{50} as determined by probit analysis was reported to be 282 cysts; however, the 95 percent confidence interval for the ID_{50} estimate was 60 to 1,096 cysts (Schaeffer *et al.*, 1991).

In summary, the data reported here corroborates that of Wallis *et al.* (1990) who noted that there was little difference between *G. muris* and *G. lamblia* Ct values for three log-units inactivation under similar ozonation conditions for temperatures ranging from 6.7 to 9.0°C. In contrast Wickramanayake *et al.* (1985) reported *G. muris* to be 1.5 times more resistant to ozone than *G. lamblia* at 25°C on the basis of calculated Ct_{99} values. Further research is warranted.

5.6 PILOT-SCALE RESULTS

5.6.1 Objective

The objective of this experiment was to evaluate bench-scale results at pilot-scale using NSR water because:

1. Scale-up effects can seriously affect the performance of full-scale facilities, making bench-scale information questionable for use in design.
2. Kinetic models for full-scale design can be limited by the actual detention time in reactors. Larger-scale evaluation is especially important if bench-scale data are to be used for regulatory purposes.

The pilot-scale disinfection procedure was used with NSR water seeded with *G. muris* cysts. HPC bacteria indigenous to the test water were enumerated using R2A agar. Infectivity was determined using the C3H/HeN mouse-*G. muris* model.

5.6.2 Discussion

Experimental conditions and *G. muris* inactivation results are presented in Table 5.11. Further details on experimental conditions and animal responses for the trials can be found in Appendix F (Table F.4, exp. 39A and 39B for bench-scale; Table F.4, exp. 40 and 42 for pilot-scale). HPC bacteria inactivation results are given in Table F.5 in Appendix F.

Figure 5.15 shows *G. muris* and R2A HPC bacteria inactivation results at bench- and pilot-scales. Observations from Figure 5.15 include:

1. There is no apparent scale-up effect in the *G. muris* data. Most of the bench- and pilot-scale results agree within 0.5 log. The largest difference was between pilot-scale trials B and 2, performed on 14-11-90 and 31-01-91, respectively. The 2 log-unit lower *G. muris* inactivation in trial B can be attributed to its poorer water quality. The trial B sample had twice the turbidity and 4.3 times the TOC of the trial 2 sample.

Table 5.11 North Saskatchewan River Water: Pilot- and bench-scale *G. muris* ozone inactivation results.

Date	Trial	Scale	Residual Ozone (mg/L)	Utilized Ozone (mg/L)	Contact Time (min)	Inactivation, $\log \frac{N}{N_0}$
14-01-91	1	1	0.25	0.71	2	2.7
	2	1	0.70	1.88	2	3.7
	3	1	0.83	1.87	5	>5.0
	4	1	0.24	2.79	5	2.0
13-02-91	1	1	0.16	0.65	2	3.7
	2	1	0.67	1.63	5	4.6
	3	1	0.30	1.41	5	>5.2
	4	1	0.69	0.98	2	3.2
14-11-90	A	2	0.70	0.98	6.3	3.1
	B	2	0.38	0.51	3.2	1.6
31-01-91	1	2	0.21	1.26	4	2.7
	2	2	0.35	2.09	6	3.6
	3	2	0.80	2.87	10	>5.0
	4	2	1.23	3.72	13.5	>5.0

> Exceeded detection limit;
Scale - 1, bench-scale; 2, pilot-scale.

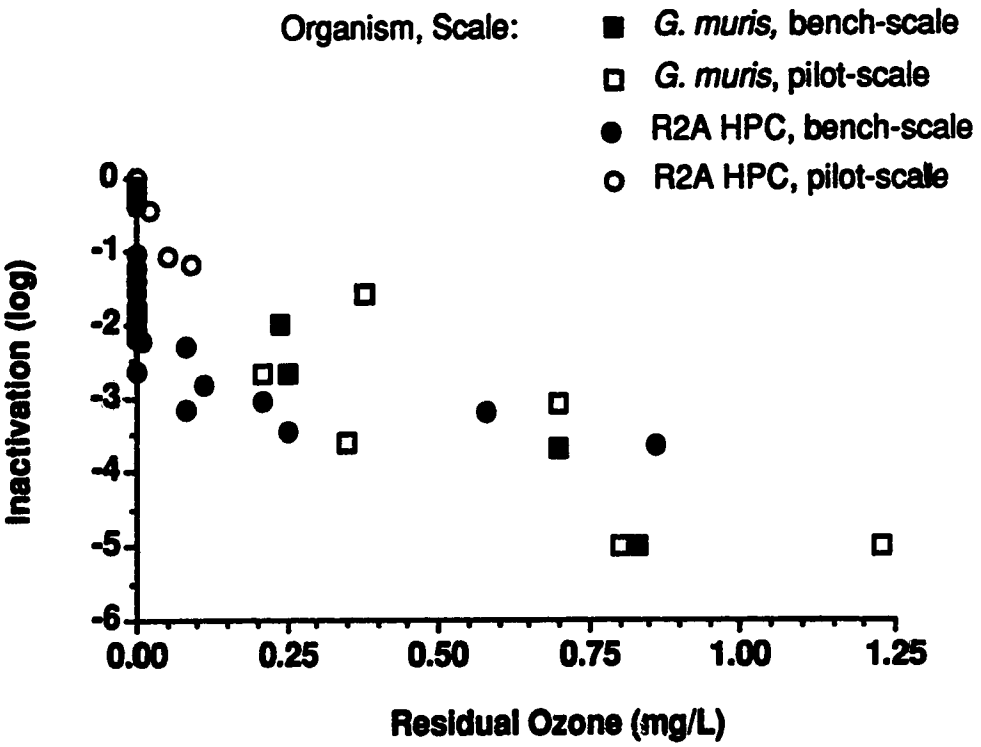

Figure 5.15 Inactivation of *G. muris* and heterotrophic plate count bacteria as a function of ozone residual at bench-scale and pilot-scale in North Saskatchewan River water.

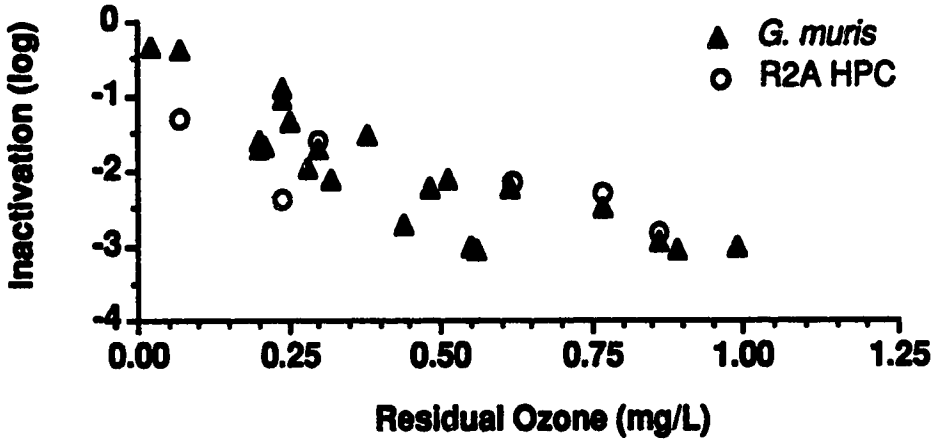

Figure 5.16 Inactivation of *G. muris* and heterotrophic plate count bacteria as a function of ozone residual at pilot-scale in Colorado River and State Project waters (R. Wolfe, Metropolitan Water District of Southern California, Los Angeles, Calif., 1990, Personal communication).

2. At first, it appears as though there may be a difference between the pilot- and bench-scale R2A HPC bacteria inactivation results. However, by interpreting the results in conjunction with the water quality characteristics of the samples (Table 4.1 and Table F.5, Appendix F), it can be seen that the pilot-scale sample was taken on 31-01-91 (pristine winter water quality) while the bench-scale data was sampled 22-04-91 (poor spring runoff water quality).

3. There is general agreement between the inactivation patterns of *G. muris* and R2A HPC bacteria.

4. Some of the variability in the inactivation results may be due to inactivation being functionally dependent on more parameters than just ozone residual.

The pilot-scale inactivation data shown in Figure 5.16 is for contact times of 3, 6 and 9 min and two surface waters (R. Wolfe, Metropolitan Water District of Southern California, Los Angeles, Calif., 1990, Personal communication). A number of additional observations can be made from Figures 5.15 and 5.16:

1. Agreement between *G. muris* and R2A HPC bacteria inactivation patterns is evident in Figures 5.16. Analysis by the MWDSC indicated that there was agreement between the inactivation of R2A HPC bacteria and *G. muris* up to 2.5 log-units (Scott *et al.*, 1992).

2. Three log-units of *G. muris* inactivation occurred at an ozone residual of 0.50 mg/L in Figure 5.15 (bench-scale data; pilot-scale trials A and B do not meet SWTR turbidity requirement), and approximately 0.80 mg/L in Figure 5.16. The biggest difference between the two studies was that infectivity was used in this study while the MWDSC used excystation. Another difference was that the current study used a Rushton turbine semi-batch, stirred-tank reactor for pilot-scale work while a countercurrent flow reactor was used by the MWDSC.

Pilot-scale trials were performed at 22°C. The four bench-scale trials at 1°C (exp. 39B data) were not included in Figure 5.15 because these results appeared highly variable and were the only trials performed at such a low temperature. A difference between the bench- and pilot-scale protocols was that ozone residual decreased at bench-scale but increased at pilot-scale. It should be noted that the dynamic forces which govern gas bubble formation in a reactor cannot be scaled. Therefore bubble size and bubble rise-rate will be the same at all scales. Because bench-scale reactors do not have the same mass-transfer characteristics as pilot- and full-scale reactors, bubbling ozone through a reactor at bench-scale will actually be a poorer simulation of full-scale performance than side-stream injection in a batch reactor (Finch and Smith, 1989).

5.7 ENGINEERING SIGNIFICANCE OF RESULTS

5.7.1 Process Design Protocol

Figure 5.17 uses quadratically fitted contours to show *G. muris* inactivation as a function of ozone residual and contact time for the viability comparison, specific ozone residual and natural water data sets. The response surface is not linear (i.e. it is not a family of straight lines) and cannot be adequately modelled by the Chick-Watson model. When ozone residual was less than 0.5 mg/L and contact time was less than 5 min, inactivation was less than 3 log. However, once the contact time was 5 min or more, and residuals were at least 0.5 mg/L, more than 3 log of *G. muris* cyst inactivation was consistently obtained. This "envelope" would yield an apparent Ct of 2.5 mg min/L, and is largely defined by the natural waters in the combined data set.

The U.S. disinfection regulations are given in terms of Ct and temperature (USEPA, 1991). The Ct system assumes that the pseudo first-order rate constant, k, follows the van't Hoff–Arrhenius relationship and that the disinfectant concentration is constant during the course of an experiment. The data reported here does not appear to support such a simple kinetic model largely due to the complexity of competing ozone

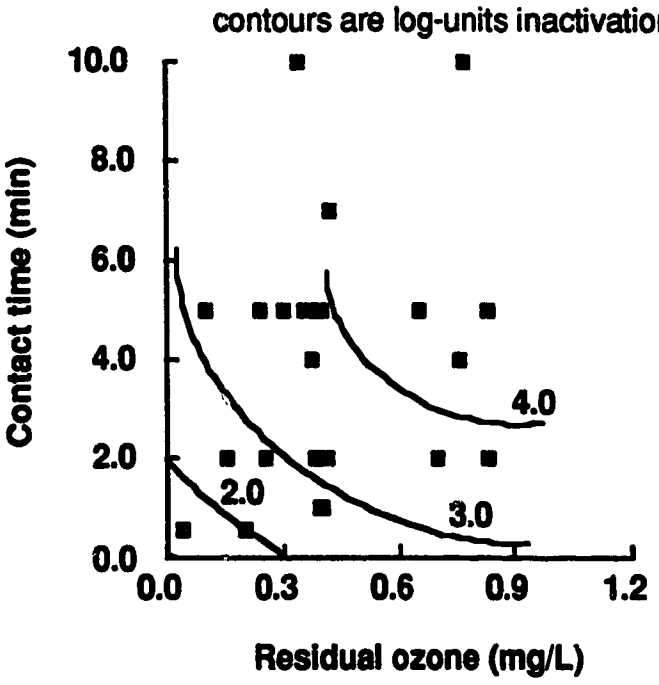

Figure 5.17 *Giardia muris* inactivation contours as a function of contact time and residual ozone in specific ozone residual, viability comparison and natural waters experiments.

reactions in natural water. Furthermore, the extrapolation of design criteria based on the Chick-Watson model beyond observed experimental conditions can result in gross errors (Hoff, 1987). The U.S. National Research Council (1980) originally adopted the Ct concept for comparing biocidal efficiency in their review of the disinfection literature; however, they cautioned that comparisons be made at the 99 percent inactivation level to minimize problems associated with initial lags and "tailing off". Other researchers have proposed alternate statistical techniques because Ct does not adequately describe nonidealities or the complete mathematics of kinetic expressions (Haas, 1987; Severin and Churn, 1987).

Even though the Chick-Watson model was found to be inadequate, kinetic data from selected natural water and laboratory water observations were used to produce the Watson plots shown in Figures 5.18 and 5.19 for comparative purposes only. Data for all temperatures (5–22°C) were pooled due to the limited number of observations available. The coefficient of dilution, n, is shown on the plots. In the Chick-Watson model n is an empirical factor, and n being greater than one suggests that residual ozone concentration may be more important than contact time, while n being less than one suggests that contact time may be more important. The n values were less than one in all cases except for 3 log inactivation in laboratory water, suggesting that contact time was more important than residual ozone concentration. The results of the screening experiment indicated that the opposite was true. The reason for the apparent contradiction is the assumption that the kinetics were pseudo first-order in nature. Wickramanayake *et al.* (1984b) reported n values of 1.1–1.2 for 3 log ozone inactivation of *G. muris* cysts in laboratory water.

Ct values from Figures 5.18 and 5.19 along with regulatory values for comparison (USEPA, 1991) are summarized in Table 5.12. Although the regulatory values contain a safety factor of two, they were just adequate for 3 log inactivation of *G. muris* cysts at 5°C for the natural waters used in this study, and inadequate for 3 log inactivation at 22°C.

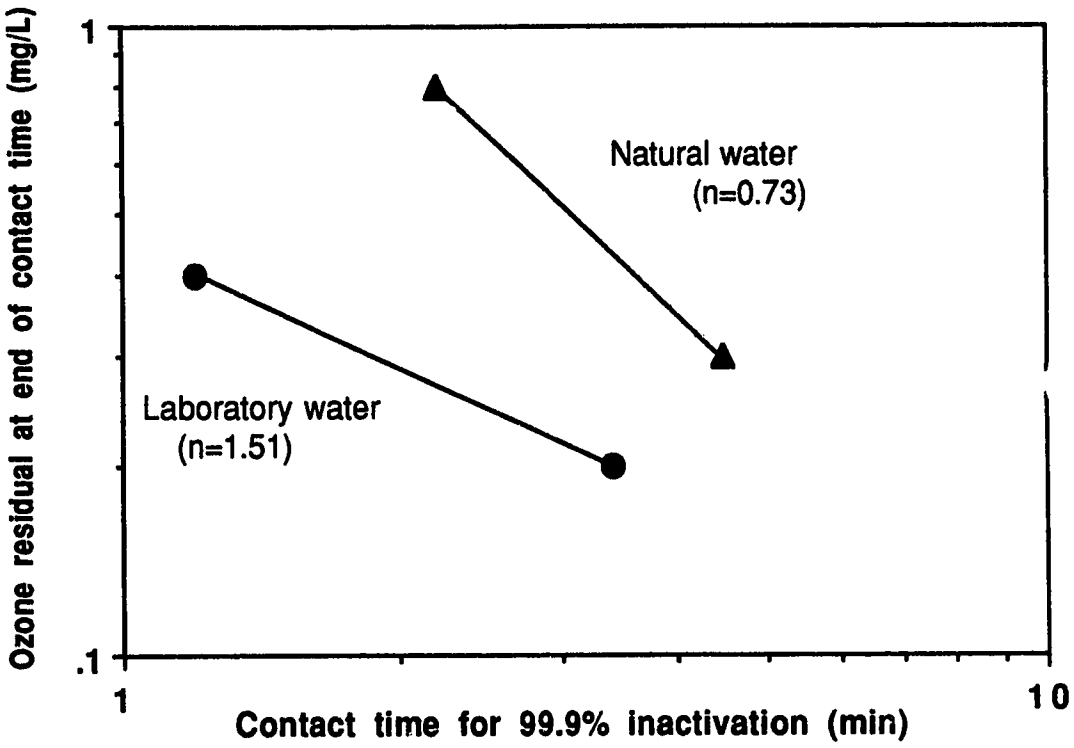

Figure 5.18 Watson plot of selected data from laboratory and natural water experiments for 99.9 percent inactivation of *G. muris* cysts.

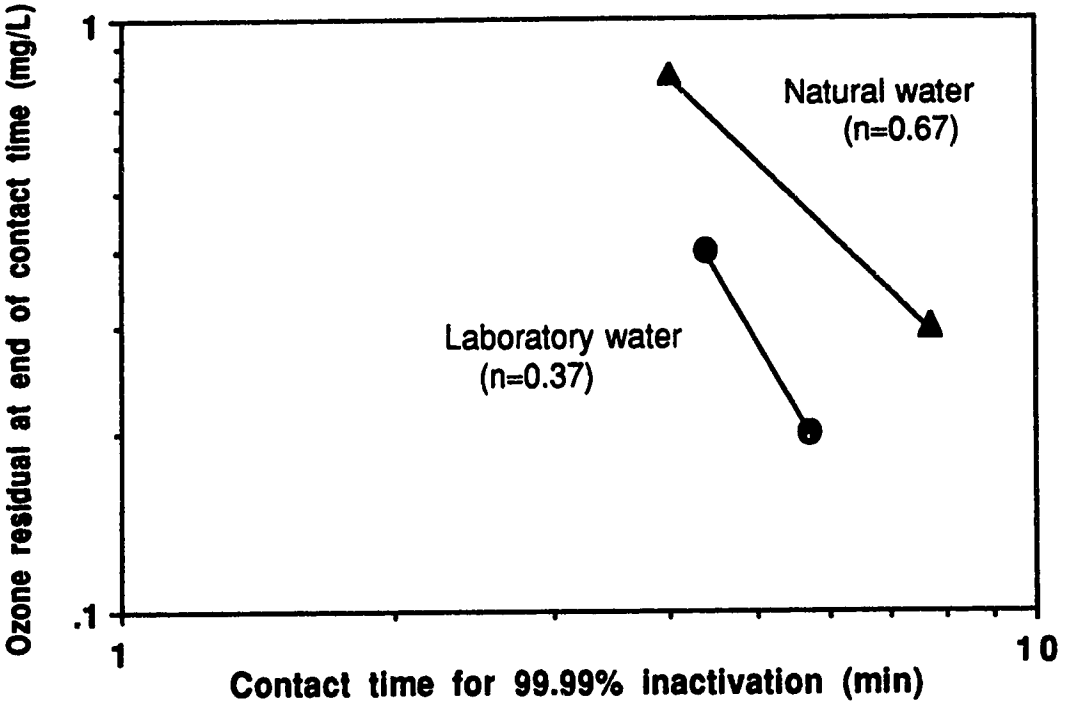

Figure 5.19 Watson plot of selected data from laboratory and natural water experiments for 99.99 percent inactivation of *G. muris* cysts.

Table 5.12 Experimental Ct values for inactivation of *G. muris* cysts by ozone (pH 5.7 to 8.3) and regulatory Ct values for *G. lamblia* cyst inactivation by ozone (pH 6 to 9)

Water type	Inactivation $\log \frac{N}{No}$	Estimated Ct values			
		This study†	EPA* 5°C	This study†	EPA* 22°C
Laboratory	3	0.30	1.9	0.30	0.62
	4	3.1	2.6††	3.1	1.22††
Natural	3	1.9	1.9	1.9	0.62
	4	3.4	2.6††	3.4	1.22††

*Table O-2, *Guidance Manual* (USEPA, 1991)

†This study pooled temperature data

††Extrapolation from Table O-2 (USEPA, 1991)

difficult to achieve 3 log inactivation of *G. muris* cysts in the natural waters at 22°C than at 5°C (Figure 5.13, p. 127). Further extrapolation of the recommended Ct values to 4 log inactivation results in theoretical Ct values which are 1.2–2.8 times too low when compared with the values determined here. Table 5.12 also indicates that it is approximately six times more difficult to achieve 3 log inactivation of *G. muris* cysts in the natural waters used in this study than in the laboratory water. This result supports the observation that carefully prepared microorganisms in demand-free buffers may underestimate the chemical dose necessary to achieve disinfection in natural waters (Berg, 1987).

