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# Effects of Sequential Exposure of *Cryptosporidium* Oocysts to Chemical Disinfectants

By

Nicola Lewin 

A thesis

submitted to the Faculty of Graduate Studies and Research in partial fulfillment of the requirements for the degree of Master of Sciences

in

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# ABSTRACT

The applications of chemical disinfectants sequentially have been shown to provide superior microorganism reduction for encysted parasites when compared to the same disinfectants used singly. This finding may be of great importance in setting new regulations under the Enhanced Surface Water Treatment rule.

Sequential treatments using chlorine dioxide followed by free chlorine or monochloramine were evaluated for their effect on the inactivation of *Cryptosporidium parvum* oocysts at different water temperatures. Animal infectivity using neonatal CD-1 mice, was used to determine the infectiousness of parasites after treatment for all experiments. Experiments were conducted in 0.05 M phosphate buffer at pH 6 for free chlorine and pH 8 for monochloramine and at 1, 10 and 22°C. Chlorine dioxide followed by free chlorine was found to be more effective in inactivating *C. parvum* than chlorine dioxide followed by monochloramine. Higher temperatures and high free chlorine CT's were essential for obtaining synergy.

Sequential treatment using ozone followed by free chlorine in modified natural water was also conducted and effects on the inactivation of *C. parvum* oocysts were evaluated. Experiments were conducted at pH 6.0, pH 8.5 and 22°C. Natural water did not appear to have an effect on the inactivation kinetics of ozone or free chlorine on *C. parvum*. A synergistic effect may be obtained in modified natural water at pH 6 after a high level of ozone pretreatment and a high level of free chorine CT but not at pH 8.5.

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# INTRODUCTION

## INTRODUCTION

Waterborne cryptosporidiosis outbreaks from surface water supplies have been documented in the US and Great Britain (Moore *et al.* 1993; Goldstein *et al.* 1996; Atherton *et al.* 1995). Occurrence of *Cryptosporidium* spp. has been reported in Alaska and the Yukon (Pollen *et al.* 1996; Roach *et al.* 1993), where source waters can reach temperatures as low as 0°C. Several studies have shown that free chlorine and monochloramine at practical plant doses are ineffective in controlling *C. parvum* oocysts at room temperature (Peeters *et al.* 1989: Korich *et al.* 1990b). Microorganism inactivation is even more difficult at lower temperatures. This has created the need for alternative disinfectants and disinfection methods. Ozone and chlorine dioxide are alternative disinfectants that have been reported to be effective against *C. parvum* (Peeters *et al.* 1989). As these disinfectants are expensive in comparison to free chlorine and monochloramine, it is desirable to reduce the quantity of ozone and chlorine dioxide used at treatment facilities but still maintain the required inactivation of *C. parvum*. Sequential disinfection, where different disinfectants are applied sequentially to the water, has been investigated as a potential solution to this problem. Although a number of studies have suggested that there may be advantages to using combinations of chemicals for controlling microorganisms (Berman *et al.* 1992; Finch *et al.* 1997), the concept of synergism and control of *C. parvum* oocysts is poorly understood and requires further investigation.

## GOALS AND OBJECTIVES

This study examines the exposure of *C. parvum* to sequential disinfectants under high and low temperature conditions. The primary goal of the research was to determine

the effect of chlorine dioxide on *C. parvum* in laboratory water, when used in combination with free chlorine or monochloramine at 1, 10 and 22°C. After analyzing the results from the first part of the project, the work required to achieve the next goal could be performed.

The second goal required applying the findings from part one of this study and the findings of a separate study to natural water to determine if there is a natural water effect on the inactivation of *C. parvum*. The external study was part of an American Water Works Association Research Foundation (AWWARF) funded project. The trials conducted for the project used the same experimental methods as this study but investigated sequential chemical combinations of ozone/free chlorine and ozone/monochloramine.