It is suggested that the Ct concept may be inappropriate for regulating the design of ozone disinfection systems for unfiltered water supplies. An alternative empirical approach would be to use the 3 log contour "envelope" in Figure 5.17 (p. 142) to make predesign decisions concerning primary ozone disinfection systems. Figure 5.20 is a three dimensional portrayal of the data in Figure 5.17. Trials which had ozone residuals greater than or equal to 0.5 mg/L and contact times of at least 5 min had greater than 3 log-units of *G. muris* cyst inactivation (shaded area). A response surface was not fitted in Figure 5.20 to let the "data speak for itself". However, in the region $0.5 \leq C_T \leq 1.0$ mg/L and $t \leq 5$ min there is a plateau in the data. This is illustrative of some of the difficulties with kinetic modelling of ozone disinfection. The plateau would have a tailing appearance in a two dimensional plot of inactivation versus ozone residual.

The results of this work indicate that increased contact time cannot compensate for inadequacies in ozone dose in the ozone inactivation of *Giardia*. Contact time beyond 2 min was found to be unimportant in the laboratory water and also in the NSR water. In the GVRD water, which had twice the turbidity and nine times the DOC as the NSR water, contact time beyond 5 min was unimportant. Competing reactions caused by the greater amount of organic matter in the GVRD water could account for the greater contact time necessary to achieve *G. muris* inactivation in that water. However, contact times greater than

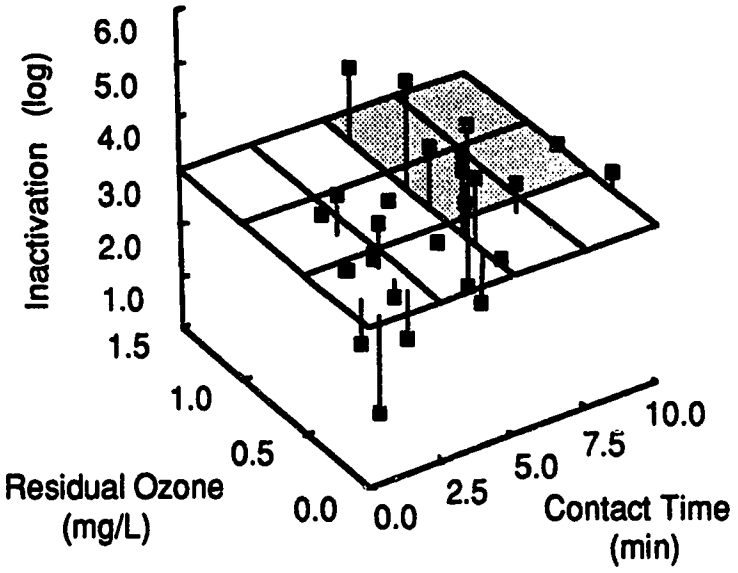

Figure 5.20 *Giardia muris* inactivation relative to 3 log-units as a function of contact time and residual ozone in specific ozone residual, viability comparison and natural waters experiments. Shaded area exceeds the empirical process design protocol.

in waters meeting the SWTR turbidity and coliform requirements. The understanding of interactions between temperature, pH, ozone dose and ozone decomposition kinetics is essential for the successful design of an ozone system for inactivation of *Giardia* cysts.

5.7.2 Ozone Inactivation Modelling and Analysis

Modelling is an iterative, circular process (Box *et al.*, 1978). The primary objective of this section was to consolidate previous results and use modelling as a tool to gain further insight into the relative importance of residual and utilized ozone when inactivating *Giardia* cysts. The numerical prediction of high levels of *G. muris* cyst inactivation was of secondary importance. Detailed variance analysis, joint confidence regions of parameters, and sum of squares plots, or equivalent, were beyond the scope of this section.

5.7.2.1 Data selection

The data used for modelling was developed by considering all C3H/HeN mouse-*G. muris* model results except the screening experiment (Tween 20 not used, see Section 4.4.1) so that the data set would be as large as possible; however, criteria based on experimental findings were applied so that the data would be homogeneous. The first criterion was that only laboratory and NSR water results with contact times of 2 min or more, and GVRD water results with contact times of at least 5 min were included. This eliminated the need for kinetic considerations in the analysis but still retained the majority of the data. Figure 5.21 illustrates the lack of time dependence beyond 2 min in the ozone inactivation of *G. muris* cysts in laboratory water.

The second criterion for inclusion in the data set for modelling was that temperature be in the range of 5–22°C. The four bench-scale trials at 1°C were excluded because these results appeared erratic, temperature significantly affected the ozone inactivation of *G. muris* cysts ($P \leq 0.05$) and temperature may affect the *G. muris* organism itself (Wickramanayake *et al.*, 1985).

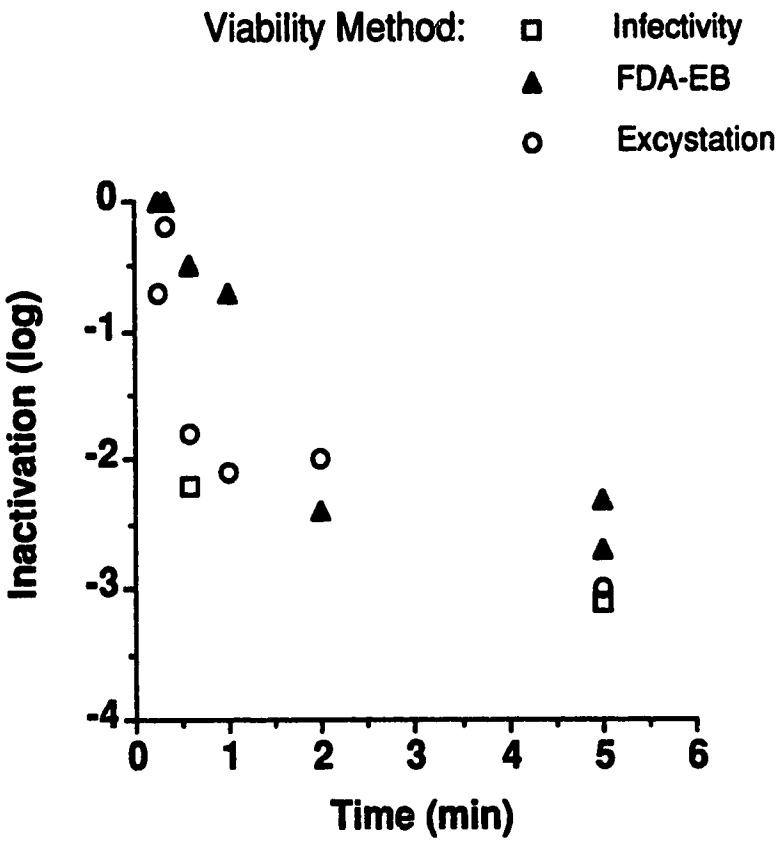

Figure 5.21 *Giardia muris* cyst ozone inactivation as a function of contact time in viability comparison experiment (ozone residual 0.1–0.2 mg/L; pH 6.7; 22°C). Viability determined by C3H/HeN mouse infectivity, fluorescein diacetate-ethidium bromide (FDA-EB) staining and *in vitro* excystation.

More ozone was utilized in natural waters than in laboratory waters. Utilized ozone values were 0.1 and 0.3 mg/L, respectively, for 3 and 4 log-units inactivation in the specific ozone residual experiment (Figure 5.12, p. 125); whereas in the natural waters these inactivations were observed when utilized ozone was 0.7 and 1.5 mg/L (Figure 5.14, p. 128). The higher utilized ozone in the natural waters was due to ozone demand. Table 4.1 indicated that ozone demand ranged from about 0.5 mg/L for the GVRD water to 1.0 mg/L for NSR water at 5°C. Figures 5.22 and 5.23 summarize the relationship between applied ozone dose, ozone residual and ozone demand in the natural waters. Ozone demand was calculated as the sum of immediate demand and the nonlinear portion of 5 min decomposition (Section 4.1.2). Ozone decomposition is a physical property of aqueous ozone and laboratory water first-order ozone decomposition plots were typically linear (Sections 2.3.2 and 5.1.1). Thus, ozone demand is considered a property of a natural water matrix and would be zero in a properly prepared laboratory water. This is somewhat different than the definition used in the recently published proposed standard method, which includes all decomposition as part of ozone demand and does not specify a standard temperature or contact time for the test (APHA *et al.*, 1991). It was hypothesized that if the ozone demand was subtracted from utilized ozone values in natural waters, *G. muris* cyst inactivation results from laboratory and natural waters could be pooled for modelling purposes.

Figure 5.24 presents *G. muris* inactivation as a function of ozone utilized per cyst in selected experiments (refer to Table I.1 in Appendix I). The objectives in preparing Figure 5.24 were to calculate an overall ozone demand and to determine if some early experiments (exps. 18 and 24, Table H.5, Appendix H) conducted using deionized water as the suspending medium could be pooled with the rest of the data. Using Figure 5.24, the ozone demand in the natural water can be calculated as $[(0.100 - 0.015)10^{-9}$g/cyst] x [2 x 10^6 cyst/0.2L] or 0.85 mg/L. This is a reasonable average value for the NSR and GVRD waters (Table 4.1, p.68). Although the deionized water data appeared comparable with the phosphate buffer data (Figure 5.24), it was not included in the

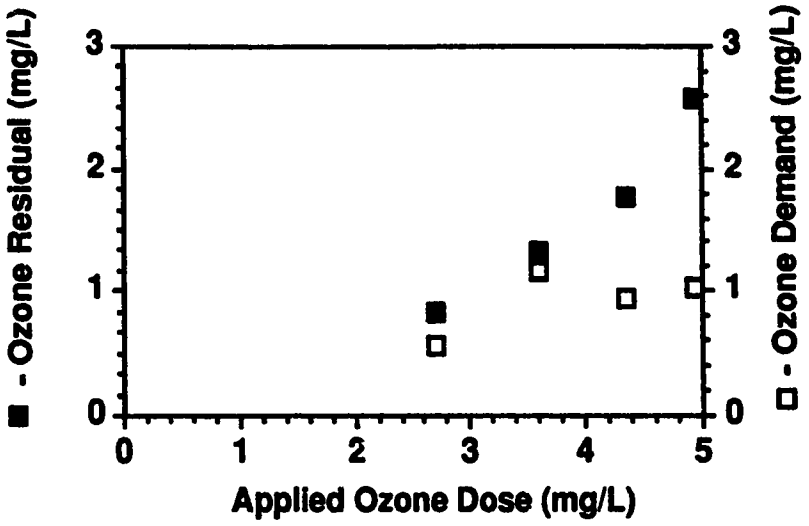

Figure 5.22 Relationship between applied ozone dose, ozone residual and ozone demand in North Saskatchewan River water for a contact time of 5 min (5°C; pH 8.3).

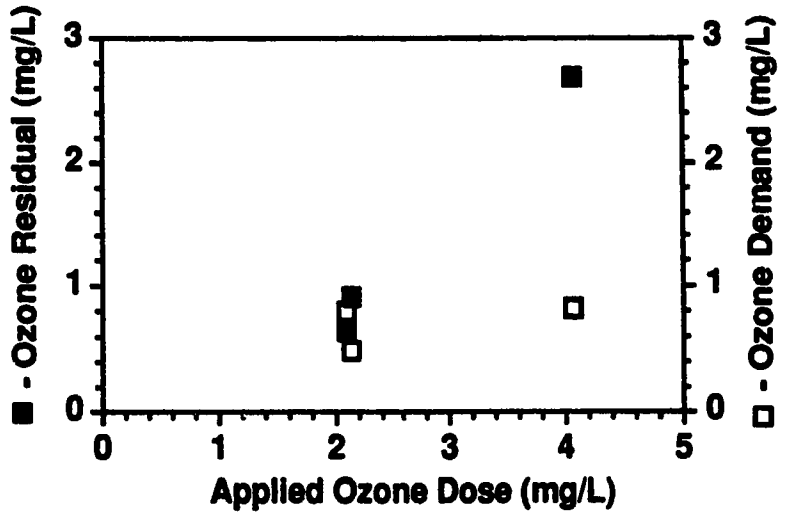

Figure 5.23 Relationship between applied ozone dose, ozone residual and ozone demand in Greater Vancouver Regional District water for a contact time of 5 min (5°C; pH 6.3).

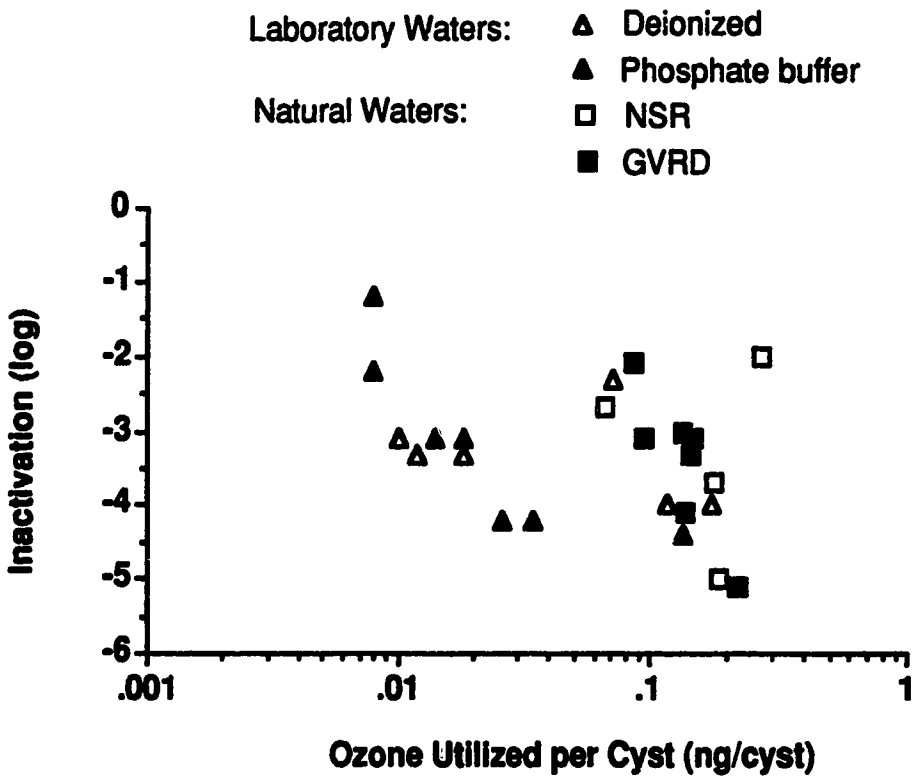

Figure 5.24 Effect of ozone utilized per cyst on *G. muris* inactivation in laboratory, North Saskatchewan River (NSR) and Greater Vancouver Regional District (GVRD) waters.

data set for modelling because there were too many differences in experimental protocol (refer to Sections 4.1.1, 4.3.8.1 and 4.4.1) and the lack of ionic strength in deionized water was suspected to cause very high *G. muris* cyst lysing. The open outlying square in Figure 5.24 is from a trial that had a large ozone demand relative to the other NSR water trials (trial 39A-4, Table J.1, Appendix J).

The results from the comparison of ozone and ozone–hydrogen peroxide experiment were not included in the data set for modelling because they were conducted in laboratory buffer systems engineered to completely utilize ozone or preserve applied ozone almost entirely. In addition, the purpose of modelling was to confirm the ozone and ozone–hydrogen peroxide experiment conclusion independently. The importance of residual ozone in disinfection is assumed to be through the direct action of the ozone molecule while utilized ozone acts through ozone decomposition products such as the hydroxyl radical.

The pooling of pilot- and bench-scale results was supported by the lack of scale-up effects as indicated in Figure 5.15. An ozone demand of 1.20 mg/L in exp. 40 was estimated from the TOC and turbidity values of the sample relative to a NSR sample taken two weeks earlier which had the ozone demand determined. Exp. 42 pilot-scale results were not used because the turbidity and TOC values were higher than for other samples and it was not possible to accurately predict the ozone demand.

Combining all results gave a data set with 29 cases, 15 from laboratory waters and 14 from natural waters (Table 5.13). The data consists of 18 trials at low pH (pH 5.7, 6.3 or 6.7) and 11 trials at high pH (pH 7.6 or 8.3). Of the 29 trials, 10 were at 5°C, 4 at 14 or 15°C, and 15 at 22°C. The temperature and pH subsets were too small to permit modelling. Plots of *G. muris* cyst inactivation versus residual ozone, utilized ozone less ozone demand and average ozone are shown in Figures 5.25, 5.26 and 5.27. There is less variation in the ozone residual plot (Figure 5.25) than in the utilized ozone less ozone demand plot (Figure 5.26). A single trial in Figure 5.26 appears to be an outlier; however, the trial had a very small ozone residual and a large utilized

Table 5.13 Summary of *G. muris* cyst inactivation data for modelling.

Trial	Residual Ozone C_r (mg/L)	Utilized Ozone C_u (mg/L)	Ozone Demand C_d (mg/L)	Average Ozone C_{av}# (mg/L)	DOC (mg/L)	Inactivation (log N/N_0)
33-A1	0.30	0.32	-	0.44	0.09	4.2
33-A2	0.40	0.24	-	0.50	0.09	4.2
33-B1	0.10	0.16	-	0.16	0.09	3.1
33-B2	0.10	0.20	-	0.18	0.09	3.1
33-C1	1.30	1.25	-	1.90	0.09	4.4
36-1	0.35	0.24	-	0.45	0.09	2.1
36-2	0.35	0.24	-	0.45	0.09	>5.1
36-3	0.40	0.33	-	0.54	0.09	4.5
36-4	0.36	0.46	-	0.57	0.09	>5.1
36-5	0.37	0.29	-	0.49	0.09	3.6
36-6	0.37	0.34	-	0.52	0.09	3.6
41-3	0.38	0.09	-	0.40	0.09	3.8
41-4	0.41	0.07	-	0.42	0.09	3.1
41-5	0.42	0.35	-	0.57	0.09	3.5
41-6	0.41	0.12	-	0.45	0.09	3.2
39A-1	0.25	0.71	0.32	0.45	0.99	2.7
39A-2	0.70	1.88	0.89	1.20	0.99	3.7
39A-3	0.83	1.87	0.55	1.49	0.99	>5.0
39A-4	0.24	2.79	1.54	0.87	0.99	2.0
39C-1	0.65	1.45	0.80	0.98	9.15	4.1
39C-3	0.35	2.27	1.37	0.80	9.15	>5.1
39D-1	0.37	0.91	0.37	0.64	9.15	3.1
39D-2	0.76	0.90	0.30	1.06	9.15	3.1
39D-3	0.77	1.28	0.49	1.17	9.15	3.0
39D-4	0.34	1.37	0.50	0.78	9.15	3.3
40-1	0.21	1.26	1.20*	0.24	1.62	2.7
40-2	0.35	2.09	1.20*	0.80	1.62	3.6
40-3	0.80	2.87	1.20*	1.64	1.62	>5.0
40-4	1.23	3.72	1.20*	2.49	1.62	>5.0

C_{av}, $C_{av} = C_r + 0.5 \times (C_u - C_d)$

*, estimate;

>, detection limit of C3H/HeN mouse model.

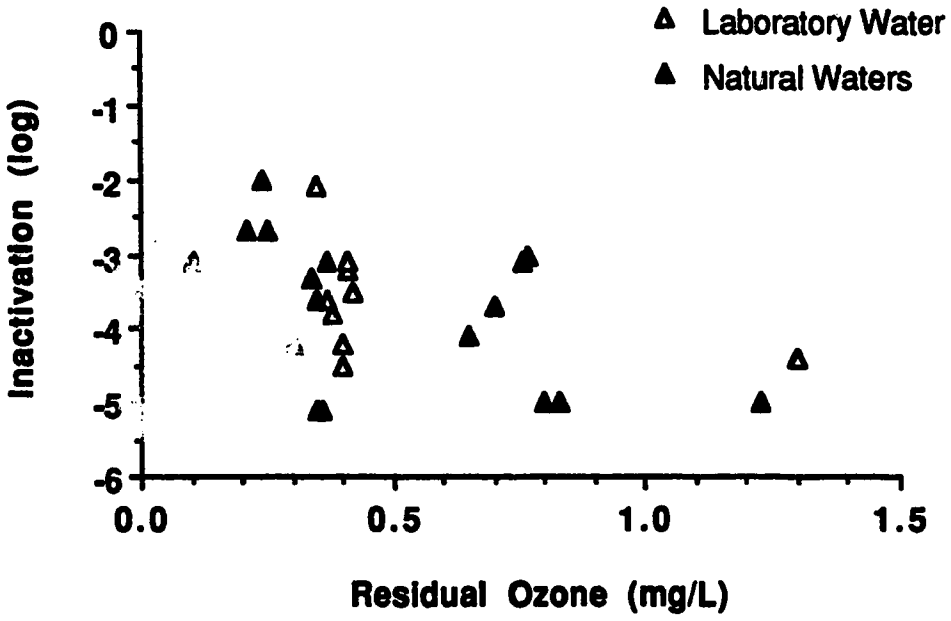

Figure 5.25 Effect of residual ozone and water type on *G. muris* inactivation in the modelling data set

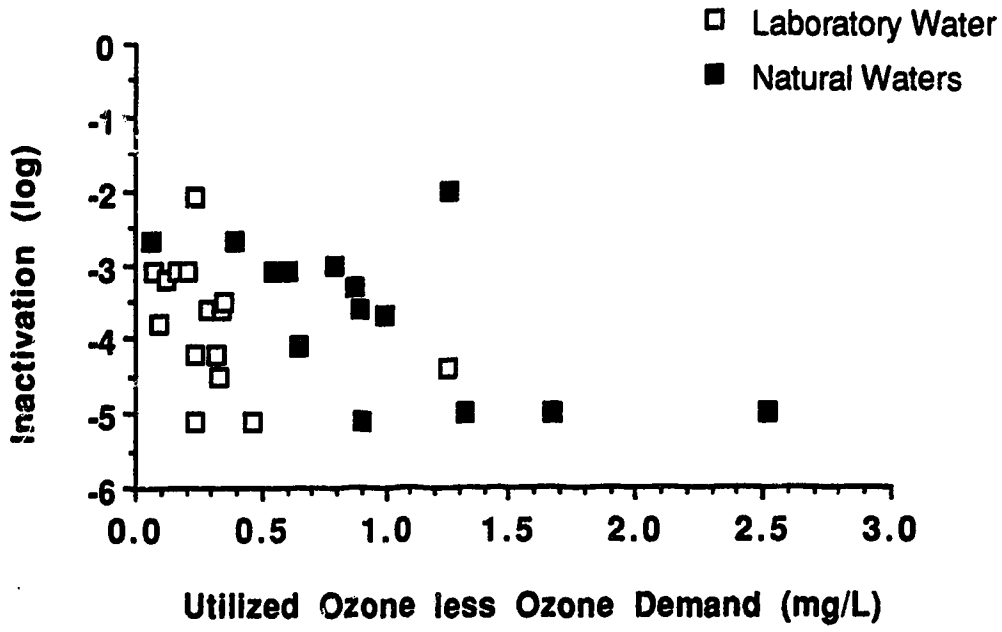

Figure 5.26 Effect of utilized ozone less ozone demand and water type on *G. muris* inactivation in the modelling data set

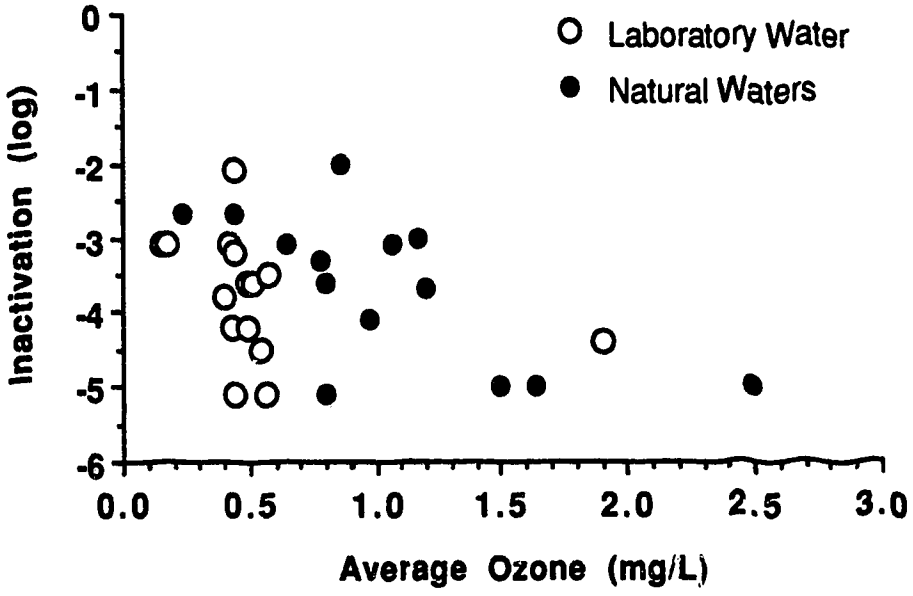

Figure 5.27 Effect of average ozone and water type on G. *muris* inactivation in the modelling data set

ozone value (trial 39A-4, Table 5.13). Figure 5.28 shows *G. muris* inactivation contours as a function of residual ozone and utilized ozone less ozone demand for this data. Figure 5.28 indicates that the data set for modelling has a cluster of data with an ozone residual of approximately 0.4 mg/L.

5.7.2.2 Model formulation

The model was identified on the basis of:

residual ozone = f(applied ozone, pH) [5.3]

utilized ozone = f(applied ozone, competing reactions, temperature) [5.4]

$$\log \frac{N}{N_0} = f(\text{residual ozone, utilized ozone}) \quad [5.5]$$

Thus the independent variables included temperature and pH effects. Contact time also influences residual and utilized ozone, however data was selected to eliminate the need for kinetic considerations (Section 5.7.2.1). The form of model used was:

$$Y = f(X_1 + X_2 + X_1X_2) \quad [5.6]$$

where Y is log cyst survival, X_1 is a residual ozone term, X_2 is an utilized ozone term and X_1X_2 is an interaction term. The initial model was:

$$\log \frac{N}{N_0} = - [\theta_1 C_r^{\theta_2} + \theta_3 (C_u - C_d)^{\theta_4} + \{\theta_1 C_r^{\theta_2}\} \times \{\theta_3 (C_u - C_d)^{\theta_4}\}] \quad [5.7]$$

where: N_0 is the initial concentration of microorganisms;

N is the concentration of surviving microorganisms when C_u ozone is utilized;

C_r is the ozone residual in mg/L;

C_u is utilized ozone in mg/L;

C_d is the ozone demand in mg/L; and

θ_1, θ_2, θ_3 and θ_4 are nonlinear model parameters.

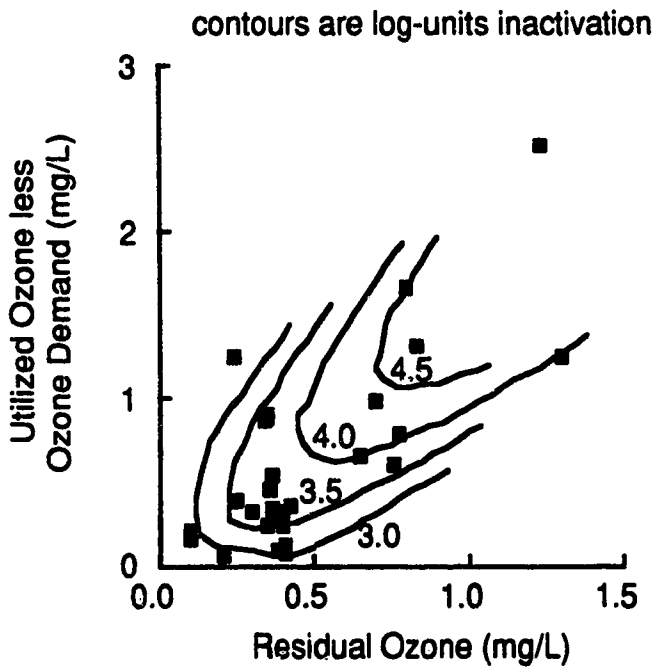

Figure 5.28 *Giardia muris* inactivation contours as a function of residual ozone and utilized ozone less ozone demand in the modelling data set.

Equation [5.7] is very general and includes a coefficient and an exponent for each independent variable. Utilized ozone less ozone demand was used for the second independent variable to permit the pooling of laboratory and natural water results (Section 5.7.2.1).

The next step in model building is parameter estimation. In 6 of the 29 trials in the data set, no mice became positive and inactivation was greater than the detection limit (Table 5.13, p. 155). One of the poorer approaches to model parameter estimation would be to simply delete this data (Helsel, 1990). However, because squaring a large value increases its impact, the use of ordinary least squares for parameter estimation with censored data is prohibited (Helsel, 1990). Instead, the selected loss function used the least absolute values of deviations in observed dependent variables from their estimates (Wilkinson and Leland, 1989):

$$\text{Minimize} \quad | y - y^* | \qquad [5.8]$$

where: y is the dependent variable; and

y^* is the model-predicted dependent variable.

Other researchers have reported relative least squares to be more suitable than ordinary least squares in some environmental engineering applications (Saez and Rittmann, 1992).

Model parameters and diagnostics including the confidence limits of parameters, asymptotic correlation matrix of parameters and residual plots are provided in Appendix K. Table 5.14 lists observed and estimated inactivations. Diagnostic analysis was generally satisfactory except that the 95 percent confidence interval of θ_2 included zero (Appendix K.1). However, at this point it was realized that the variances of utilized ozone less ozone demand in the laboratory and natural waters may not be homogeneous (Figure 5.26, p. 157). An *F*-test confirmed that $(C_u - C_d)$ variances in the laboratory and natural waters were not homogeneous. The variances of these two waters appear equivalent in Figure 5.24 (p. 153); however logarithmic plots can be deceptive.

Table 5.14 Observed and estimated *G. muris* cyst inactivation by ozone

Trial	Inacti-vation ($\log N/N_0$)	Model equation number [5.7]		Model equation number [5.9]	
		Estimate	Residual	Estimate	Residual
	y	y^*	ε	y^*	ε
33-A1	4.2	3.52	0.68	3.69	0.51
33-A2	4.2	3.49	0.71	3.70	0.50
33-B1	3.1	3.02	0.08	3.10	0.00
33-B2	3.1	3.09	0.01	3.17	-0.07
33-C1	4.4	4.58	-0.18	4.92	-0.52
36-1	2.1	3.45	-1.35	3.65	-1.55
36-2	>5.1	3.45	>1.65	3.65	>1.45
36-3	4.5	3.61	0.89	3.81	0.69
36-4	>5.1	3.71	>1.39	3.90	>1.20
36-5	3.6	3.54	0.06	3.74	-0.14
36-6	3.6	3.60	0.00	3.80	-0.20
41-3	3.8	3.15	0.65	3.35	0.45
41-4	3.1	3.10	0.00	3.30	-0.20
41-5	3.5	3.64	-0.14	3.86	-0.36
41-6	3.2	3.26	-0.06	3.47	-0.27
39A-1	2.7	3.55	-0.85	3.29	-0.59
39A-2	3.7	4.26	-0.56	4.00	-0.30
39A-3	>5.0	4.47	>0.53	4.18	>0.82
39A-4	2.0	4.06	-2.06	3.67	-1.67
39C-1	4.1	4.03	0.07	3.44	0.66
39C-3	>5.1	4.01	>1.09	3.32	>1.78
39D-1	3.1	3.79	-0.69	3.19	-0.09
39D-2	3.1	4.04	-0.94	3.47	-0.37
39D-3	3.0	4.18	-1.18	3.56	-0.56
39D-4	3.3	3.98	-0.68	3.30	0.00
40-1	2.7	2.91	-0.21	2.68	0.02
40-2	3.6	4.00	-0.40	3.60	0.00
40-3	>5.0	4.59	>0.41	4.16	>0.84
40-4	>5.0	4.99	>0.01	4.53	>0.47
Mean		3.76		3.64	
SD		0.52		0.45	

y, observed dependent variable; SD, standard deviation;

y^*, dependent variable predicted from nonlinear model;

>, detection limit of C3H/HeN mouse model.

The ozone demand calculation method used in this work is affected by pH. This is the reason that although GVRD water had nine times the DOC as the NSR water, its ozone demand was lower than that of the NSR water. A transformation was desired which would compensate for this deficiency as well as stabilize variances. The selected transformation was $(C_u - C_d)/\sqrt{DOC}$. Ozone metameters are frequently normalized by the DOC or TOC values of natural waters (Chang and Singer, 1991). The square root transformation is relatively mild (Box *et al.*, 1978), and in this application improves the applicability of the model to both laboratory and natural waters. The factor $1/\sqrt{DOC}$ is a surrogate for competing reactions not included in C_d. Variances of $(C_u - C_d)/\sqrt{DOC}$ in the laboratory and natural waters are homogeneous (Table J.1, Appendix J). The revised model was:

$$\log \frac{N}{N_o} = - [\theta_1 C_r^{\theta_2} + \theta_3 \left(\frac{C_u - C_d}{\sqrt{DOC}}\right)^{\theta_4} + \{\theta_1 C_r^{\theta_2}\} \times \{\theta_3 \left(\frac{C_u - C_d}{\sqrt{DOC}}\right)^{\theta_4}\}] \qquad [5.9]$$

where: DOC is dissolved organic carbon in mg/L, and the other parameters are as defined for equation [5.7].

Model parameters and diagnostics for equation [5.9] are provided in Appendix K.2. There were no obvious lack-of-fit of the data to the model. The high negative correlation between θ_1 and θ_3 is a reflection of the fact that residual ozone and utilized ozone are often negatively correlated. A challenge in the data being modelled is that trials 36-1 and 36-2 had identical C_r and C_u values, yet N/N_o values were 2.1 and >5.1 log-units, respectively. There would be questions regarding the validity of the data except that trial 36-1 was conducted at 5°C and trial 36-2 was conducted at 22°C. At the same time, a weakness of the model is that temperature is not explicitly included.

The ratio of the first to second terms in equations [5.7] and [5.9] gives a general idea of the relative importance of residual and utilized ozone when inactivating *Giardia* cysts. A t-test was used to confirm if the two terms were statistically different. A summary of the contribution of each term in the models is presented in Table 5.15. Note

Table 5.15 Summary of *G. muris* cyst inactivation by ozone in the modelling data set as predicted by two models

Model equation number	Final value of loss function†	Mean value of model term number			Mean model-predicted inactivation y*	Ratio: Term 1 / Term 2
		1	2	3		
[5.7]	17.540	1.81#	0.69	1.26	3.76	2.62
[5.9]	16.252	1.27#	1.04	1.33	3.64	1.22

† Loss function used was least absolute values;
# Statistically significant difference from term 2 value, $P \leq 0.001$;
y*, dependent variable predicted from nonlinear model.

that equation [5.9] is a preferable model compared with equation [5.7] because the final value of the loss function is 16.252, more than a full unit lower than previously.

5.7.2.3 Discussion

The data presented in Table 5.15 indicates that ozone residual appears to be more important than utilized ozone when inactivating *G. muris* cysts. In both models the C_r term was greater than the $(C_u - C_d)$ or $(C_u - C_d)/\sqrt{DOC}$ terms, and the C_r term accounted for a significantly larger amount of model-predicted inactivation ($P \leq 0.001$). This supports the conclusion of the ozone and ozone–hydrogen peroxide experiment.

The sensitivity of the dependent variable in equation [5.9] to various values of C_r, C_u, C_d and DOC is presented in Table 5.16. Figure 5.29 is a plot of the simulated inactivations. Note the general similarity between the observed data in Figure 5.20 and the simulated data in Figure 5.29. The major difference is that the range of the simulated data in not as large. Conditions in Figure 5.29 which satisfy the SWTR *Giardia* requirement are ozone residuals greater than or equal to 0.5 mg/L and a $(C_u - C_d)/\sqrt{DOC}$ value of at least 0.10.

A difficulty with models is that they are sometimes very specific to the data for which they are developed. Data from Table 4.13 of Metropolitan Water District of Southern California and James M. Montgomery Consulting Engineers (1991) was used to compare model-predicted and observed *G. muris* cyst ozone inactivations. These data were for State Project Water tests with a temperature range of 13–21°C and TOC values of 2.4–2.6 mg/L. DOC was estimated to be 2.5 mg/L and C_d was estimated as 0.63 mg/L. Model-predicted inactivation was 2.74 log-units (simulation 100, Table 5.16) compared with an observed inactivation of 2.03 log-units (mean of 10 tests) for C_r of 0.3 mg/L (mean). The predicted inactivation was within the range of the observations. Differences may be attributable to imprecision of C_d estimate, excystation used by MWDSC while equation [5.9] is based on infectivity, and t_{10} values of 3.9 min in the MWDSC tests, although HDT was 12 min.

Table 5.16 Sensitivity analysis of *G. muris* cyst inactivation by ozone as predicted by equation [5.9]

Simulation	Independent Variables				Transformed Variable	Dependent Variable Estimate
	C_r (mg/L)	C_u (mg/L)	C_d (mg/L)	DOC (mg/L)	$\frac{C_u - C_d}{\sqrt{DOC}}$	y^*
100	0.3	0.7	0.63	2.5	0.04	2.74
101	0.2	1.3	0.5	2.5	0.51	3.30
102	0.2	1.3	0.5	10.0	0.25	3.09
103	0.2	1.3	1.0	2.5	0.19	3.01
104	0.2	1.3	1.0	10.0	0.09	2.83
105	0.2	1.3	1.2	2.5	0.06	2.73
106	0.2	1.3	1.2	10.0	0.03	2.58
107	0.3	1.2	0.5	2.5	0.44	3.39
108	0.3	1.2	0.5	10.0	0.22	3.18
109	0.3	1.2	1.0	2.5	0.13	3.02
110	0.3	1.2	1.0	10.0	0.06	2.85
111	0.4	1.1	0.5	2.5	0.38	3.44
112	0.4	1.1	0.5	10.0	0.19	3.23
113	0.4	1.1	1.0	2.5	0.06	2.94
114	0.4	1.1	1.0	10.0	0.03	2.77
115	0.5	1.0	0.5	2.5	0.32	3.46
116	0.5	1.0	0.5	10.0	0.16	3.25
117	0.5	1.0	0.9	2.5	0.06	3.01
118	0.5	1.0	0.9	10.0	0.03	2.84
119	0.5	2.0	0.5	2.5	0.95	3.85
120	0.5	2.0	0.5	10.0	0.47	3.60
121	0.5	2.0	1.0	2.5	0.63	3.70
122	0.5	2.0	1.0	10.0	0.32	3.46
123	0.5	2.0	1.8	2.5	0.13	3.19
124	0.5	2.0	1.8	10.0	0.06	3.01
125	0.8	1.7	0.5	2.5	0.76	3.95
126	0.8	1.7	0.5	10.0	0.38	3.70
127	0.8	1.7	1.0	2.5	0.44	3.75
128	0.8	1.7	1.0	10.0	0.22	3.52
129	0.8	1.7	1.6	2.5	0.06	3.16
130	0.8	1.7	1.6	10.0	0.03	2.99

C_r, residual ozone; C_u, utilized ozone; C_d, ozone demand;
DOC, dissolved organic carbon;
y^*, log-units inactivation predicted by model equation [5.9].