In order to achieve the two goals mentioned the following objectives were established:

- determine the effects of free chlorine or monochloramine on *C. parvum* at 1, 10 and 22°C in laboratory water;
- develop a kinetic model for the inactivation of *C. parvum* by free chlorine or monochloramine at 1, 10 and 22°C;
- determine the effects of chlorine dioxide followed by free chlorine or monochloramine on *C. parvum* at 1, 10 and 22°C in laboratory water; and
- determine the effects of ozone followed by free chlorine on *C. parvum* at 22°C in natural water.

The conditions for conducting the experiments were chosen to reflect a maximum potential for synergy and conventional water treatment plant conditions.

## SCOPE OF PROJECT

The first part of the project used laboratory waters at 1°C, 10°C and 22°C in a controlled bench-scale setting. This was necessary to control extraneous sources of experimental error so that the fundamental factors affecting *Cryptosporidium spp.* could be determined reliably. Animal infectivity was used to measure the effectiveness of the disinfectants in the inactivation of *C. parvum*.

The natural water experiments used modified North Saskatchewan River water at 22°C in a controlled bench-scale setting. The laboratory methods followed were the same as those used in the first part of the project to reduce sources of error and enable proper comparisons of laboratory water and natural water data.

## LITERATURE REVIEW

### Water Quality Standards

Two findings have resulted in significant reevaluation of disinfection practices. The first finding was that disinfection byproducts can form when disinfectants react with certain organics in water and the second was the discovery of waterborne disease-causing organisms that could not be properly controlled by conventional water treatment processes. These concerns require that new regulations be developed for the removal of newly recognized pathogenic microorganisms, while controlling the formation of disinfection by-products.

According to US Environmental Protection Agency (EPA) (1994) the water treatment process must be capable of removing 99.9% *Giardia* spp. cysts and 99.99% bacteria and viruses. US EPA has set drinking water regulations, known as the Surface Water Treatment Rule (SWTR), using enteric viruses and *Giardia lamblia* as the most restrictive pathogen categories (Bryant *et al.* 1992). The basic assumption of this rule is that a specific combination of disinfectant dose and contact time (Ct) will result in a

certain degree of inactivation of the specified pathogen. An important factor for establishing compliance with Ct criteria is the actual distribution of contact times within the system. For batch systems the actual contact time (t) can be used. For continuous flow systems, however, short-circuiting of a portion of the flow can occur. To account for this, the contact time used for this system corresponds to the time for 10% of the flow to exit the contact chamber and is referred to as $t_{10}$.

Removal requirements for *Giardia* spp. may be increased, however, additionally more stringent regulations need to be developed to control *Cryptosporidium* spp. Recently, *C. parvum* oocysts have proven to be more difficult to physically remove and inactivate by disinfection than *G. lamblia* cysts (Finch *et al.* 1994). Water suppliers have been concerned that conventional treatment methods may not be sufficient to reduce *Cryptosporidium* spp. According to Fayer *et al.* (1996), in order to meet the $10^{-4}$ annual risk of *Cryptosporidium* spp. infection goal, the average plant will have to provide 2 log reduction, as well as, effective particle removal. The enhanced SWTR, currently under development in the US, will include requirements for *Cryptosporidium* spp.

In addition to protecting against microbial contaminants, rules must be designed to reduce potential health risks of oxidative chemical byproducts called disinfection byproducts (DBP's). Disinfectants can react with natural organics in water to form potentially harmful DBP's. Some studies have indicated carcinogenic effects in animals from chloroform, dichlorobromomethane and bromoform (Bull and Kopfler 1991). The disinfection byproducts on the US EPA 1991 Drinking Water Priority List for future consideration include the total trihalomethanes (TTHM's); bromoform and chloroform, chlorine by-products; haloacetic acids and haloketones, and the ozonization by-products: bromate and aldehydes (Bryant *et al.* 1992). Existing regulations under the Safe Drinking Water Act (U.S. Environmental Protection Agency 1994) have been established for TTHM's, haloacetic acids and bromate. The disinfection by-product rule requires that maximum concentration limits (MCL's) are at levels of 0.08 mg/L for trihalomethanes (TTHM's), 0.06 mg/L for haloacetic acids (HAA's) and 0.01 mg/L for bromate. In addition, maximum residual disinfectant levels (MRDL's) have been set for chlorine,

chloramine and chlorine dioxide at 4.0 mg/L as $Cl_2$, 4.0 mg/L as $Cl_2$ and 0.8 mg/L as $ClO_2$, respectively.

## Microorganisms

### *Bacteria and Viruses*

Bacteria are single celled prokaryotic organisms. They are generally spherical, cylindrical or helical in shape and vary in size from 0.5 to 15 μm in length. Temperature and pH have a significant effect on the survival and growth of bacteria (Metcalf and Eddy, 1991). Optimal growth occurs within a range of temperatures depending on whether bacteria are psycrophillic (12 to 18°C), mesophillic (25 to 40°C) or thermophillic (55 to 65°C). Most bacteria cannot survive at pH levels above 9.5 or below 4.0. Waterborne bacteria that are pathogenic to humans are usually transmitted through contamination of water supplies by human or animal feces. Examples of waterborne diseases that are of bacterial origin include cholera, typhoid and salmonellosis (Bryant *et al.* 1992).

Viruses are obligate parasites as they cannot propagate outside of their host. Waterborne viruses that are pathogenic to humans range from 21 to 80 nm. Viruses are generally more resistant than bacteria to inactivation by disinfectants and while bacteria may require hundreds or thousands of viable organisms to achieve infection, viruses can be infective at doses as low as 1 to 100 virus particles (Bryant *et al.* 1992). The Ct for 99% inactivation of E. coli bacteria at 5°C and pH 6 to 7 using free chlorine is 0.034 to 0.05 mg·min/L, whereas, the Ct for 99% inactivation of Polio 1 virus under the same conditions is 1.1 to 2.5 mg·min/L (Hoff 1987).

### *Protozoa*

Protozoa are single celled eukaryotic cells approximately 1 to 150 μm in size. They are either free-living or parasitic. The parasitic protozoa usually tend toward the

lower end of the size range. In general, protozoa have short generation times and high rates of reproduction and hosts that become infected by the protozoa and survive tend to become immune to it. However, parasitic protozoa can also develop adaptations that allow them to survive in their hosts and evade the immune response (Metcalf and Eddy, 1991).

There are two stages in the life cycle of *Giardia* spp. First it inhabits the small intestine of warm blooded vertebrates in the form of trophozoites. Later environmentally resistant cysts are formed that pass out of the host in the feces. This allows the parasite to infect a new host through the ingestion of contaminated water or food. The parasitic protozoan *Giardia lamblia* acts in this way, and is the most frequently identified cause of waterborne disease in North America (Wallis *et al.* 1996). *G. lamblia* causes a disease called giardiasis. The cyst is the normal infective stage in the transmission of giardiasis to new hosts.

*C. parvum* is smaller than *G. lamblia* (approximately 2 to 6 μm in size) and has been found more frequently and in greater numbers than *G. lamblia* (Rose 1988). Studies have shown that *C. parvum* oocysts are more resistant to inactivation by chemical disinfection than *Giardia spp.* cysts (Korich *et al.* 1990a; Smith *et al.* 1988).

*C. parvum* invades and replicates within epithelial cells that line the gastrointestinal tract of mammals. Consumption of oocysts through contaminated food or water causes the disease cryptosporidiosis, which is a potentially life threatening diarrheal illness in immunocompromised individuals (Smith *et al.* 1995).

Fayer *et al.* (1997) describes the life cycle of *Cryptosporidium* spp. According to Fayer *et al.* (1997) the exogenous stage of the oocyst consists of four sporozites within a tough, thick-wall. It is excreted from the body of an infected host in the feces. The endogenous phase begins after the oocyst is ingested by a suitable host. Sporozoites that exist within the ingested oocyst excyst from it and invade and replicate within cells that line the digestive and respiratory tracts. After this gamete formation, fertilization and oocyst wall formation occurs. The first wall formation of the oocyst is the thin-walled form. This form is auto-infective within the host and is not believed to exist outside the

host. The thick-walled oocysts later form that exit the host and can survive prolonged periods of time in the environment due to a protective bilayer cyst wall. The exogenous oocyst stage of this parasite is much more resistant to conventional water treatment disinfection processes than bacteria or viruses (Fayer *et al.* 1997).