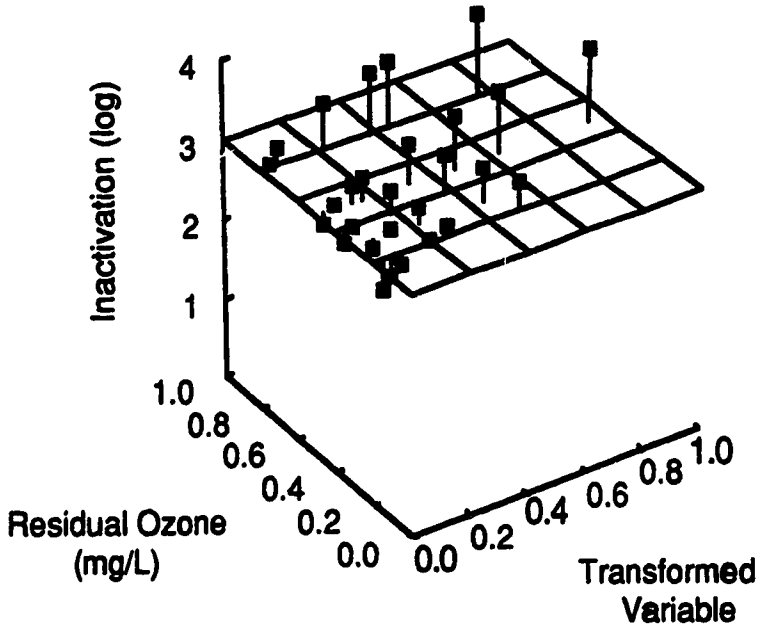

Figure 5.29 Simulated *G. muris* inactivation relative to 3 log-units as a function of residual ozone and a transformed variable that depends on utilized ozone, ozone demand and dissolved organic carbon [$(C_u - C_d)/\sqrt{DOC}$].

Equation [5.9] was developed as an inferential statistical tool and it may be overparameterized for general prediction of *G. muris* cyst ozone inactivation. Equation [5.9] should not be used for design. Additional modelling work is required to develop design equations which should be validated using independent data. Further analysis may indicate that the ozone demand subtracted in equations [5.7] and [5.9] may in fact account for some inactivation. Until more work is done, the process design "envelope" developed in Section 5.7.1 can be used to achieve the objective of inactivating 3 log-units of *Giardia*.

5.7.3 Integration of Proposed Protocol with *Guidance Manual*

Appendix O in the "Guidance Manual for Compliance with the Filtration and Disinfection Requirements for Public Water Systems Using Surface Water Sources" provides recommendations on the definitions of C and t in ozone disinfection (USEPA, 1991).

The completely mixed bench-scale and semi-batch stirred tank pilot-scale reactors used in this work were theoretically perfect contactors. Each particle in the water was exposed to the same concentration of ozone for the same period of time. To compensate for nonidealities such as short-circuiting in full-scale systems, the t of 5 min in this work should be interpreted as the t_{10} requirement of flow-through reactors in the *Guidance Manual*.

The definition of ozone concentration is complicated because of ozone decomposition and various possible flow regimes depending on contactor configuration (Section 2.5.3). Ozone contactor configurations favored for primary disinfection in water treatment are multi-chamber bubble diffusers because plug-flow conditions are increased minimizing contactor volumes and short-circuiting (IOA, 1990). The bench-scale ozonation protocol used herein may conceptually be regarded as a very short CSTR stage during ozone addition followed by a reactive stage for the specified contact time. The ozone concentration C reported in this work was generally C_r, the ozone residual at the end of the specified

contact time. C_r, or C_{out} as it is designated in Appendix O of the *Guidance Manual* has the advantages of being readily observable and indicating a surplus level of ozone over competing ozone reactions.

The use of average ozone for the characteristic concentration C is also discussed in Appendix O of the *Guidance Manual.* Various definitions have been used for average ozone. The MWDSC in their pilot-scale work reported average ozone as [(applied ozone dose + ozone residual)/2] (Wolfe *et al.*, 1989b). To exclude the effect of ozone demand, the definition of average ozone used in this work was (Table 5.13, p.155):

$$C_{av} = C_r + 0.5 \times (C_u - C_d) \qquad [5.10]$$

or, $$C_{av} = C_r + 0.5 \times [(C_o - C_r) - C_d] \qquad [5.11]$$

where: C_{av} is the calculated average ozone value in mg/L;

C_r is the ozone residual in mg/L;

C_u is utilized ozone in mg/L;

C_d is the ozone demand in mg/L; and

C_o is the applied ozone dose in mg/L.

Equation [5.11] reduces to $[C_{av} = (C_o + C_r)/2]$ when C_d is zero.

Average ozone was found to be less suitable than other ozone metameters in bench-scale work performed here. Difficulties include:

1. there is a loss of information by averaging (i.e. Figure 5.27, p. 158 has less information than Figures 5.25 and 5.26, p. 156 and p. 157);

2. weighting ozone dose and residual ozone equally (C_{in} and C_{out} as defined in *Guidance Manual*; C_o and C_r as defined in this document) may not be justified because C_r appears to be more important in disinfection;

3. comparing average ozone values is not meaningful unless waters are of similar temperature, pH and are ozonated for similar contact times; and

4. high ozone demand waters could have high average ozone values, but little inactivation (i.e. trial 39A-4, Table 5.13, p. 155).

5.7.4 Microflocculation Effects of Ozone

In the screening experiment three ozonated trials with turbidity artificially introduced had cyst recovery that was an order of magnitude larger than in the controls. This phenomenon was presumed to be due to the effects of microflocculation. In these trials inactivation was estimated by using the actual ozonated trial inoculum size for the denominator term in equation [4.8].

Microflocculation is the flocculation of soluble micropollutants from water which can then be removed by filtration (Rice, 1989). Polar groups such as the carboxyl and hydroxyl groups are capable of hydrogen bonding in the presence of polyvalent metal cations and yield complexed higher molecular weight materials that are insoluble (Rice *et al.*, 1981).

A related phenomenon is the complex effect ozone has on particle stability in natural waters. It has been shown that for optimal ozone-induced coagulation, the hardness to TOC ratio should be greater than 25 mg hardness as calcium carbonate per mg of carbon with ozone doses of about 0.4 to 0.8 mg per mg carbon (Chang and Singer, 1991). These ratios for the two surface waters used were: hardness to TOC ratio of 145 and ozone to TOC ratio of 0.8-2.5 for NSR water, and hardness to TOC ratio of 0.5 and ozone to TOC ratio of 0.1-0.2 for GVRD water. Consequently, significant ozone-induced coagulation could be expected for the NSR water.

Hoff and Akin (1986) have reported that particulates associated with cell debris and wastewater effluent solids markedly decreased virus and bacteria inactivation rates by chlorine, chlorine dioxide and ozone.

When viruses and bacteria were adsorbed on clays, inactivation rates were affected only minimally. A comprehensive study on the effect of particulates on ozone disinfection included bacteria and viruses, but not *Giardia* (Sproul *et al.*, 1979).

Clearly, when ozone is added to natural waters there are many interacting variables. Pilot-scale experiments using the natural waters to be treated under a variety of seasonal conditions should be conducted prior to the design of full-scale facilities.

5.7.5 Public Health Protection

Although this work shows that ozone alone can achieve 3 log-units of *G. muris* cyst inactivation, operational criteria of actual contact time (HDT and t_{10}), ozone residual, applied ozone dose and influent water quality would have to be closely monitored to ensure successful operation. Should any part of the ozonation system fail, or short-circuiting occur in the contactor, public health could be compromised. A multi-barrier approach of chemical pretreatment prior to granular media filtration would have less inherent risk.

6. CONCLUSIONS

Ozone disinfection of water containing *Giardia* is complicated due to problems in determining *Giardia* viability, the definition of ozone concentration, and ultimately, in a gas-liquid reactor, defining the contact time. The use of the C3H/HeN mouse-*G. muris* model appears to be an effective means for determining the appropriate ozonation conditions for preventing transmission of infective *Giardia* cysts. The labor intensive nature of the C3H/HeN mouse-*G. muris* model currently restricts its use to researchers; however, the results have practical application. The conclusions of this work are:

1. The mouse model is highly sensitive and can detect greater than 5 log-units inactivation of *G. muris* cysts. In addition to being capable of detecting high level inactivation, the C3H/HeN mouse-*G. muris* model is capable of detecting inactivation as low as 1.2 log-units.

2. There were no significant differences ($P \leq 0.05$) between infectivity and *in vitro* excystation, or between fluorogenic staining and excystation for up to 99.9 percent inactivation of *G. muris* cysts. Fluorogenic staining significantly overestimated viability compared with infectivity ($P \leq 0.05$).

3. The C3H/HeN mouse-*G. muris* model was capable of measuring 1.5 to 2 log inactivation higher than *in vitro* excystation or fluorogenic staining. Should *Giardia* inactivation requirements be increased, infectivity may have to be used exclusively due to its high sensitivity.

4. The phosphate-bicarbonate buffer system, which had negligible decomposition of ozone, had significantly greater ($P \leq 0.05$) inactivation of *G. muris* cysts than that observed in the phosphate buffer system with peroxide where the ozone was completely decomposed in less than 60 s. By inference, the direct action of the ozone molecule was the most significant factor for inactivation of *G. muris*.

5. Ozone alone is a better disinfectant than ozone–hydrogen peroxide.

6. Temperature, pH, applied ozone dose and ozone dose x pH and temperature x pH interactions had statistically significant ($P \leq 0.05$) effects on *G. muris* cyst infectivity.

7. It may be more difficult to achieve 3 log inactivation of *G. muris* cysts at 22°C than at 5°C in certain waters. Higher temperatures increase the dissipation of ozone while colder temperatures tend to preserve ozone residual.

8. Contact time had more effect in some waters than in others. Contact time in demand-free 0.05 M phosphate buffer and North Saskatchewan River water was not significant beyond two min ($P \leq 0.05$). Contact times greater than five min were not significant in Greater Vancouver Regional District water.

9. The Ct concept, a simplification of the Chick-Watson model, should be reexamined for the purposes of predicting ozone inactivation of *G. muris* cysts. The data presented here indicates that the ozone inactivation of *G. muris* cysts is very complex and is not adequately predicted by Ct.

10. The use of a design "envelope" such as a minimum contact time of 5 min and a minimum ozone residual of 0.5 mg/L is recommended as a means of achieving the Surface Water Treatment Rule objective of inactivating 3 log-units of *Giardia*. The 5 min contact time should be interpreted as the t_{10} requirement of flow-through reactors for integration with values published in the *Guidance Manual*.

11. Some of the Ct values published in the *Guidance Manual* for ozone inactivation of *Giardia* may be too small when applied to natural waters.

12. There were no apparent differences between the bench- and pilot-scale *G. muris* cyst inactivation results.

13. Nonlinear modelling indicated that ozone inactivation of *G. muris* cysts is a function of residual and utilized ozone. The utilized ozone value used in the model was transformed to $(C_u - C_d)/\sqrt{DOC}$ to account for competing reactions in waters with ozone demands up to approximately 1 mg/L and DOC values up to 10 mg/L. pH and temperature effects were implicitly included by their influence on residual and utilized ozone levels. The residual ozone term accounted for a significantly larger amount of model-predicted inactivation ($P \leq 0.001$) than the utilized ozone term. This is additional inferential evidence of the importance of the direct action of the ozone molecule in disinfection. The model also indicates that the Surface Water Treatment Rule *Giardia* requirement is met if the ozone residual is at least 0.5 mg/L and the value of $(C_u - C_d)/\sqrt{DOC}$ is at least 0.10.

7. RECOMMENDATIONS

Recommendations for future work include:

1. Additional work should be done to expand the infectivity data set, particularly at pilot-scale using more natural surface waters. Sufficient replicates should be made to permit calculation of the 95 percent confidence limits of inactivation estimates.

2. At colder temperatures (5°C and less) results were more variable. Work should be done to try to ascertain why this is so. In addition, a set of inactivation data at 1°C should be developed.

3. A study should be made on the mode of action of ozone on *Giardia* cysts. This understanding would permit inactivation data to be applied more generally rather than being species-specific and would assist in the development of chemical kinetic models.

4. Further work should be done on *Giardia* cyst inactivation kinetics, the results of which could be used to optimize ozone reactor design. The maintenance of optimized ozone residual levels would decrease operating costs, and the design of contactors for minimum contact times would reduce capital costs.

5. A well designed comparison between the susceptibilities of *G. muris* and *G. lamblia* cysts to ozone in both laboratory and natural waters is required. There may be less difference between the susceptibilities of *G. muris* and *G. lamblia* cysts to ozone than has been previously thought and further investigation is warranted.

6. Additional microbial indicator studies should be done for *G. lamblia*. Heterotrophic plate count bacteria might be useful.

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APPENDIX A: HYDROGEN PEROXIDE DETERMINATION USING MASSCHELEIN'S METHOD

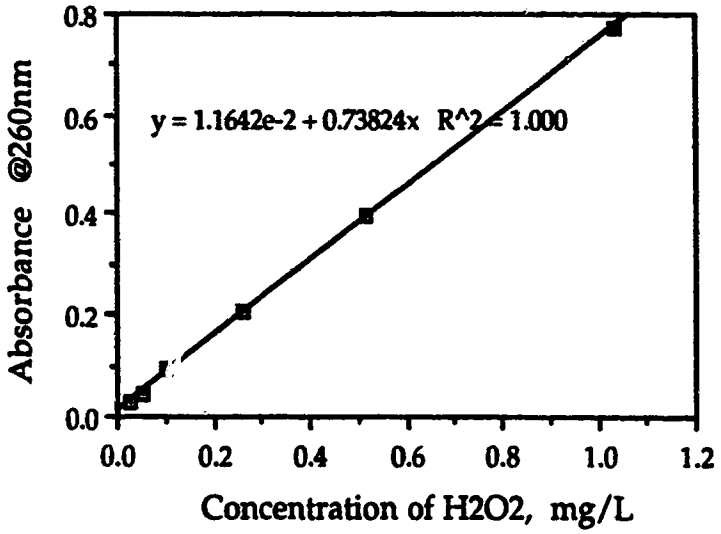


Figure A.1 Hydrogen peroxide residual calibration curve using Masschelein's method (solution 1)

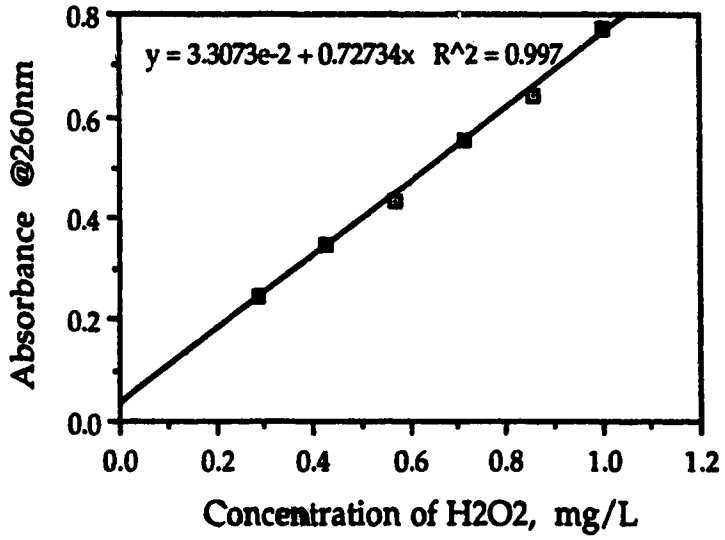


Figure A.2 Hydrogen peroxide residual calibration curve using Masschelein's method (solution 2)

The results from Masschelein's method using the two different hydrogen peroxide solutions agree within 4 percent. The overall regression equation (12 points) was $y = 0.0156+0.745x$, $R^2=0.998$.

APPENDIX B: EXAMPLE CALCULATION OF *GIARDIA MURIS* INACTIVATION USING THE MOUSE MODEL

EXAMPLE CALCULATION OF *GIARDIA MURIS* INACTIVATION USING THE MOUSE MODEL

The relation used for determining inactivation (N/N_O) from mouse model data was:

$$\frac{N}{N_o} = \frac{n \times I}{n_o \times I_o} \qquad [4.8]$$

where: n is the number of positive mice in an ozonated trial;

I is the number of infectious cysts per positive mouse after ozonation, determined using the latent period (if the inoculum had 10^5, 10^4, 10^3, 10^2, or 10 infectious cysts, mice became positive on days 3, 4, 5, 6 or 8, respectively);

n_O is the number of positive mice in the control; and

I_O is the inoculum size in the control, determined by hemocytometer count.

As an example, *G. muris* inacitvation (N/N_O) in trial A1 of experiment 33 was calculated using equation [4.8] and:

$n = 4$;

$n_O = 5$;

$I = 10$ (latent period is day 8); and

$I_O = 100{,}000$ (latent period is day 3).

thus: $$\frac{N}{N_o} = \frac{4 \times 10}{5 \times 120{,}000} = 0.000067 \text{ or } 4.2 \text{ log-units}$$

APPENDIX C: SAMPLE CALCULATION OF HOMOGENEITY CHECK USING REPLICATE HEMOCYTOMETER CYST COUNTS

Table C.1 Viability comparison experiment: Homogeneity check of replicate hemocytometer cyst count data.

Trial	Cyst Counts Before Centrifugation (Four Replicates)				
	Mean (9 squares)	S^2	D^2	Poisson† Yes / No	Concentration (cysts/mL)
A1	6.88	2.23	0.97	Yes	7.6E+03
A2	4.75	1.42	0.90	Yes	5.3E+03
B1	11.38	7.06	1.86	Yes	1.3E+04
B2	10.50	8.17	2.33	Yes	1.2E+04
C1	4.63	3.56	2.31	Yes	5.1E+03
C2	7.63	2.06	0.81	Yes	8.5E+03
C3	8.00	1.50	0.56	Yes	8.9E+03
C4	11.50	8.33	2.17	Yes	1.3E+04
D1	3.63	2.40	1.98	Yes	4.0E+03
D2	7.50	1.17	0.47	Yes	8.3E+03
D3	6.38	1.23	0.58	Yes	7.1E+03
D4	8.13	3.23	1.19	Yes	9.0E+03
AC*	7.63	1.73	0.68	Yes	8.5E+03
BC	11.13	5.40	1.46	Yes	1.2E+04
CC	7.38	2.23	0.91	Yes	8.2E+03
DC	8.88	3.23	1.09	Yes	9.9E+03

*Control - Cyst Batch A;

†χ^2 (0.05, 3) = 7.81;

S^2 is the estimate of the population variance;

D^2 is the index of dispersion determined as [(4-1) x S^2]/mean.

APPENDIX D: AQUEOUS RESIDUAL OZONE DETERMINATION USING THE INDIGO TRISULPHONATE METHOD

Table D.1 Aqueous residual ozone determination using the indigo trisulphonate method.

Method	Absorbance Measured at (nm)	Absorbance (1/cm)	Concentration of Ozone (mg/L)	Percent Difference (%)
u.v.	260	1.28719	18.73	
u.v.	260	1.27228	18.51	
Indigo	600	0.18000-0.01714	19.39	+4.1
u.v.	260	1.23384	17.95	
u.v.	260	1.19855	17.43	
Indigo	600	0.18000-0.02678	18.24	+3.1
u.v.	260	1.24963	18.18	
u.v.	260	1.23305	17.94	
Indigo	600	0.17949-0.02409	18.50	+2.4
u.v.	260	1.10361	16.06	
u.v.	260	1.13188	16.47	
Indigo	600	0.17949-0.03426	17.29	+6.3
u.v.	260	1.13773	16.55	
u.v.	260	1.13087	16.45	
Indigo	600	0.17949-0.03218	17.54	+6.3
u.v.	260	1.11562	16.23	
u.v.	260	1.06317	15.47	
Indigo	600	0.17949-0.03871	16.76	+5.7

Mean percent difference (6 trials) is +4.7 %

APPENDIX E: SELECTED OZONE DECOMPOSITION RESULTS

Table E.1 - Data Set A: Half-Life Summary

50 mL Erlenmeyer Flask					
0.5 mg/L O3 in O.D.F. PO4			0.5 mg/L O3 in O.D.F. Milli-Q		
	Applied O3, mg/L	Half-Life, s		Applied O3, mg/L	Half-Life, s
	0.47	180		0.57	420
	0.52	660		0.63	600
Ave.	0.50	420	Ave.	0.60	510
1.0 mg/L O3 in O.D.F. PO4			1.0 mg/L O3 in O.D.F. Milli-Q		
	Applied O3, mg/L	Half-Life, s		Applied O3, mg/L	Half-Life, s
	1.51	340		1.01	480
	0.75	480		1.15	480
Ave.	1.13	410	Ave.	1.08	480

250 mL Erlenmeyer Flask					
0.5 mg/L O3 in O.D.F. PO4			0.5 mg/L O3 in O.D.F. Milli-Q		
	Applied O3, mg/L	Half-Life, s		Applied O3, mg/L	Half-Life, s
	0.55	840		0.51	2640
	0.65	780		0.59	1620
Ave.	0.60	810	Ave.	0.55	2130
1.0 mg/L O3 in O.D.F. PO4			1.0 mg/L O3 in O.D.F. Milli-Q		
	Applied O3, mg/L	Half-Life, s		Applied O3, mg/L	Half-Life, s
	1.04	1320		1.04	3360
	0.88	840		1.02	3120
Ave.	0.96	1080	Ave.	1.03	3240

2.0 L Reaction Vessel					
0.5 mg/L O3 in O.D.F. PO4			0.5 mg/L O3 in O.D.F. Milli-Q		
	Applied O3, mg/L	Half-Life, s		Applied O3, mg/L	Half-Life, s
	0.48	900		0.66	5880
	0.65	1020		0.60	6180
Ave.	0.57	960	Ave.	0.63	6030
1.0 mg/L O3 in O.D.F. PO4			1.0 mg/L O3 in O.D.F. Milli-Q		
	Applied O3, mg/L	Half-Life, s		Applied O3, mg/L	Half-Life, s
	0.99	1920		1.20	7500
	0.92	1620		1.35	10490
Ave.	0.96	1770	Ave.	1.28	8995

Table E.2 ANOVA for ozone decomposition Data Set 3

Source		Sum of Squares	d.f.	Mean Square	*F*-ratio
Between levels of factor	A	9,113.3	2	4,556.7	54.8*
	B	604.9	1	604.9	7.3
	C	5,878.6	1	5,878.6	70.7*
Interactions	AB	516.6	2	258.3	3.1
	AC	5,460.2	2	2,730.1	32.9*
	BC	205.0	1	205.0	2.5
Error	ABC	166.2	2	83.1	-
Total		21,944.8	11		

* $P \leq 0.05$;

A, size of ozone reactor;

B, applied ozone dose;

C, type of laboratory water.

Table E.3 Regression summary for Data Set B, assuming first-order ozone decomposition

Source	Sum of Squares	d.f.	Mean Square	*F*-ratio
Regression*	55.36	3	18.45	35.7*
Residual	6.21	12	0.52	-
Total	61.57	15		

* $P \leq 0.001$;
R^2 is 89.9 percent; R^2(adjusted) is 87.4 percent;
s is 0.7193 with 16 - 4 = 12 d.f.;
R^2, coefficient of determination;
s, standard deviation of the dependent variable about the regression line.

Table E.4 Regression coefficients for Data Set B, assuming first-order ozone decomposition

Variable	Coefficient	s.e. of coeff	t-ratio
Constant	-6.54	0.18	-36.4*
temperature x pH	0.31	0.18	1.7
pH	-1.80	0.18	-10.0*
temperature	-0.34	0.18	-1.9*

* $P \leq 0.10$;
s.e., standard error.

DATA SET B: Ozone Decomposition in 0.05M Phosphate Buffer

Trial 1 - 5°C, 0 NTU, pH 5.7, 250 mL Erlenmeyer reaction vessel

Time (s)	Absorbance (1/cm)	C (mg/L)	C/C_0 (mg/L)	log C/C_0
0	0.1221	1.78	1.000	0.000
30	0.1193	1.74	0.977	-0.010
60	0.1171	1.70	0.959	-0.018
90	0.1145	1.67	0.938	-0.028
120	0.1111	1.62	0.910	-0.041
150	0.1071	1.56	0.878	-0.057
180	0.1044	1.52	0.855	-0.068
210	0.1022	1.49	0.837	-0.077
240	0.1005	1.46	0.823	-0.085
270	0.0981	1.43	0.804	-0.095
300	0.0958	1.39	0.785	-0.105
330	0.0940	1.37	0.770	-0.113
360	0.0931	1.35	0.763	-0.118
390	0.0917	1.33	0.751	-0.124
420	0.0899	1.31	0.737	-0.133
450	0.0877	1.28	0.719	-0.143
480	0.0859	1.25	0.704	-0.153
510	0.0843	1.23	0.691	-0.161
540	0.0822	1.20	0.673	-0.172
570	0.0804	1.17	0.658	-0.182
600	0.0786	1.14	0.644	-0.191
630	0.0772	1.12	0.633	-0.199
660	0.0760	1.11	0.623	-0.206
690	0.0747	1.09	0.612	-0.213
720	0.0742	1.08	0.608	-0.216
750	0.0677	0.98	0.554	-0.256
780	0.0650	0.95	0.532	-0.274
810	0.0633	0.92	0.518	-0.285
840	0.0619	0.90	0.507	-0.295
870	0.0600	0.87	0.492	-0.308
900	0.0586	0.85	0.480	-0.318

DATA SET B: Ozone Decomposition in 0.05M Phosphate Buffer

Trial 2 - 5°C, 0 NTU, pH 5.7, 250 mL Erlenmeyer reaction vessel

Time (s)	Absorbance (1/cm)	C (mg/L)	C/C_o (mg/L)	log C/C_o
0	0.2043	2.97	1.000	0.000
30	0.2020	2.94	0.989	-0.005
60	0.1984	2.89	0.971	-0.013
90	0.1948	2.83	0.954	-0.020
120	0.1911	2.78	0.936	-0.029
150	0.1870	2.72	0.915	-0.038
180	0.1830	2.66	0.896	-0.048
210	0.1788	2.60	0.875	-0.058
240	0.1750	2.55	0.857	-0.067
270	0.1703	2.48	0.834	-0.079
300	0.1663	2.42	0.814	-0.089
330	0.1617	2.35	0.792	-0.101
360	0.1575	2.29	0.771	-0.113
390	0.1529	2.23	0.749	-0.126
420	0.1485	2.16	0.727	-0.138
450	0.1441	2.10	0.706	-0.151
480	0.1396	2.03	0.684	-0.165
510	0.1349	1.96	0.661	-0.180
540	0.1304	1.90	0.638	-0.195
570	0.1258	1.83	0.616	-0.210
600	0.1212	1.76	0.593	-0.227
630	0.1165	1.70	0.571	-0.244
660	0.1122	1.63	0.549	-0.260
690	0.1080	1.57	0.529	-0.277
720	0.1037	1.51	0.508	-0.294
750	0.0995	1.45	0.487	-0.312

DATA SET B: Ozone Decomposition in 0.05M Phosphate Buffer

Trial 3 - 22°C, 0 NTU, pH 5.7, 250 mL Erlenmeyer reaction vessel

Time (s)	Absorbance (1/cm)	C (mg/L)	C/C_o (mg/L)	log C/C_o
0	0.1167	1.70	1.000	0.000
30	0.1148	1.67	0.984	-0.007
60	0.1120	1.63	0.960	-0.018
90	0.1091	1.59	0.935	-0.029
120	0.1061	1.54	0.909	-0.041
150	0.1033	1.50	0.885	-0.053
180	0.1001	1.46	0.858	-0.067
210	0.0970	1.41	0.832	-0.080
240	0.0938	1.37	0.804	-0.095
270	0.0906	1.32	0.777	-0.110
300	0.0876	1.27	0.751	-0.125
330	0.0844	1.23	0.724	-0.141
360	0.0814	1.18	0.698	-0.156
390	0.0784	1.14	0.672	-0.173
420	0.0752	1.09	0.644	-0.191
450	0.0720	1.05	0.617	-0.210
480	0.0688	1.00	0.590	-0.229
510	0.0655	0.95	0.561	-0.251
540	0.0625	0.91	0.535	-0.271
570	0.0589	0.86	0.505	-0.297
600	0.0556	0.81	0.476	-0.322
630	0.0524	0.76	0.449	-0.348
660	0.0493	0.72	0.423	-0.374

DATA SET B: Ozone Decomposition in 0.05M Phosphate Buffer

Trial 4 - 22°C, 0 NTU, pH 5.7, 250 mL Erlenmeyer reaction vessel

Time (s)	Absorbance (1/cm)	C (mg/L)	C/C_o (mg/L)	log C/C_o
0	0.1842	2.68	1.000	0.000
30	0.1817	2.64	0.986	-0.006
60	0.1786	2.60	0.969	-0.014
90	0.1752	2.55	0.951	-0.022
120	0.1716	2.50	0.932	-0.031
150	0.1681	2.45	0.912	-0.040
180	0.1642	2.39	0.891	-0.050
210	0.1606	2.34	0.872	-0.060
240	0.1566	2.28	0.850	-0.071
270	0.1528	2.22	0.829	-0.081
300	0.1488	2.17	0.808	-0.093
330	0.1448	2.11	0.786	-0.105
360	0.1409	2.05	0.765	-0.117
390	0.1370	1.99	0.743	-0.129
420	0.1328	1.93	0.721	-0.142
450	0.1288	1.87	0.699	-0.156
480	0.1248	1.82	0.677	-0.169
510	0.1206	1.76	0.655	-0.184
540	0.1165	1.69	0.632	-0.199
570	0.1121	1.63	0.608	-0.216
600	0.1079	1.57	0.585	-0.232
630	0.1035	1.51	0.562	-0.250
660	0.0995	1.45	0.540	-0.268
690	0.0950	1.38	0.515	-0.288
720	0.0906	1.32	0.492	-0.308
750	0.0864	1.26	0.469	-0.329
780	0.0822	1.20	0.446	-0.350
810	0.0782	1.14	0.425	-0.372
840	0.0741	1.08	0.402	-0.396
870	0.0701	1.02	0.381	-0.420
900	0.0662	0.96	0.359	-0.445
930	0.0625	0.91	0.339	-0.469
960	0.0585	0.85	0.317	-0.498

DATA SET B: Ozone Decomposition in 0.05M Phosphate Buffer

Trial 5 - 5°C, 5 NTU, pH 5.7, 250 mL Erlenmeyer reaction vessel

Time (s)	Absorbance (1/cm)	C (mg/L)	C/C_o (mg/L)	log C/C_o
0	0.2475	1.43	1.000	0.000
30	0.2463	1.41	0.987	-0.006
60	0.2457	1.40	0.981	-0.008
90	0.2437	1.37	0.960	-0.018
120	0.2427	1.36	0.951	-0.022
150	0.2411	1.33	0.934	-0.030
180	0.2401	1.32	0.924	-0.034
210	0.2379	1.29	0.902	-0.045
240	0.2363	1.26	0.886	-0.053
270	0.2346	1.24	0.868	-0.061
300	0.2327	1.21	0.849	-0.071
330	0.2310	1.19	0.831	-0.080
360	0.2286	1.15	0.807	-0.093
390	0.2263	1.12	0.783	-0.106
420	0.2240	1.08	0.760	-0.119
450	0.2214	1.05	0.734	-0.134
480	0.2185	1.00	0.703	-0.153
510	0.2159	0.97	0.677	-0.169
540	0.2175	0.99	0.694	-0.159
570	0.2100	0.88	0.617	-0.209
600	0.2077	0.85	0.594	-0.227
630	0.2048	0.80	0.564	-0.249
660	0.2027	0.77	0.542	-0.266
690	0.2106	0 89	0.623	-0.205
720	0.1964	0.68	0.478	-0.320

DATA SET B: Ozone Decomposition in 0.05M Phosphate Buffer

Trial 6 - 5°C, 5 NTU, pH 5.7, 250 mL Erlenmeyer reaction vessel

Time (s)	Absorbance (1/cm)	C (mg/L)	C/C_o (mg/L)	log C/C_o
0	0.3502	3.11	1.000	0.000
30	0.3471	3.07	0.985	-0.006
60	0.3438	3.02	0.970	-0.013
90	0.3403	2.97	0.953	-0.021
120	0.3367	2.92	0.937	-0.028
150	0.3337	2.87	0.923	-0.035
180	0.3284	2.80	0.898	-0.047
210	0.3233	2.72	0.874	-0.058
240	0.3185	2.65	0.852	-0.070
270	0.3133	2.58	0.828	-0.082
300	0.3082	2.50	0.804	-0.095
330	0.3029	2.43	0.779	-0.109
360	0.2971	2.34	0.752	-0.124
390	0.2925	2.27	0.730	-0.137
420	0.2865	2.19	0.702	-0.153
450	0.2811	2.11	0.677	-0.169
480	0.2753	2.02	0.650	-0.187
510	0.2695	1.94	0.623	-0.205
540	0.2635	1.85	0.595	-0.225
570	0.2581	1.77	0.570	-0.244
600	0.2522	1.69	0.542	-0.266
630	0.2462	1.60	0.514	-0.289

DATA SET B: Ozone Decomposition in 0.05M Phosphate Buffer

Trial 7 - 22°C, 5 NTU, pH 5.7, 250 mL Erlenmeyer reaction vessel

Time (s)	Absorbance (1/cm)	C (mg/L)	C/C_o (mg/L)	log C/C_o
0	0.2477	1.47	1.000	0.000
30	0.2433	1.41	0.957	-0.019
60	0.2399	1.36	0.923	-0.035
90	0.2355	1.30	0.880	-0.056
120	0.2329	1.26	0.854	-0.069
150	0.2292	1.20	0.817	-0.088
180	0.2262	1.16	0.787	-0.104
210	0.2232	1.12	0.758	-0.120
240	0.2196	1.07	0.723	-0.141
270	0.2168	1.02	0.695	-0.158
300	0.2139	0.98	0.666	-0.177
330	0.2113	0.94	0.641	-0.193
360	0.2077	0.89	0.605	-0.218
390	0.2055	0.86	0.583	-0.234
420	0.2026	0.82	0.555	-0.256
450	0.1996	0.77	0.525	-0.280
480	0.1967	0.73	0.497	-0.304
510	0.1944	0.70	0.474	-0.325

DATA SET B: Ozone Decomposition in 0.05M Phosphate Buffer

Trial 8 - 22°C, 5 NTU, pH 5.7, 250 mL Erlenmeyer reaction vessel

Time (s)	Absorbance (1/cm)	C (mg/L)	C/C_o (mg/L)	log C/C_o
0	0.3385	2.93	1.000	0.000
30	0.3338	2.86	0.976	-0.010
60	0.3296	2.80	0.956	-0.020
90	0.3262	2.75	0.939	-0.027
120	0.3219	2.68	0.917	-0.038
150	0.3164	2.60	0.890	-0.051
180	0.3113	2.53	0.865	-0.063
210	0.3081	2.48	0.849	-0.071
240	0.3042	2.43	0.829	-0.081
270	0.2997	2.36	0.807	-0.093
300	0.2957	2.30	0.787	-0.104
330	0.2917	2.24	0.767	-0.115
360	0.2878	2.19	0.748	-0.126
390	0.2901	2.22	0.759	-0.120
420	0.2786	2.05	0.702	-0.154
450	0.2745	1.99	0.682	-0.166
480	0.2696	1.92	0.657	-0.182
510	0.2665	1.88	0.642	-0.193
540	0.2610	1.80	0.614	-0.212
570	0.2570	1.74	0.594	-0.226
600	0.2518	1.66	0.569	-0.245
630	0.2473	1.60	0.546	-0.263
660	0.2420	1.52	0.520	-0.284
690	0.2376	1.46	0.498	-0.303
720	0.2330	1.39	0.475	-0.323
750	0.2289	1.33	0.455	-0.342
780	0.2240	1.26	0.430	-0.366
810	0.2201	1.20	0.411	-0.386
840	0.2155	1.14	0.388	-0.411
870	0.2108	1.07	0.365	-0.438
900	0.2103	1.06	0.362	-0.441
930	0.2032	0.96	0.327	-0.486
960	0.2001	0.91	0.311	-0.507

DATA SET B: Ozone Decomposition in 0.05M Phosphate Buffer

Trial 9 - 5°C, 0 NTU, pH 7.6, 250 mL Erlenmeyer reaction vessel

Time (s)	Absorbance (1/cm)	C (mg/L)	C/C_o (mg/L)	log C/C_o
0	0.1026	1.49	1.000	0.000
30	0.0948	1.38	0.924	-0.034
60	0.0889	1.29	0.866	-0.062
90	0.0833	1.21	0.812	-0.090
120	0.0784	1.14	0.765	-0.117
150	0.0737	1.07	0.718	-0.144
180	0.0695	1.01	0.678	-0.169
210	0.0647	0.94	0.631	-0.200
240	0.0614	0.89	0.599	-0.223
270	0.0580	0.84	0.565	-0.248
300	0.0548	0.80	0.534	-0.272
330	0.0518	0.75	0.505	-0.297
360	0.0491	0.71	0.479	-0.320
390	0.0464	0.67	0.452	-0.345
420	0.0437	0.64	0.426	-0.370
450	0.0414	0.60	0.404	-0.394
480	0.0394	0.57	0.384	-0.416
510	0.0370	0.54	0.361	-0.442
540	0.0353	0.51	0.344	-0.463
570	0.0335	0.49	0.326	-0.486
600	0.0318	0.46	0.310	-0.509
630	0.0303	0.44	0.295	-0.530
660	0.0288	0.42	0.280	-0.552

DATA SET B: Ozone Decomposition in 0.05M Phosphate Buffer

Trial 10 - 5°C, 0 NTU, pH 7.6, 250 mL Erlenmeyer reaction vessel

Time (s)	Absorbance (1/cm)	C (mg/L)	C/C_0 (mg/L)	log C/C_0
0	0.2042	2.97	1.000	0.000
30	0.1858	2.70	0.910	-0.041
60	0.1711	2.49	0.838	-0.077
90	0.1587	2.31	0.777	-0.110
120	0.1475	2.15	0.722	-0.141
150	0.1381	2.01	0.676	-0.170
180	0.1293	1.88	0.633	-0.198
210	0.1224	1.78	0.599	-0.222
240	0.1132	1.65	0.554	-0.256
270	0.1176	1.71	0.576	-0.240
300	0.1119	1.63	0.548	-0.261
330	0.0951	1.38	0.466	-0.332
360	0.0899	1.31	0.440	-0.357
390	0.0828	1.20	0.405	-0.392
420	0.0778	1.13	0.381	-0.419
450	0.0733	1.07	0.359	-0.445
480	0.0835	1.22	0.409	-0.388
510	0.0646	0.94	0.316	-0.500
540	0.0608	0.88	0.297	-0.527

DATA SET B: Ozone Decomposition in 0.05M Phosphate Buffer

Trial 11 - 22°C, 0 NTU, pH 7.6, 250 mL Erlenmeyer reaction vessel

Time (s)	Absorbance (1/cm)	C (mg/L)	C/C_0 (mg/L)	log C/C_0
0	0.1208	1.76	1.000	0.000
30	0.1114	1.62	0.923	-0.035
60	0.1037	1.51	0.858	-0.066
90	0.0966	1.41	0.800	-0.097
120	0.0905	1.32	0.749	-0.126
150	0.0852	1.24	0.706	-0.151
180	0.0804	1.17	0.666	-0.177
210	0.0762	1.11	0.631	-0.200
240	0.0723	1.05	0.599	-0.223
270	0.0688	1.00	0.569	-0.245
300	0.0650	0.95	0.538	-0.269
330	0.0613	0.89	0.508	-0.294
360	0.0582	0.85	0.482	-0.317

DATA SET B: Ozone Decomposition in 0.05M Phosphate Buffer

Trial 12 - 22°C, 0 NTU, pH 7.6, 250 mL Erlenmeyer reaction vessel

Time (s)	Absorbance (1/cm)	C (mg/L)	C/C_o (mg/L)	log C/C_o
0	0.1595	2.32	1.000	0.000
30	0.1475	2.15	0.925	-0.034
60	0.1365	1.99	0.856	-0.068
90	0.1271	1.85	0.797	-0.099
120	0.1192	1.73	0.747	-0.126
150	0.1127	1.64	0.707	-0.151
180	0.1066	1.55	0.668	-0.175
210	0.1010	1.47	0.634	-0.198
240	0.0963	1.40	0.604	-0.219
270	0.0920	1.34	0.577	-0.239
300	0.0881	1.28	0.552	-0.258
330	0.0844	1.23	0.529	-0.277
360	0.0810	1.18	0.508	-0.294
390	0.0777	1.13	0.487	-0.312
420	0.0748	1.09	0.469	-0.329
450	0.0716	1.04	0.449	-0.347
480	0.0688	1.00	0.432	-0.365
510	0.0661	0.96	0.415	-0.382