## Animal Infectivity Assay

Animal infectivity is commonly used for studying the viability of *Giardia* spp. cysts and *Cryptosporidium* spp. oocysts before and after disinfection. Animal infectivity can detect up to 4 log-units inactivation and has been reported to be the best method currently available for detecting high levels of *G. muris* cyst (Labatiuk *et al.* 1991) and *C. parvum* oocyst (Finch *et al.* 1993) inactivations. It is also, presently, the only accepted method for determining the efficacy of chemical disinfectants on *C. parvum* oocysts because it gives direct information about the ability of the parasite to infect the host (Neumann *et al.* 2000). Animal infectivity is, however, a tedious and difficult protocol.

The CD-1 mouse data from a study by Finch *et al.* (1993) showed that the CD-1 strain is susceptible to *C. parvum*. Dose response characteristics of the infectivity of *C. parvum* oocysts in neonatal CD-1 mice were developed by Finch *et al.* (1993) in that study. The dose response of *C. parvum* was modeled using a logistic dose response model with 90% confidence limits. The 50% infective dose (ID50) was estimated to be 79 oocysts with infection occurring within 7 days postinoculation. This method, however, is only an estimation of the infectivity of oocysts in humans.

## Disinfectants

### *Chlorine Dioxide*

The most common method of chlorine dioxide ($ClO_2$) generation involves reacting sodium chlorite with chlorine to produce gaseous chlorine dioxide. $ClO_2$ is produced as a result of chlorous acid formation from chlorite and hydrogen followed by a

subsequent reaction with chlorine species to produce dichlorine dioxide, which dissociates to produce $ClO_2$ (Sawyer *et al.* 1994).

The active agent in a chlorine dioxide ($ClO_2$) system is free dissolved chlorine dioxide. It has an extremely high oxidation potential, which most likely accounts for its potent germicidal powers. In aqueous solutions the major reactions $ClO_2$ undergoes include oxidation, disproportionation and photolysis (Metcalf and Eddy, 1991). In oxidation chlorine dioxide reacts to form chloride ($Cl^-$) at pH 2 or less or chlorite ($ClO_2^-$) at neutral pH:

$$ClO_2 + 4H^+ + 5e^- \rightarrow Cl^- + 2H_2O \qquad (1.1)$$

$$ClO_2 + e^- \rightarrow ClO_2^- \qquad (1.2)$$

Chlorine dioxide can disproportionate by hydrolysis into chlorite and chlorate ($ClO_3^-$) at pH 10 or greater or to chlorous ($HClO_2$) and chloric ($HClO_3$) acids at pH 6 to 8:

$$2ClO_2 + 2OH^- \rightarrow ClO_2^- + ClO_3^- + H_2O \qquad (1.3)$$

$$2ClO2 + H_2O \rightarrow HClO_2 + HClO_3 \qquad (1.4)$$

Chlorine dioxide undergoes photolysis in a complex manner in aqueous solutions. The main end-products of this reaction are chlorate, chloride and hypochlorite ions. Of these three reactions the most significant in drinking water treatment is oxidation at neutral pH conditions. Approximately 50 to 70 % applied chlorine dioxide is converted to chlorite under typical water treatment conditions (Aieta *et al.* 1984; Rav-Acha *et al.* 1985).

Chlorine dioxide has better selective reactivity than most chemicals used for microorganism reduction and a low production of halogenated organics. It does not react with ammonia to produce potentially toxic chloramines as does chlorine (Sawyer *et al.* 1994). $ClO_2$ reduces to chlorate and chlorite, which are potentially toxic end-products, however, chlorine dioxide end-products are believed to degrade more quickly than

chlorine residuals. Chlorite is typically the product of concern in drinking water. Due to the formation of chlorate and chlorite it may not be possible to get more than 1 log-unit inactivation by $ClO_2$ under normal water treatment plant conditions. A by-product level of less than 1.0 mg/L has been set (US EPA, 1998). With this limitation a maximum of 1.4 mg/L chlorine dioxide can be applied (US EPA, 1998). Chlorine dioxide is also a fairly expensive chemical and requires complicated generation and monitoring techniques.