DATA SET B: Ozone Decomposition in 0.05M Phosphate Buffer

Trial 13 - 5°C, 5 NTU, pH 7.6, 250 mL Erlenmeyer reaction vessel

Time (s)	Absorbance (1/cm)	C (mg/L)	C/C_0 (mg/L)	log C/C_0
0	0.2570	1.23	1.000	0.000
30	0.2509	1.14	0.927	-0.033
60	0.2457	1.07	0.866	-0.062
90	0.2413	1.00	0.814	-0.090
120	0.2378	0.95	0.772	-0.112
150	0.2339	0.89	0.726	-0.139
180	0.2300	0.84	0.680	-0.168
210	0.2270	0.79	0.644	-0.191
240	0.2242	0.75	0.612	-0.213
270	0.2219	0.72	0.585	-0.233
300	0.2197	0.69	0.559	-0.253
330	0.2171	0.65	0.528	-0.277
360	0.2152	0.62	0.505	-0.297
390	0.2130	0.59	0.479	-0.320
420	0.2110	0.56	0.455	-0.342
450	0.2092	0.53	0.434	-0.362
480	0.2078	0.51	0.417	-0.380
510	0.2062	0.49	0.399	-0.399
540	0.2044	0.46	0.377	-0.424
570	0.2034	0.45	0.365	-0.437
600	0.2018	0.43	0.346	-0.460
630	0.2003	0.40	0.329	-0.483

DATA SET B: Ozone Decomposition in 0.05M Phosphate Buffer

Trial 14 - 5°C, 5 NTU, pH 7.6, 250 mL Erlenmeyer reaction vessel

Time (s)	Absorbance (1/cm)	C (mg/L)	C/C_o (mg/L)	log C/C_o
0	0.3296	2.54	1.000	0.000
30	0.3236	2.45	0.966	-0.015
60	0.3158	2.34	0.921	-0.036
90	0.3084	2.23	0.878	-0.056
120	0.3014	2.13	0.838	-0.077
150	0.2947	2.03	0.800	-0.097
180	0.2885	1.94	0.764	-0.117
210	0.2822	1.85	0.728	-0.138
240	0.2761	1.76	0.693	-0.159
270	0.2698	1.67	0.657	-0.183
300	0.2644	1.59	0.626	-0.204
330	0.2577	1.49	0.588	-0.231
360	0.2518	1.40	0.554	-0.257
390	0.2464	1.33	0.522	-0.282
420	0.2401	1.23	0.486	-0.313
450	0.2350	1.16	0.457	-0.340
480	0.2297	1.08	0.427	-0.370
510	0.2247	1.01	0.398	-0.400
540	0.2201	0.94	0.372	-0.430
570	0.2160	0.88	0.348	-0.458

DATA SET B: Ozone Decomposition in 0.05M Phosphate Buffer

Trial 15 - 22°C, 5 NTU, pH 7.6, 250 mL Erlenmeyer reaction vessel

Time (s)	Absorbance (1/cm)	C (mg/L)	C/C_o (mg/L)	log C/C_o
0	0.2690	1.56	1.000	0.000
30	0.2593	1.42	0.910	-0.041
60	0.2509	1.29	0.831	-0.080
90	0.2438	1.19	0.764	-0.117
120	0.2373	1.10	0.704	-0.153
150	0.2331	1.03	0.665	-0.177
180	0.2294	0.98	0.630	-0.201
210	0.2252	0.92	0.591	-0.228
240	0.2216	0.87	0.557	-0.254
270	0.2180	0.81	0.523	-0.281
300	0.2160	0.79	0.505	-0.297
330	0.2130	0.74	0.477	-0.322
360	0.2108	0.71	0.456	-0.341
390	0.2087	0.68	0.436	-0.360
420	0.2068	0.65	0.418	-0.378
450	0.2042	0.61	0.395	-0.404
480	0.2000	0.55	0.355	-0.450
510	0.1989	0.54	0.345	-0.462
540	0.1957	0.49	0.315	-0.502

DATA SET B: Ozone Decomposition in 0.05M Phosphate Buffer

Trial 16 - 22°C, 5 NTU, pH 7.6, 250 mL Erlenmeyer reaction vessel

Time (s)	Absorbance (1/cm)	C (mg/L)	C/C_o (mg/L)	log C/C_o
0	0.3376	2.89	1.000	0.000
30	0.3145	2.55	0.884	-0.054
60	0.2970	2.30	0.796	-0.099
90	0.2839	2.11	0.730	-0.137
120	0.2737	1.96	0.678	-0.169
150	0.2651	1.83	0.635	-0.197
180	0.2585	1.74	0.601	-0.221
210	0.2522	1.65	0.570	-0.244
240	0.2473	1.57	0.545	-0.263
270	0.2416	1.49	0.516	-0.287
300	0.2370	1.43	0.494	-0.307
330	0.2324	1.36	0.470	-0.328
360	0.2286	1.30	0.451	-0.346

APPENDIX F: SUMMARY OF TREATMENT CONDITIONS IN THE EXPERIMENTAL TRIALS

Table F.1 - Treatment conditions in the viability comparison experiment

Reference		Ozone (mg/L)				Experimental Conditions					Infectivity Results								
Exp.	Trial	Applied (mg/L)	Peak Obs. (mg/L)	Residual (mg/L)	Utilized (mg/L)	Water	Temp. (°C)	Time (min)	pH	Turbidity (NTU)	No. of Mice per trial	Inoculum cysts/mouse	Days After Infection (No. of mice cyst pos.) 3	4	5	6	7	8	8T	N/No (log)
33	A1	0.62	0.47	0.3	0.32	PB	22	5	6.7	0	5	1.2E+05	0	0	2	2	2	4	4	4.2
33	A2	0.64	0.52	0.4	0.24	PB	22	5	6.7	0	5	1.2E+05	0	0	2	3	3	4	4	4.2
33	B1	0.26	0.18	0.1	0.16	PB	22	5	6.7	0	5	1.3E+05	0	0	3	5	-	-	-	3.1
33	B2	0.30	0.22	0.1	0.20	PB	22	5	6.7	0	4	1.6E+05	0	0	1	4	-	-	-	3.1
33	C1	2.55	2.14	1.3	1.25	PB	22	5	6.7	0	5	1.4E+05	0	0	0	0	1	1	1	4.4
33	C2	0.13	0.07	0.04	0.09	PB	22	0.58	6.7	0	5	1.3E+05	2	5	-	-	-	-	-	1.2
33	C3	0.27	0.17	0.2	0.07	PB	22	0.25	6.7	0	-	-	-	-	-	-	-	-	-	-
33	C4	0.13	0.07	0.1	0.03	PB	22	0.33	6.7	0	-	-	-	-	-	-	-	-	-	-
33	D1	0.28	0.15	0.2	0.08	PB	22	0.58	6.7	0	5	7.2E+04	2	2	5	-	-	-	-	2.2
33	D2	0.23	0.15	0.1	0.13	PB	22	1	6.7	0	-	-	-	-	-	-	-	-	-	-
33	D3	0.21	0.12	0.1	0.11	PB	22	2	6.7	0	-	-	-	-	-	-	-	-	-	-
33	D4	0.18	0.07	0.02	0.16	PB	22	1.53	6.7	0	-	-	-	-	-	-	-	-	-	-
33	AC	-	-	-	-	PB	22	5	6.7	0	5	1.2E+05	5	-	-	-	-	-	-	0.0
33	BC	-	-	-	-	PB	22	5	6.7	0	5	1.2E+05	5	-	-	-	-	-	-	0.0
33	CC	-	-	-	-	PB	22	5	6.7	0	5	1.6E+05	5	-	-	-	-	-	-	0.0
33	DC	-	-	-	-	PB	22	2	6.7	0	2	1.5E+05	2	-	-	-	-	-	-	0.0

Reference		Ozone (mg/L)				FDA-EB Staining Results							Excystation Results						FDA	Infect.
						Cyst Counts				Stained	% of Stained	N/No					%	N/No	N/No	N/No
Exp.	Trial	Applied (mg/L)	Peak Obs. (mg/L)	Residual (mg/L)	Utilized (mg/L)	FDA-pos.	EB-pos.	Not stained	Total	Cyst Total	Cysts that are FDA-positive	(log)	ECW	PET	IC	Total	Excyst.	(log)	(log)	(log)
33	A1	0.62	0.47	0.3	0.32	0	140	3	143	140	0.0	>2.1	0	0	669	669	0.0	>2.8	>2.1	4.2
33	A2	0.64	0.52	0.4	0.24	0	165	4	169	165	0.0	>2.2	0	0	546	546	0.0	>2.7	>2.2	4.2
33	B1	0.26	0.18	0.1	0.16	4	962	52	1018	966	0.41	2.3	0	1	999	1000	0.1	3.0	2.3	3.1
33	B2	0.30	0.22	0.1	0.20	1	719	26	746	720	0.14	2.7	0	0	1000	1000	0.0	>3.0	2.7	3.1
33	C1	2.55	2.14	1.3	1.25	0	566	109	675	566	0.0	>2.8	0	0	782	782	0.0	>2.9	>2.8	4.4
33	C2	0.13	0.07	0.04	0.09	332	263	90	685	595	55.8	0.2	37	252	711	1000	28.9	0.5	0.2	1.2
33	C3	0.27	0.17	0.2	0.07	533	212	108	853	745	71.5	0.1	34	171	805	1000	19.5	0.7	0.1	-
33	C4	0.13	0.07	0.1	0.03	566	195	129	890	761	74.4	0.04	168	387	445	1000	55.5	0.2	0.04	-
33	D1	0.28	0.15	0.2	0.08	109	331	13	453	440	24.8	0.5	4	11	985	1000	1.5	1.8	0.5	2.2
33	D2	0.23	0.15	0.1	0.13	122	708	17	847	830	14.7	0.7	1	7	992	1000	0.8	2.1	0.7	-
33	D3	0.21	0.12	0.1	0.11	2	689	52	743	691	0.29	2.4	1	9	990	1000	1.0	2.0	2.4	-
33	D4	0.18	0.07	0.02	0.16	197	565	15	777	762	25.9	0.5	1	3	996	1000	0.4	2.4	0.5	-
33	AC	-	-	-	-	64	18	115	197	82	78.0	0.0	877	86	37	1000	96.3	0.0	0.0	0.0
33	BC	-	-	-	-	95	30	209	334	125	76.0	0.0	881	110	9	1000	99.1	0.0	0.0	0.0
33	CC	-	-	-	-	139	32	173	344	171	81.3	0.0	882	76	42	1000	95.8	0.0	0.0	0.0
33	DC	-	-	-	-	175	45	150	370	220	79.5	0.0	831	99	70	1000	93.0	0.0	0.0	0.0

C-control, PB is 0.05 M phosphate buffer, FDA-fluorescein diacetate, EB-ethidium bromide, ECW-empty cyst walls, PET-partially excysted trophozoites, IC-intact cysts. Peak Obs. ozone is the highest observed ozone concentration using the diode-array spectrophotometer.

Table F.2 - Treatment conditions in the screening experiment

Reference		Ozone (mg/L)				Experimental Conditions					Infectivity Results								
Exp.	Trial	Applied (mg/L)	Peak Obs. (mg/L)	Residual (mg/L)	Utilized (mg/L)	Water	Temp. (°C)	Time (min)	pH	Turbidity (NTU)	No. of Mice per trial	Inoculum Size (cysts/mouse)	Days After Infection - No. of positive mice 3	4	5	6	7	8	ST	N/No (log)
30	1	1.58	1.47	1.09	0.49	PB	5	2	7.6	0	5	7.4E+03	-	0	0	1	1	1	2	4.3
30	2	3.35	3.09	2.97	0.38	PB	5	2	5.7	0	5	5.0E+02	-	0	0	0	-	0	0	>4.4
30	3	1.61	1.34	1.28	0.33	PB	22	2	5.7	0	4	1.2E+04	-	0	2	4	-	-	-	2.4
30	4	2.73	2.10	1.36	1.37	PB	22	2	7.6	0	5	1.1E+04	-	0	1	1	2	2	2	3.4
30	5	1.76	1.77	1.29	0.47	PB	5	7	5.7	0	5	3.2E+04	-	0	3	4	4	5	-	3.5
30	R5	1.48	1.48	1.14	0.34	PB	5	7	5.7	0	5	8.0E+03	-	0	2	3	4	4	5	3.5
30	6	2.90	2.81	1.64	1.26	PB	5	7	7.6	0	5	2.8E+03	-	0	2	3	3	3	5	3.8
30	7	1.54	1.48	0.29	1.25	PB	22	7	7.6	0	5	3.2E+04	-	0	1	2	2	2	3	4.0
30	8	3.15	2.97	1.77	1.38	PB	22	7	5.7	0	5	6.0E+03	-	1	3	3	3	4	5	3.2
30	R8	2.88	2.94	1.95	0.93	PB	22	7	5.7	0	5	1.6E+04	-	0	2	3	3	5	5	3.1
30	9	1.60	1.38	1.21	0.39	PB	5	2	5.7	5	5	5.0E+04	-	0	2	3	4	4	5	3.7
30	10	2.88	2.60	1.95	0.93	PB	5	2	7.6	5	5	2.0E+05	-	0	1	3	3	4	4	4.4
30	R10	2.54	2.20	1.57	0.97	PB	5	2	7.6	5	5	1.2E+05	-	0	2	5	-	-	-	3.1
30	11	1.75	1.62	1.17	0.58	PB	22	2	7.6	5	5	8.4E+03	-	0	0	1	1	1	3	4.0
30	R11	1.56	1.31	0.73	0.83	PB	22	2	7.6	5	5	1.1E+05	-	0	0	0	0	0	0	>5.0
30	12	3.15	2.87	2.76	0.39	PB	22	2	5.7	5	5	4.4E+04	-	0	0	0	0	0	0	>4.3
30	13	1.56	1.09	0.55	1.01	PB	5	7	7.6	5	5	5.0E+04	-	0	1	1	-	3	5	3.8
30	14	2.71	2.47	2.22	0.49	PB	5	7	5.7	5	5	4.0E+04	-	0	0	1	1	1	3	4.1
30	15	1.46	1.43	0.83	0.63	PB	22	7	5.7	5	5	4.6E+04	-	0	3	5	-	-	-	2.5
30	16	3.27	2.61	0.94	2.33	PB	22	7	7.6	5	5	3.6E+04	-	0	0	0	-	0	0	>4.4
30	C1	-	-	-	-	PB	22	5	5.7	0	3	8.4E+02	-	2	3	-	-	-	-	0.0
30	RC1	-	-	-	-	PB	22	5	5.7	0	5	1.4E+04	-	5	-	-	-	-	-	0.0
30	C2	-	-	-	-	PB	22	5	5.7	5	5	2.0E+04	-	5	-	-	-	-	-	0.0
30	C3	-	-	-	-	PB	22	5	7.6	0	5	2.4E+04	-	5	-	-	-	-	-	0.0
30	C4	-	-	-	-	PB	22	5	7.6	5	5	2.6E+04	-	5	-	-	-	-	-	0.0
30	RC4	-	-	-	-	PB	22	5	7.6	5	5	1.1E+04	-	5	-	-	-	-	-	0.0
30	C5	-	-	-	-	PB	22	5	5.7	0	5	2.6E+04	-	5	-	-	-	-	-	0.0
30	C6	-	-	-	-	PB	22	5	5.7	5	5	3.2E+04	-	5	-	-	-	-	-	0.0
30	C7	-	-	-	-	PB	22	5	7.6	0	5	3.5E+04	-	5	-	-	-	-	-	0.0
30	C8	-	-	-	-	PB	22	5	7.6	5	5	2.4E+04	-	5	-	-	-	-	-	0.0

C-control, PB is 0.05 M phosphate buffer.
Peak Obs. ozone is the highest observed ozone concentration using the diode-array spectrophotometer.

Table F.3 - Treatment conditions in the ozone and ozone-hydrogen peroxide, specific ozone residual and G. lamblia experiments

Reference		Ozone (mg/L)				Experimental Conditions					Infectivity Results								
Exp.	Trial	Applied (mg/L)	Peak Obs. (mg/L)	Residual (mg/L)	Utilized (mg/L)	Water	Temp. (°C)	Time (min)	pH	Turbidity (NTU)	No. of Mice per trial	Inoculum Size (cysts/mouse)	Days After Infection - No. of positive mice 3	4	5	6	7	8	8T	N/No (log)
31	1	1.55	0.85	0.0	1.55	PBP	22	2	5.7	0	5	2.0E+04	-	0	1	2	2	2	2	2.5
31	2	1.62	0.74	0.0	1.62	PBP	22	2	5.7	0	5	5.0E+04	0	0	5	-	-	-	-	2.2
31	3	1.68	0.94	0.0	1.68	PBP	22	2	5.7	0	5	6.5E+04	1	1	5	-	-	-	-	2.2
31	4	1.62	1.44	1.4	0.18	PCB	22	2	5.7	0	5	1.1E+03	-	0	1	1	1	2	2	3.4
31	5	1.68	1.58	1.5	0.10	PCB	22	2	5.7	0	5	5.0E+04	0	1	3	4	4	4	4	3.3
31	6	1.84	1.71	1.6	0.13	PCB	22	2	5.7	0	5	4.2E+04	0	0	0	0	1	1	1	4.9
31	C1	-	-	-	-	PBP	22	2	5.7	0	5	1.4E+04	-	5	-	-	-	-	-	0.0
31	C2	-	-	-	-	PBP	22	2	5.7	0	5	1.5E+05	5	-	-	-	-	-	-	0.0
31	C3	-	-	-	-	PCB	22	2	5.7	0	5	1.1E+04	-	5	-	-	-	-	-	0.0
31	C4	-	-	-	-	PCB	22	2	5.7	0	4	1.6E+05	4	-	-	-	-	-	-	0.0

Reference		Ozone (mg/L)				Experimental Conditions					Infectivity Results								
Exp.	Trial	Applied (mg/L)	Peak Obs. (mg/L)	Residual (mg/L)	Utilized (mg/L)	Water	Temp. (°C)	Time (min)	pH	Turbidity (NTU)	No. of Mice per trial	Inoculum Size (cysts/mouse)	Days After Infection - No. of positive mice 3	4	5	6	7	8	8T	N/No (log)
36	1	0.59	0.46	0.35	0.24	PB	5	5	5.7	0	4	6.6E+04	0	2	4	-	-	-	-	2.1
36	2	0.59	0.51	0.35	0.24	PB	22	5	5.7	0	5	9.4E+03	0	0	0	0	0	0	0	>5.1
36	3	0.73	0.57	0.40	0.33	PB	5	5	7.6	0	4	1.0E+05	0	0	0	1	2	2	4	4.5
36	4	0.82	0.70	0.36	0.46	PB	22	5	7.6	0	5	6.4E+04	0	0	0	0	0	0	0	>5.1
36	5	0.66	0.54	0.37	0.29	PB	14	5	6.7	0	5	1.1E+05	0	0	4	4	5	5	-	3.6
36	6	0.71	0.57	0.37	0.34	PB	15	5	6.7	0	5	1.4E+05	0	0	1	4	5	5	-	3.6
36	C	-	-	-	-	PB	22	5	7.6	0	5	1.3E+05	5	-	-	-	-	-	-	0.0
41	1	0.42	0.40	0.39	0.03	PB	5	1	5.7	0	5	1.9E+04	0	1	4	5	-	-	-	3.1
41	2	0.43	0.40	0.40	0.03	PB	5	1	7.6	0	5	8.6E+04	0	0	2	5	-	-	-	3.1
41	3	0.47	0.39	0.38	0.09	PB	5	2	7.6	0	5	8.4E+04	0	0	1	1	3	3	3	3.8
41	4	0.48	0.42	0.41	0.07	PB	14	2	6.7	0	5	1.0E+05	0	2	4	5	-	-	-	3.1
41	5	0.77	0.77	0.42	0.35	PB	14	7	6.7	0	5	1.0E+05	0	0	1	2	2	2	2	3.5
41	6	0.53	0.48	0.41	0.12	PB	22	2	5.7	0	5	9.2E+04	0	0	2	4	4	4	4	3.2
41	C	-	-	-	-	PB	22	2	6.7	0	4	1.3E+05	4	-	-	-	-	-	-	0.0

Reference		Ozone (mg/L)				Experimental Conditions					Infectivity Results							
Exp.	Trial	Applied (mg/L)	Peak Obs. (mg/L)	Residual (mg/L)	Utilized (mg/L)	Water	Temp. (°C)	Time (min)	pH	Turbidity (NTU)	Gerbils per trial	Inoculum Size (cysts/gerbil)	Days After Infection - No. of positive gerbils 10	11	12	13	14	14 trophozoites
43	1	1.44	1.38	0.84	0.60	PB	22	5	6.8	0	5	7.8E+04	0	0	0	0	0	0
43	2	0.95	0.78	0.53	0.42	PB	22	5	6.8	0	5	1.7E+04	0	0	0	0	0	0
43	3	0.54	0.34	0.20	0.34	PB	22	2	6.8	0	4	2.0E+04	1	1	1	1	1	1
43	C	-	-	-	-	PB	22	5	6.8	0	5	6.2E+04	1	5	-	-	-	-

C-control, PBP is 0.05 M phosphate buffer with 10:1 peroxide to ozone ratio. PCB is 0.05 M phosphate-0.01 M carbonate buffer, PB is 0.05 M phosphate buffer.
Peak Obs. ozone is the highest observed ozone concentration using the diode-array spectrophotometer.

Table F.4 - Treatment conditions in the natural waters experiment

Reference			Ozone (mg/L)			Experimental Conditions						Infectivity Results								
Exp.	Trial	Date	Applied (mg/L)	Residua (mg/L)	Utilized (mg/L)	Scale	Water	Temp. (°C)	Time (min)	pH	Turbidity (NTU)	No. of Mice per trial	Inoculum Size (cysts/mouse)	Days After Infection - No. of positive mice 3	4	5	6	7	8	8T	N/No (log)
39A	1	14-01-91	0.96	0.25	0.71	1	NSR	5	2	8.3	1.5	5	5.4E+04	0	0	1	1	1	1	1	2.7
39A	2	14-01-91	2.58	0.70	1.88	1	NSR	22	2	8.3	1.5	4	2.4E+05	0	0	0	1	1	1	1	3.7
39A	3	14-01-91	2.70	0.83	1.87	1	NSR	5	5	8.3	1.5	5	7.2E+04	0	0	0	0	0	0	0	>5.0
39A	4	14-01-91	3.03	0.24	2.79	1	NSR	22	5	8.3	1.5	5	2.2E+05	0	4	5	-	-	-	-	2
39A	C	14-01-91	-	-	-	1	NSR	22	5	8.3	1.5	5	9.6E+04	5	-	-	-	-	-	-	0.0
39B	1	13-02-91	0.81	0.16	0.65	1	NSR	1	2	8.1	2.6	5	1.6E+05	0	0	1	4	5	-	-	3.7
39B	2	13-02-91	2.30	0.67	1.63	1	NSR	1	5	8.1	2.6	5	1.3E+05	0	0	0	1	1	2	2	4.6
39B	3	13-02-91	1.71	0.30	1.41	1	NSR	1	5	8.1	2.6	5	9.8E+03	0	0	0	0	0	0	0	>5.2
39B	4	13-02-91	1.67	0.69	0.98	1	NSR	1	2	8.1	2.6	5	3.3E+05	0	2	3	5	-	-	-	3.2
39B	C	13-02-91	-	-	-	1	NSR	1	2	8.1	2.6	4	1.7E+05	4	-	-	-	-	-	-	0.0
39C	1	22-02-91	2.10	0.65	1.45	1	GVRD	5	5	6.3	3.8	4	5.7E+04	0	0	1	3	3	3	4	4.1
39C	2	22-02-91	1.09	0.15	0.94	1	GVRD	5	2	6.3	3.8	5	1.5E+05	0	2	5	-	-	-	-	2.1
39C	3	22-02-91	2.62	0.35	2.27	1	GVRD	22	5	6.3	3.8	5	2.5E+05	0	0	0	0	0	0	0	>5.1
39C	4	22-02-91	2.37	0.83	1.54	1	GVRD	22	2	6.3	3.8	5	6.6E+04	0	0	4	5	-	-	-	3.1
39C	C	22-02-91	-	-	-	1	GVRD	22	2	6.3	3.8	4	1.2E+05	4	-	-	-	-	-	-	0.0
39D	1	13-03-91	1.28	0.37	0.91	1	GVRD	5	4	6.3	3.9	5	2.8E+04	0	0	0	1	1	1	1	3.1
39D	2	13-03-91	1.66	0.76	0.90	1	GVRD	5	4	6.3	3.9	5	5.4E+04	0	0	0	2	2	2	2	3.1
39D	3	13-03-91	2.05	0.77	1.28	1	GVRD	5	10	6.3	3.9	5	1.0E+04	0	0	0	4	4	5	-	3.0
39D	4	13-03-91	1.71	0.34	1.37	1	GVRD	5	10	6.3	3.9	4	3.6E+04	0	0	0	1	1	1	1	3.3
39D	C	13-03-91	-	-	-	1	GVRD	5	4	6.3	3.9	5	1.2E+04	2	5	-	-	-	-	-	0.0
42	A	14-11-90	1.68	0.70	0.98	2	NSR	22	6.3	8.2	6.0	4	1.0E+04	-	0	0	0	1	1	1	3.1
42	B	14-11-90	0.89	0.38	0.51	2	NSR	22	3.2	8.2	6.0	4	1.0E+04	-	0	1	1	1	1	2	1.6
42	C	14-11-90	-	-	-	2	NSR	22	-	8.2	6.0	3	1.0E+04	-	3	-	-	-	-	-	0.0
40	1	31-01-91	1.47	0.21	1.26	2	NSR	22	4	8.3	3.0	5	1.0E+05	0	0	1	1	1	1	1	2.7
40	2	31-01-91	2.44	0.35	2.09	2	NSR	22	6	8.3	3.0	4	1.0E+05	0	0	0	1	1	1	1	3.6
40	3	31-01-91	3.67	0.80	2.87	2	NSR	22	10	8.3	3.0	4	1.0E+05	0	0	0	0	0	0	0	>5.0
40	4	31-01-91	4.95	1.23	3.72	2	NSR	22	13.5	8.3	3.0	5	1.0E+05	0	0	0	0	0	0	0	>5.0
40	C	31-01-91	-	-	-	2	NSR	22	-	8.3	3.0	4	1.0E+05	4	4	-	-	-	-	-	0.0

C-control, NSR-North Saskatchewan River, GVRD-Greater Vancouver Regional District; Scale-1, bench-scale, 2, pilot-scale.

Table F.5 - Treatment conditions for heterotrophic plate count (HPC) bacteria at bench- and pilot-scale

Exp.&Trial	Water&Date	Scale	HPCkill	G.muriskill	App.O3	DOC	O3:DOC	pH	Turb	Temp	Conduct.	Cont.Time	Res.O3
42B	NSR141190	2		1.6	0.89	2.46	0.36	8.2	6.0	22	325	3.2	0.38
42A		2		3.1	1.68	2.46	0.68	8.2	6.0	22	325	6.3	0.7
39A1	NSR140191	1		2.7	0.96	0.99	0.97	8.3	1.5	5	310	2.0	0.25
39A2		1		3.7	2.58	0.99	2.61	8.3	1.5	22	310	2.0	0.70
39A3		1		>5	2.70	0.99	2.73	8.3	1.5	5	310	5.0	0.83
39A4		1		2.0	3.03	0.99	3.06	8.3	1.5	22	310	5.0	0.24
40-C	NSR310191	2			0.00	1.62	0.00	8.3	3.0	22	280	0.3	0.00
40-1		2		2.7	1.47	1.62	0.91	8.3	3.0	22	280	4.0	0.21
40-2		2		3.6	2.44	1.62	1.51	8.3	3.0	22	280	6.0	0.35
40-3		2		>5	3.67	1.62	2.27	8.3	3.0	22	280	10.0	0.80
40-4		2		>5	4.95	1.62	3.06	8.3	3.0	22	280	13.5	1.23
44-C	NSR130291	2	0.00		0.00	1.61	0.00	8.1	2.6	11	265	0.0	0.00
44-P3		2	0.00		0.14	1.61	0.09	8.1	2.6	11	265	1.8	0.00
44-P5		2	0.45		0.28	1.61	0.17	8.1	2.6	11	265	3.6	0.02
44-P6		2	1.08		0.34	1.61	0.21	8.1	2.6	11	265	4.4	0.05
44-P7		2	1.21		0.45	1.61	0.28	8.1	2.6	11	265	5.7	0.09
39B1		1		3.7	0.81	1.61	0.50	8.1	2.6	1	300	2.0	0.16
39B2		1		4.6	2.30	1.61	1.43	8.1	2.6	1	300	5.0	0.67
39B3		1		>5.2	1.71	1.61	1.06	8.1	2.6	1	300	5.0	0.30
39B4		1		3.2	1.67	1.61	1.04	8.1	2.6	1	300	2.0	0.69
45-1	NSR270291	1	1.84		0.22	1.20	0.18	8.0	13.0	22	298	0.3	0.00
45-2		1	2.23		0.22	1.20	0.18	8.0	1.5	22	298	0.3	0.01
45-3		1	1.93		0.21	1.20	0.18	7.5	6.7	22	298	0.3	0.00
45-4		1	1.76		0.22	1.20	0.18	7.0	14.0	22	298	0.3	0.00
45-5		1	2.20		0.22	1.20	0.18	7.5	6.8	22	298	0.3	0.00
45-6		1	2.17		0.22	1.20	0.18	7.0	1.3	22	298	0.3	0.00
45-7		1	1.23		0.21	1.20	0.18	8.0	1.3	22	298	0.5	0.00
45-8		1	1.56		0.22	1.20	0.18	8.1	7.3	22	298	0.5	0.00
45-9		1	1.42		0.22	1.20	0.18	8.1	7.2	22	298	0.5	0.00
45-10		1	1.87		0.21	1.20	0.18	8.0	1.3	22	298	0.5	0.00
45-11		1	1.23		0.22	1.20	0.18	8.0	20.0	22	298	0.5	0.00

Table F.5 - Treatment conditions for heterotrophic plate count (HPC) bacteria at bench- and pilot-scale, continued

Exp.&Trial	Water&Date	Scale	HPCkill	G.muriskill	App.O3	DOC	O3:DOC	pH	Turb	Temp	Conduct.	Cont.Time	Res.O3
45-12	NSR270291	1	1.74		0.21	1.20	0.18	8.0	19.0	22	298	0.5	0.00
45-13		1	1.85		0.22	1.20	0.19	8.0	1.5	22	298	0.5	0.00
45-14		1	0.17		0.04	1.20	0.04	8.0	1.5	22	298	0.5	0.00
45-15		1	2.64		0.23	1.20	0.19	7.0	1.5	22	298	0.5	0.00
45-16		1	0.12		0.04	1.20	0.04	7.0	1.5	22	298	0.5	0.00
45-17		1	0.37		0.14	1.20	0.11	7.5	1.5	22	298	0.5	0.00
45-18		1	0.33		0.13	1.20	0.11	7.5	1.5	22	298	0.5	0.00
45-20		1	1.06		0.05	1.20	0.04	8.0	14.0	22	298	0.5	0.00
45-21		1	1.23		0.23	1.20	0.19	8.0	2.5	22	298	0.5	0.00
45-23		1	0.30		0.13	1.20	0.11	8.0	6.4	22	298	0.5	0.00
45-24		1	0.32		0.13	1.20	0.11	8.0	6.4	22	298	0.5	0.00
45-25		1	1.25		0.22	1.20	0.18	8.0	14.0	22	298	0.5	0.00
46-7	NSR220491	1	2.09		0.84	2.96	0.28	8.0	9.3	22	315	0.5	0.00
46-8		1	2.82		0.86	2.22	0.39	8.0	7.1	22	315	0.5	0.11
46-10		1	2.30		0.86	2.22	0.39	8.0	6.9	22	315	0.5	0.08
46-13		1	3.06		1.42	2.10	0.68	8.1	14.0	22	315	0.5	0.21
46-14		1	3.17		1.42	2.88	0.49	8.1	20.0	22	315	0.5	0.08
46-15		1	3.65		1.47	1.34	1.10	7.9	7.8	4	315	0.5	0.86
46-16		1	3.48		1.45	2.10	0.69	8.0	14.0	22	315	0.5	0.25
46-18		1	3.21		1.55	1.34	1.16	7.9	8.5	22	315	0.5	0.58
39C1	GVRD220291	1		4.1	2.10	9.15	0.23	6.3	3.8	5	10	5.0	0.65
39C2		1		2.1	1.09	9.15	0.12	6.3	3.8	5	10	2.0	0.15
39C3		1		>5.1	2.62	9.15	0.29	6.3	3.8	22	10	5.0	0.35
39C4		1		3.1	2.37	9.15	0.26	6.3	3.8	22	10	2.0	0.83
39D1	GVRD130391	1		3.1	1.28	9.15	0.14	6.3	3.9	5	10	4.0	0.37
39D2		1		3.1	1.66	9.15	0.18	6.3	3.9	5	10	4.0	0.76
39D3		1		3.0	2.05	9.15	0.22	6.3	3.9	5	10	10.0	0.77
39D4		1		3.3	1.71	9.15	0.19	6.3	3.9	5	10	10.0	0.34

C-control, NSR-North Saskatchewan River, GVRD-Greater Vancouver Regional District; Scale-1, bench-scale, 2, pilot-scale.

APPENDIX G: HEMOCYTOMETER COUNT DATA AND ANIMAL RESPONSES IN THE VIABILITY COMPARISON EXPERIMENT

Table G.1 Viability comparison experiment: Ozone inactivation of *G. muris* as estimated by fluorescein diacetate-ethidium bromide staining (pH 6.7; 22°C).

Trial	Cyst Counts				Stained Cyst Total	Stained Cysts that are FDA-positive(%)	Inactivation (log N/N_0)†
	FDA-positive	EB-positive	Un-stained	Total			
A1	0	140	3	143	140	0.0	>2.1
A2	0	165	4	169	165	0.0	>2.2
B1	4	962	52	1018	966	0.41	2.3
B2	1	719	26	746	720	0.14	2.7
C1	0	566	109	675	566	0.0	>2.8
C2	332	263	90	685	595	55.8	0.2
C3	533	212	108	853	745	71.5	0.006
C4	566	195	129	890	761	74.4	0.004
D1	109	331	13	453	440	24.8	0.5
D2	122	708	17	847	830	14.7	0.7
D3	2	689	52	743	691	0.29	2.4
D4	197	565	15	777	762	25.9	0.5
AC*	64	18	115	197	82	78.0	-
BC	95	30	209	334	125	76.0	-
CC	139	32	173	344	171	81.3	-
DC	175	45	150	370	220	79.5	-

† >, detection limit;

* control, cyst batch A;

FDA, fluorescein diacetate; EB, ethidium bromide.

Table G.2 Viability comparison experiment: Ozone inactivation of *G. muris* as estimated by *in vitro* excystation (pH 6.7; 22°C).

Trial	Cyst Counts				% excystation	Inactivation (log N/N_0)†
	ECW	PET	IC	Total		
A1	0	0	669	669	0.0	>2.8
A2	0	0	546	546	0.0	>2.7
B1	0	1	999	1000	0.1	3.0
B2	0	0	1000	1000	0.0	>3.0
C1	0	0	782	782	0.0	>2.9
C2	37	252	711	1000	28.9	0.5
C3	24	171	805	1000	19.5	0.7
C4	168	387	445	1000	55.5	0.2
D1	4	11	985	1000	1.5	1.8
D2	1	7	992	1000	0.8	2.1
D3	1	9	990	1000	1.0	2.0
D4	1	3	996	1000	0.4	2.4
AC*	877	86	37	1000	96.3	-
BC	881	110	9	1000	99.1	-
CC	882	76	42	1000	95.8	-
DC	831	99	70	1000	93.0	-

† >, detection limit;

* control, cyst batch A;

ECW, empty cyst walls; PET, partially excysted trophozoites; IC, intact cysts.

Table G.3 Viability comparison experiment: Number of C3H/HeN mice infected with *G. muris* as a function of days after infection and inoculum size

Trial	No. of mice in trial	Inoculum (cysts per mouse)	Days After Infection† (No. of mice positive for cysts) 3	4	5	6	7	8	Inactivation (log N/N_O)
A1	5	1.2E+05	0	0	2	2	2	4	4.2
A2	5	1.2E+05	0	0	2	3	3	4	4.2
B1	5	1.3E+05	0	0	3	5	-	-	3.1
B2	4	1.6E+05	0	0	1	4	-	-	3.1
C1	5	1.4E+05	0	0	0	0	1	1	4.4
C2	5	1.3E+05	2	5	-	-	-	-	1.2
D1	5	7.2E+04	2	2	5	-	-	-	2.2
AC*	5	1.2E+05	5	-	-	-	-	-	-
BC	5	1.2E+05	5	-	-	-	-	-	-
CC	5	1.6E+05	5	-	-	-	-	-	-
DC	2	1.5E+05	2	-	-	-	-	-	-

† Data collected until day 8 or when all mice in trial became positive. No trophozoites were detected on day 8 in mice that were negative for cysts.

* control, cyst batch A.

APPENDIX H: SUMMARY OF ANIMAL RESPONSES IN THE EXPERIMENTAL TRIALS

Table H.1 Ozone and ozone hydrogen-peroxide experiment: Number of C3H/HeN mice infected with *G. muris* as a function of days after infection and inoculum size

Trial	Residual Ozone at 2 min (mg/L)	Buffer	Inoculum (cysts per mouse)	Days After Infection (No. of mice positive for cysts) 3	4	5	6	7	8	8T#	Inactivation (log N/N_O)
1	0.0	PBP	2.0E+04	-	0	1	2	2	2	2	2.5
2	0.0	PBP	5.0E+04	0	0	5	-	-	-	-	2.2
3	0.0	PBP	6.5E+04	1	1	5	-	-	-	-	2.2
4	1.4	PCB	1.1E+03	-	0	1	1	1	2	2	3.4
5	1.5	PCB	5.0E+04	0	1	3	4	4	4	4	3.3
6	1.6	PCB	4.2E+04	0	0	0	0	1	1	1	4.9
C1*	-	PBP	1.4E+04	-	5	-	-	-	-	-	0.0
C2	-	PBP	1.5E+05	5	-	-	-	-	-	-	0.0
C3	-	PCB	1.1E+04	-	5	-	-	-	-	-	0.0
C4	-	PCB	1.6E+05	4†	-	-	-	-	-	-	0.0

*C1, control 1;

#8T, number of mice positive for cysts or trophozoites on day 8 out of cohort of 5;

†4, cohort was 4 mice in this trial;

PBP, 0.05 M phosphate buffer with 10 to 1 peroxide to ozone ratio;

PCB, 0.05 M phosphate-0.01 M carbonate buffer.

Table H.2 Screening experiment: Number of C3H/HeN mice infected with *G. muris* as a function of days after infection and inoculum size

Trial	Inoculum (cysts per mouse)	No. of mice per trial	Days After Infection (Number of mice positive for cysts)						Inactivation (log N/N_0)†
			4	5	6	7	8	8T#	
1	7.4E+03	5	0	0	1	1	1	2	4.3
2	5.0E+02	5	0	0	0	-	0	0	>4.4
3	1.2E+04	4	0	2	4	-	-	-	2.4
4	1.1E+04	5	0	1	1	2	2	2	3.4
5	3.2E+04	5	0	3	4	4	5	-	3.5
R5	8.0E+03	5	0	2	3	4	4	5	3.5
6	2.8E+03	5	0	2	3	3	3	5	3.8
7	3.2E+04	5	0	1	2	2	2	3	4.0
8	6.0E+03	5	1	3	3	3	4	5	3.2
R8	1.6E+04	5	0	2	3	3	5	5	3.1
9	5.0E+04	5	0	2	3	4	4	5	3.7
10	2.0E+05	5	0	1	3	3	4	4	4.4
R10	1.2E+05	5	0	3	5	-	-	-	3.1
11	8.4E+03	5	0	0	1	1	1	3	4.0
R11	1.1E+05	5	0	0	0	0	0	0	>5.0
12	4.4E+04	5	0	0	0	0	0	0	>4.3
13	5.0E+04	5	0	1	1	-	3	5	3.8
14	4.0E+04	5	0	0	1	1	1	3	4.1
15	4.6E+04	5	0	3	5	-	-	-	2.5
16	3.6E+04	5	0	0	0	-	0	0	>4.4
C1	8.4E+02	3	2	3	-	-	-	-	0.0
RC1*	1.4E+04	5	5	-	-	-	-	-	0.0
C2	2.0E+04	5	5	-	-	-	-	-	0.0
C3	2.4E+04	5	5	-	-	-	-	-	0.0
C4	2.6E+04	5	5	-	-	-	-	-	0.0
RC4	1.1E+04	5	5	-	-	-	-	-	0.0
C5	2.6E+04	5	5	-	-	-	-	-	0.0
C6	3.2E+04	5	5	-	-	-	-	-	0.0
C7	3.5E+04	5	5	-	-	-	-	-	0.0
C8	2.4E+04	5	5	-	-	-	-	-	0.0

† >, detection limit;

*RC1, repeat of control 1;

#8T, number of mice positive for cysts or trophozoites on day 8.