### *Ozone*

Ozone is a highly reactive and relatively unstable molecule. It is generated by passing oxygen through a corona discharge, where molecular oxygen is dissociated into oxygen radicals (Langlais *et al.* 1991). The corona discharge is produced by applying high voltages across two electrodes. In an aqueous solution ozone ($O_3$) reacts with constituents in water directly or reacts to form free radicals $HO_2$ and OH, which are more reactive than ozone itself. Formation of these radicals tends to increase as pH increases, which leads to a higher rate of ozone degradation. Increased rates of decomposition become significant at pH 8 or greater and ozone residual becomes difficult to maintain at pH 9 and higher (Bryant *et al.* 1992). According to Metcalf and Eddy (1991) formation of $HO_2$ and OH is believed to occur as follows:

$$O_3 + H_2O \rightarrow OH^- + HO_3^+ \qquad (1.5\ a)$$

$$HO_3^+ + OH^- \rightarrow 2HO_2 \qquad (1.5\ b)$$

$$O_3 + HO_2 \rightarrow HO + 2O_2 \qquad (1.5\ c)$$

$$HO + HO_2 \rightarrow H_2O + O_2 \qquad (1.5\ d)$$

Ozone has been found to be a potent barrier against *Giardia* spp. and *Cryptosporidium* spp. and is one of the most effective disinfectants for controlling *Giardia* spp. and *Cryptosporidium* spp. A study by Tobin *et al.* (1987) showed that at varying pH of 6 to 9 and a temperature of less than 1°C the CT value for 99.9% removal

of *Giardia* cysts was between 165 and 500 mg·min/L for free chlorine and 2.9 mg·min/L for ozone.

It has been found that ozone can react to form varying degrees of halogenated organics. In addition, ozone reacts with bromide to form bromate, a carcinogen, at very low concentrations. Langlais *et al.* (1991) stated that to date ozone is the single primary disinfectant for the surface water treatment which is effective and doesn't form by products of health concern unless bromide ion is present.

Ozone use is fully demonstrated but not widely used in North America because of relatively high costs in generating, distributing and maintaining an ozone facility. Also, because ozone has a short half-life and does not produce a long lasting residual, the addition of chlorine or some other residual providing disinfectant, is required after ozonization.

### *Free Chlorine*

Chlorine combines with water to form hypochlorous (HOCl) and hydrochloric acids. HOCl dissociates to form hydrogen and hypochlorite ion ($OCl^-$) at pH values above 4. These reactions are as follows:

$$Cl_2 + H_2O \leftrightarrows HOCl + H^+ + Cl^- \qquad (1.6)$$

$$HOCl \leftrightarrows OCl^- + H^+ \qquad (1.7)$$

$Cl_2$, HOCl and $OCl^-$ are collectively referred to as free chlorine with HOCl and $OCl^-$ being the active agents. HOCl is a weak acid and a very effective bactericide. $OCl^-$ is also a strong oxidizing agent but not as effective as HOCl. HOCl has 40 to 80 times greater killing efficiency than $OCl^-$ (Sawyer *et al.* 1994). The relative amounts of $OCl^-$ and HOCl in solution are dependent on pH. At pH greater than 8.0 $OCl^-$ becomes the dominant chlorine form. For this reason, a lower pH, where HOCl is the more dominant species, is required for optimization of chlorine dose.

Chlorine has traditionally been used for disinfection of drinking water because it is very effective against bacteria and viruses, it maintains a residual in the distribution system, is fairly inexpensive and is easy to use. However, chlorine based disinfectants have generally had a low level of effectiveness for oocyst inactivation and toxic byproducts are formed when chlorine reacts with natural organics in water. Evidence of adverse health effects resulting from chlorination byproducts are shown in several epidemiology and toxicology studies (Bull *et al.* 1995; Suarez Varela *et al.* 1994). Korich *et al.* (1990a) showed that free chlorine at practical plant doses and contact times was ineffective against *C. parvum*.

### *Monochloramine*

Chloramines are formed when chlorine or hypochlorous acid reacts with ammonia to form monochloramine ($NH_2Cl$), dichloramine and trichloramine. The chlorine in these compounds are referred to as combined available chlorine. Monochloramine is formed as follows:

$$NH_3 + HOCl \rightarrow NH_2Cl + H_2O \qquad (1.8)$$

The combination of chloramine species is dependant on the molar ratio of chlorine to ammonia, temperature, pH and alkalinity. In order to form monochloramine a chlorine to ammonia molar ratio up to 1:1 and a pH above 8.0 is required (Sawyer *et al.* 1994).

Monochloramines have significant disinfecting power, however, are generally not as effective as free chlorine. For low levels of inactivation (greater than 0.5 log-units) of *C. parvum* free chlorine efficacy at pH 6 is much greater than that of monochloramine at pH 8 (Gyürék *et al.* 1997). On the other hand, combined chlorine has a slower degradation and reaction rate than free chlorine and, therefore, continues to disinfect for a longer period of time (Bryant *et al.* 1992). As discussed previously, chlorine based disinfectants have generally had a low level of effectiveness for oocyst inactivation and may form potentially harmful disinfection byproducts.

## Disinfection Kinetics

The rate of disinfection determines the reduction in the quantity of pathogenic organisms that occurs in a given contact time. Kinetic models have been developed that represent the inactivation rate of disinfection chemicals and the disappearance of that chemical over time. The models attempt to represent interactions of chemicals that have different cellular targets and modes of action with more complex microorganisms. Each model was developed for buffered laboratory waters.

### *Chick-Watson Model*

Chemical disinfection results have typically been expressed in terms of the product of the concentration of the disinfectant and contact time (Ct) for different levels of inactivation. Disinfection systems are designed using Ct products derived from Chick-Watson kinetics. The Ct product can be derived theoretically from the Chick-Watson pseudo first-order rate law, which can be integrated to give the Chick-Watson model:

$$\log\left(\frac{N}{N_o}\right) = -k' C^n t \qquad (1.9)$$

where, $N_0$ is the initial number of viable organisms, N is the number of viable organisms that remain after exposure to the disinfectant for a contact time, t and disinfectant concentration, C, k' is the rate coefficient that defines the rate of organism inactivation over time and n equals 1 (as assumed in practice) (Haas and Karra 1984).

This was derived first by Chick, who showed that if the concentration of disinfectant was constant the reaction is first order with respect to the surviving bacteria. The model was then expanded by Watson to account for changes in concentration of disinfectant. The $C^nt$ relationship is referred to as the Ct product. The Chick-Watson model has been reported to be adequate for describing chlorine inactivation kinetics in laboratory waters (Haas and Karra 1984; Qualls and Johnson 1983).

*Incomplete Gamma Hom Model*

Although the Chick-Watson model has proved to be adequate for describing chlorine disinfection, studies using different chemical disinfectants and microorganisms have shown deviations between the model predictions and observed kill (Gyürék and Finch 1998). This lead to the proposal of an alternate rate law by Hom (1972) to account for deviations from the Chick-Watson model:

$$\frac{dN}{dt} = -k' mNC^n t^{m-1} \tag{1.10}$$

where, m is an empirical constant.

The Hom model assumes that the rate of decomposition of the chemical disinfectant is not a function of the organisms present, chemical disinfectant decay is independent of the inactivation reaction and rate law parameter estimates are independent of water characteristics. The level of inactivation predicted by the Hom model is a nonlinear function of C and t, dependent on the model parameters.

Both the Chick-Watson and Hom models assume that the disinfectant concentration remains constant. A closed form Hom model accounts for disinfectant decay and was derived using the incomplete gamma function. This gives the Incomplete gamma Hom model. The Incomplete gamma Hom (I.g.H) model assumes, in addition to those previously mentioned, that oxidant decay rate constants do not include instantaneous oxidant demand that occurs within seconds following rapid mixing of the oxidant. The I.g.H. model, therefore, describes deviations from exponential die-off kinetics, as well as account for first-order decay rate (Haas and Joffe 1994):

$$\log\frac{N}{N_o} = \frac{-mkC_o^n}{(nk')^m} \cdot \gamma(m, nk't), \quad m > 0, \; nk't \geq 0 \tag{1.11}$$

where $\gamma(m, nk't)$ is the incomplete gamma function which requires $m>0$ and $nk't \geq 0$.