Table H.3 Specific ozone residual experiment: Number of C3H/HeN mice infected with *G. muris* as a function of days after infection and inoculum size

Trial	Inoculum (cysts per mouse)	No. of mice per trial	Days After Infection (Number of mice positive for cysts) 3	4	5	6	7	8	8T#	Inactivation (log N/N_O)†
1	6.6E+04	4	0	2	4	-	-	-	-	2.1
2	9.4E+03	5	0	0	0	0	0	0	0	>5.1
3	1.0E+05	4	0	0	0	1	2	2	4	4.5
4	6.4E+04	5	0	0	0	0	0	0	0	>5.1
5	1.1E+05	5	0	0	4	4	5	5	-	3.6
R5	1.4E+05	5	0	0	1	4	5	5	-	3.6
C1*	1.3E+05	5	5	-	-	-	-	-	-	0.0
6	1.2E+05	5	0	0	2	2	2	4	4	4.2
R6	1.2E+05	5	0	0	2	3	3	4	4	4.2
C2	1.2E+05	5	5	-	-	-	-	-	-	0.0
7	3.2E+04	5	-	0	1	2	2	2	3	4.0
C3	2.4E+04	5	-	5	-	-	-	-	-	0.0
8	1.0E+05	5	0	0	1	2	2	2	2	3.5
9	8.4E+04	5	0	0	1	1	3	3	3	3.8
10	1.0E+05	5	0	2	4	5	-	-	-	3.1
11	9.2E+04	5	0	0	2	4	4	4	4	3.2
12	1.9E+04	5	0	1	4	5	-	-	-	3.1
13	8.6E+04	5	0	0	2	5	-	-	-	3.1
C4	1.3E+05	4	4	-	-	-	-	-	-	0.0
14	7.2E+04	5	2	2	5	-	-	-	-	2.2
C5	1.5E+05	2	2	-	-	-	-	-	-	0.0

† >, detection limit;

*C1, control 1;

#8T, number of mice positive for cysts or trophozoites on day 8.

Table H.4 Natural waters experiment: Number of C3H/HeN mice infected with *G. muris* as a function of days after infection and inoculum size

Trial	Water	Inoculum (cysts per mouse)	No. of mice per trial	Days After Infection (No. of mice positive for cysts)							Inactivation (log N/N_0)†
				3	4	5	6	7	8	8T#	
1	NSR	5.4E+04	5	0	0	1	1	1	1	1	2.7
2	NSR	2.4E+05	4	0	0	0	1	1	1	1	3.7
3	NSR	7.2E+04	5	0	0	0	0	0	0	0	>5.0
4	NSR	2.2E+05	5	0	4	5	-	-	-	-	2
39AC*	NSR	9.6E+04	5	5	-	-	-	-	-	-	0.0
5	GVRD	5.7E+04	4	0	0	1	3	3	3	4	4.1
6	GVRD	1.5E+05	5	0	2	5	-	-	-	-	2.1
7	GVRD	2.5E+05	5	0	0	0	0	0	0	0	>5.1
8	GVRD	6.6E+04	5	0	0	4	5	-	-	-	3.1
39CC	GVRD	1.2E+05	4	4	-	-	-	-	-	-	0.0
9	GVRD	2.8E+04	5	0	0	0	1	1	1	1	3.1
10	GVRD	5.4E+04	5	0	0	0	2	2	2	2	3.1
11	GVRD	1.0E+04	5	0	0	0	4	4	5	-	3.0
12	GVRD	3.6E+04	4	0	0	0	1	1	1	1	3.3
39DC	GVRD	1.2E+04	5	2	5	-	-	-	-	-	0.0

† >, detection limit;

*39AC, control experiment 39A;

#8T, number of mice positive for cysts or trophozoites on day 8;

NSR, North Saskatchewan River water;

GVRD, Greater Vancouver Regional District water.

Table H.5 Summary of results from experiments 18 and 24: Number of C3H/HeN mice infected with *G. muris* as a function of days after infection and inoculum size (pH 6.9; 22°C; 50 mL reactor).

Trial	Residual Ozone at 5 min (mg/L)	No. of mice per trial	Inoculum (cysts per mouse)	Days After Infection (No. of mice positive for cysts) 3	4	6	8	10	12	20T#	Inactivation (log N/N_0)†
18-1	0.8	3	1.6E+02	-	0	2	2	2	2	2	2.3
18-2	1.7	3	3.5E+01	-	0	0	0	0	0	0	>4
18-3	4.4	3	9.0E+00	-	0	0	0	0	0	0	>4
18-C	-	1	1.3E+04	-	1	1	-	-	-	-	0
24-1	0.2	3	4.4E+04	-	0	3	3	-	-	-	3.1
24-2	0.8	3	1.3E+04	-	0	2	2	2	2	2	3.3
24-3	1.4	3	9.9E+03	-	0	2	2	2	2	2	3.3
24-C	-	1	1.4E+05	1*	1	-	-	-	-	-	0

C, control;

† >, detection limit;

#20T, number of mice positive for cysts or trophozoites on day 20.

* mice were not checked on day 3, however based on the cyst load on day 4 as well as the number of cysts in the inoculum they would have been positive on day 3.

APPENDIX I: CALCULATION OF OZONE UTILIZED PER *G. MURIS* CYST DURING OZONATION REACTIONS IN SELECTED EXPERIMENTS

Table I.1 Calculation of ozone utilized per *G. muris* cyst during ozonation reactions in selected experiments.

Trial	Applied Ozone (mg/L)	Residual Ozone (mg/L)	Utilized Ozone (mg/L)	Liquid Volume in Reactor (mL)	No. of Cysts in Reactor	Ozone Utilized per Cyst (ng/cyst)*	Inactivation (log N/N_0)†
18-1	1.39	0.79	0.60	40	3.4E+05	0.071	2.3
18-2	2.71	1.72	0.99	40	3.4E+05	0.116	>4.0
18-3	5.82	4.37	1.45	40	3.4E+05	0.176	>4.0
24-1	0.70	0.19	0.51	40	2.0E+06	0.010	3.1
24-2	1.41	0.82	0.59	40	2.0E+06	0.012	3.3
24-3	2.35	1.43	0.92	40	2.0E+06	0.018	3.3
33-A1	0.62	0.30	0.32	209.5	2.0E+06	0.034	4.2
33-A2	0.64	0.40	0.24	217.5	2.0E+06	0.026	4.2
33-B1	0.26	0.10	0.16	204.4	2.3E+06	0.014	3.1
33-B2	0.30	0.10	0.20	209.4	2.3E+06	0.018	3.1
33-C1	2.55	1.30	1.25	235.0	2.2E+06	0.134	4.4
33-C2	0.13	0.04	0.09	202.5	2.2E+06	0.008	1.2
33-D1	0.28	0.20	0.08	199.0	2.0E+06	0.008	2.2
39A-1	0.96	0.25	0.71	186.0	2.0E+06	0.066	2.7
39A-2	2.58	0.70	1.88	193.0	2.0E+06	0.181	3.7
39A-3	2.70	0.83	1.87	199.0	2.0E+06	0.186	>5.0
39A-4	3.03	0.24	2.79	196.0	2.0E+06	0.273	2.0
39C-1	2.10	0.65	1.45	190.9	2.0E+06	0.138	4.1
39C-2	1.09	0.15	0.94	185.8	2.0E+06	0.087	2.1
39C-3	2.62	0.35	2.27	193.6	2.0E+06	0.220	>5.1
39C-4	2.37	0.83	1.54	192.0	2.0E+06	0.148	3.1
39D-1	1.28	0.37	0.91	207.8	2.0E+06	0.095	3.1
39D-2	1.66	0.76	0.90	209.4	2.0E+06	0.094	3.1
39D-3	2.05	0.77	1.28	212.0	2.0E+06	0.136	3.0
39D-4	1.71	0.34	1.37	209.8	2.0E+06	0.144	3.3

C, control;
*ng/cyst, nanogram of ozone per *G. muris* cyst;
† >, detection limit of C3H/HeN mouse model.

APPENDIX J: SUMMARY OF *G. MURIS* CYST INACTIVATION DATA FOR MODELLING

Table J.1 Summary of *G. muris* cyst inactivation data for modelling.

Trial	Residual Ozone C_r (mg/L)	Utilized Ozone Demand C_u (mg/L)	C_d (mg/L)	Utilized less Demand $C_u - C_d$ (mg/L)	Average Ozone C_{av}# (mg/L)	DOC (mg/L)	$\frac{C_u - C_d}{\sqrt{DOC}}$	Inactivation (log N/N_o)
33-A1	0.30	0.32	-	0.32	0.44	0.09	1.07	4.2
33-A2	0.40	0.24	-	0.24	0.50	0.09	0.80	4.2
33-B1	0.10	0.16	-	0.16	0.16	0.09	0.53	3.1
33-B2	0.10	0.20	-	0.20	0.18	0.09	0.67	3.1
33-C1	1.30	1.25	-	1.25	1.90	0.09	4.17	4.4
36-1	0.35	0.24	-	0.24	0.45	0.09	0.80	2.1
36-2	0.35	0.24	-	0.24	0.45	0.09	0.80	>5.1
36-3	0.40	0.33	-	0.33	0.54	0.09	1.10	4.5
36-4	0.36	0.46	-	0.46	0.57	0.09	1.53	>5.1
36-5	0.37	0.29	-	0.29	0.49	0.09	0.97	3.6
36-6	0.37	0.34	-	0.34	0.52	0.09	1.13	3.6
41-3	0.38	0.09	-	0.09	0.40	0.09	0.30	3.8
41-4	0.41	0.07	-	0.07	0.42	0.09	0.23	3.1
41-5	0.42	0.35	-	0.35	0.57	0.09	1.17	3.5
41-6	0.41	0.12	-	0.12	0.45	0.09	0.40	3.2
39A-1	0.25	0.71	0.32	0.39	0.45	0.99	0.39	2.7
39A-2	0.70	1.88	0.89	0.99	1.20	0.99	0.99	3.7
39A-3	0.83	1.87	0.55	1.32	1.49	0.99	1.33	>5.0
39A-4	0.24	2.79	1.54	1.25	0.87	0.99	1.26	2.0
39C-1	0.65	1.45	0.80	0.65	0.98	9.15	0.21	4.1
39C-3	0.35	2.27	1.37	0.90	0.80	9.15	0.30	>5.1
39D-1	0.37	0.91	0.37	0.54	0.64	9.15	0.18	3.1
39D-2	0.76	0.90	0.30	0.60	1.06	9.15	0.20	3.1
39D-3	0.77	1.28	0.49	0.79	1.17	9.15	0.26	3.0
39D-4	0.34	1.37	0.50	0.87	0.78	9.15	0.29	3.3
40-1	0.21	1.26	1.20*	0.06	0.24	1.62	0.05	2.7
40-2	0.35	2.09	1.20*	0.89	0.80	1.62	0.70	3.6
40-3	0.80	2.87	1.20*	1.67	1.64	1.62	1.31	>5.0
40-4	1.23	3.72	1.20*	2.52	2.49	1.62	1.98	>5.0

C_{av}, $C_{av} = C_r + 0.5 \times (C_u - C_d)$

*, estimate;

>, detection limit of C3H/HeN mouse model.

APPENDIX K: MODEL DIAGNOSTIC ANALYSIS

K.1 $$\log \frac{N}{N_o} = -[\theta_1 C_r^{\theta_2} + \theta_3 (C_u - C_d)^{\theta_4} + \{\theta_1 C_r^{\theta_2}\} \times \{\theta_3 (C_u - C_d)^{\theta_4}\}]$$

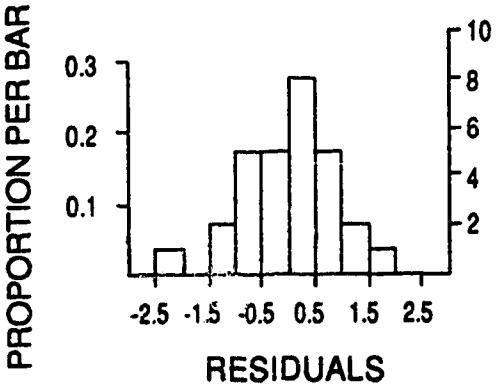
Least absolute value loss function is 17.540. Quasi-Newton estimation.

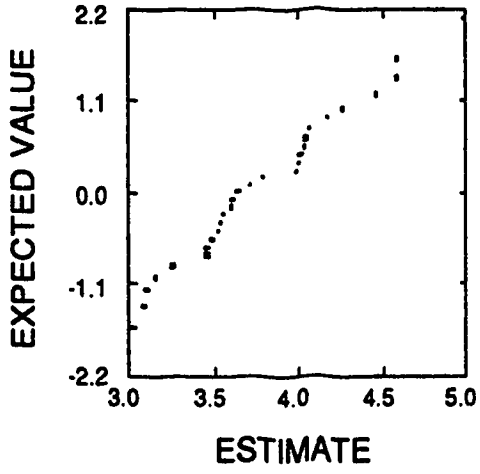
Parameter	Estimate	A.S.E.	Lower <95%>	Upper
θ_1	1.960	0.130	1.692	2.228
θ_2	0.089	0.056	-0.026	0.203
θ_3	0.816	0.038	0.739	0.894
θ_4	0.217	0.031	0.152	0.282

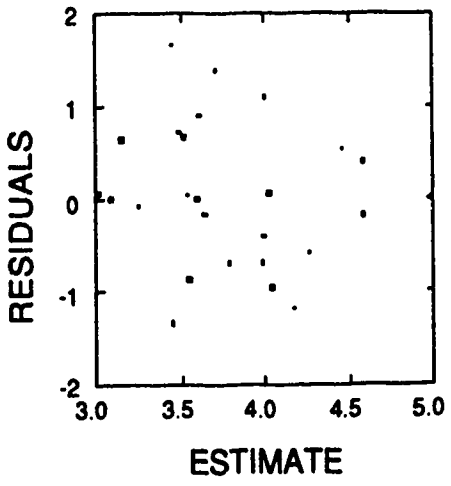
A.S.E. = asymptotic square error

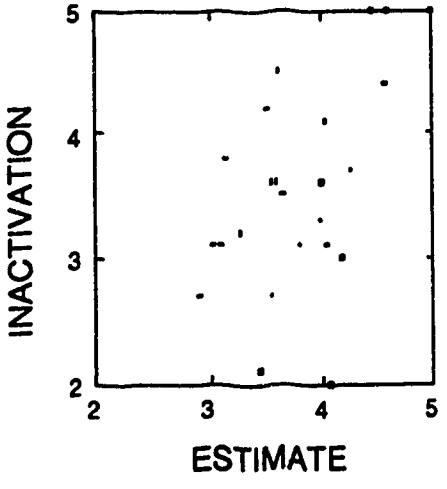
Asymptotic correlation matrix of parameters:

	θ_1	θ_2	θ_3	θ_4
θ_1	1.000			
θ_2	0.860	1.000		
θ_3	0.008	0.292	1.000	
θ_4	0.260	-0.024	0.276	1.000









K.2 $$\log \frac{N}{N_o} = -[\theta_1 C_r^{\theta_2} + \theta_3 (\frac{C_u - C_d}{\sqrt{DOC}})^{\theta_4} + \{\theta_1 C_r^{\theta_2}\} \times \{\theta_3 (\frac{C_u - C_d}{\sqrt{DOC}})^{\theta_4}\}]$$

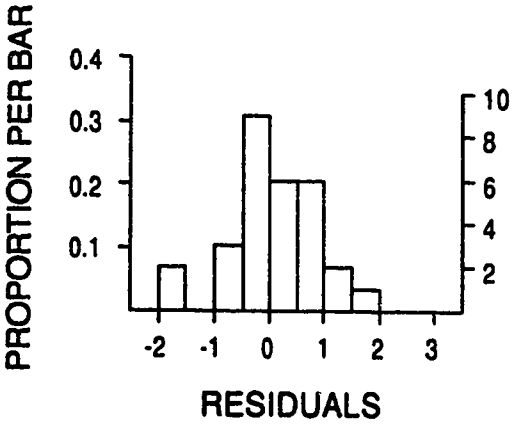
Least absolute value loss function is 16.252. Quasi-Newton estimation.

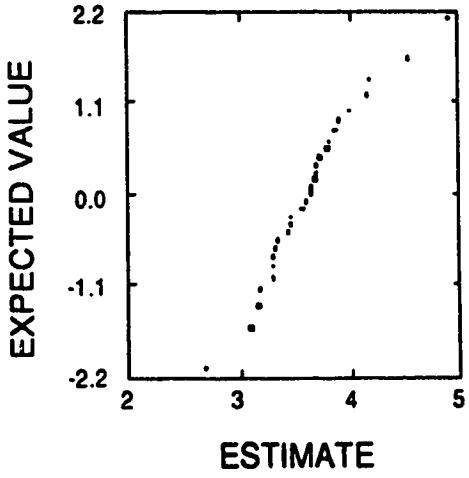
Parameter	Estimate	A.S.E.	Lower <95%>	Upper
θ_1	1.442	0.101	1.233	1.651
θ_2	0.142	0.021	0.098	0.186
θ_3	1.109	0.071	0.962	1.256
θ_4	0.148	0.028	0.090	0.205

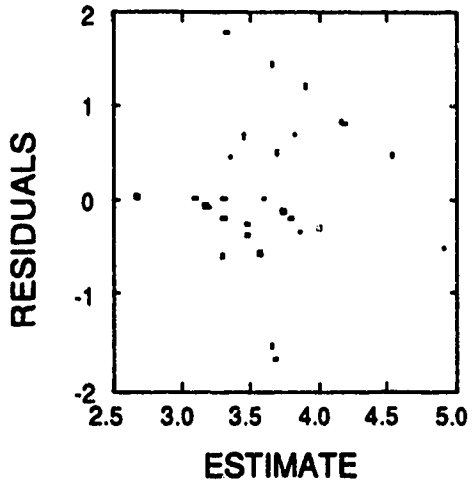
A.S.E. = asymptotic square error

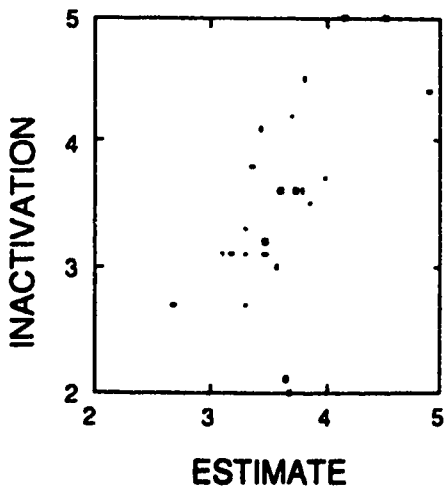
Asymptotic correlation matrix of parameters:

	θ_1	θ_2	θ_3	θ_4
θ_1	1.000			
θ_2	0.389	1.000		
θ_3	-0.896	-0.097	1.000	
θ_4	0.285	-0.014	-0.035	1.000









APPENDIX L: VITA

VITA

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PUBLICATIONS

Adamowski, K. and Labatiuk, C. 1986. Estimation of flood frequencies by nonparametric density procedures. (In) Proceedings, International Symposium on Flood Frequency and Risk Analyses, Louisiana State University, Baton Rouge, Louisiana, ed. V. P. Singh, D. Reidel Publishing Co., Dordrecht, Holland, 97-106.

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