The I.g.H. model for pH 6 to 8 was found to adequately predict the inactivation of *C. parvum* by ozone (Gyürék *et al.* 1999). Gyürék *et al.* (1999) showed that the I.g.H

model for pH 6 to 8 provided a significantly better fit to the ozone inactivation data than the Chick-Watson model.

## Previous Studies

### *Single Disinfectants*

Many studies have reported that chlorine bleach solutions are ineffective against *Cryptosporidium* spp. viability (Campbell *et al.* 1982; Ransome *et al.* 1993; Smith *et al.* 1988). Using CD-1 neonatal mice and the logit dose response model, Finch *et al.* (1997) found in a comprehensive study of *C. parvum* inactivation, that free chlorine alone could kill 1 log-unit *C. parvum* at a dose of 15 mg/L and a contact time of 160 min at pH 6 and 22°C. It was also found that monochloramine could inactivate greater than 1 log-unit *C. parvum* with a concentration of 15 mg/L for 480 min at pH 8 and 22°C. For water treatment plants, however, these high concentrations and long contact times are impractical. As discussed Korich *et al.* (1990a) showed that free chlorine at practical plant doses of 5 mg/L was ineffective.

The same study discussed above, conducted by Finch *et al.* (1997) also looked at the effects of ozone and chlorine dioxide on the inactivation of *C. parvum*. At pH 7 and 22°C an applied ozone dose of 1.7 mg/L for 0.16 min gave an inactivation of greater than 2.0 log-units. Also, Korich *et al.* (1990b, 1990a) determined the infectivity of oocysts in neonatal BALB/c mice when applying a constant ozone residual of 1 mg/L. In a semi-batch reactor containing buffer at pH 7, an ozone dose of 1 mg/L for 5 min resulted in 99% inactivation of *C. parvum*.

The study by Finch *et al.* (1997) also observed the inactivation of *C. parvum* at pH 8 and 22°C for chlorine dioxide. A chlorine dioxide dose of 2.0 mg/L for 120 min gave an inactivation of more than 1.5 log-units. Peeters *et al.* (1989) used neonatal Swiss OF1 mice to determine the infectivity of oocysts. This study showed that chlorine dioxide at a concentration of 0.43 mg/L for a contact times of 30 min resulted in 1 log-unit inactivation of *C. parvum* at pH 7 and 22°C.

### *Synergistic Effects*

The sequential application of chemical disinfectants found in large-scale drinking water treatment may use free chlorine, ozone or chlorine dioxide as the primary disinfectant followed by free chlorine or monochloramine as the final disinfectant. This sequential application may result in a synergistic effect. It is believed that synergy occurs through sequential disinfection because the stronger oxidant conditions the outer membrane of the microorganism enabling the secondary disinfectant to penetrate the oocyst wall more readily, however, few studies have examined the mechanisms of synergy. A synergistic effect was reported by Kouame and Haas (1991) on the inactivation of *E. coli* when free chlorine and monochloramine were both present in a continuous stirred tank reactor system at pH 8 and 20°C.

There have been several published reports of synergism between disinfectant species leading to more effective treatment of *C. parvum*. Although *C. parvum* is very resistant to conventional disinfection it was discovered that when free chlorine is followed by monochloramine inactivation levels greater than the sum of the individual inactivation levels of these disinfectants occur. Finch *et al.* (1997) determined that at pH 8.0 and 22°C there was a reduction in infectivity of 0.6 log-units of oocysts exposed to 4.0 mg/L of free chlorine for 15 min followed by 4 mg/L of monochloramine for 240 min. Expected inactivation of *C. parvum* by either chlorine species alone under these conditions is close to zero.

Several other sequential disinfection studies that have shown synergistic effects on *C. parvum* inactivation are summarized in Table 1-1. The table shows that evident synergy (greater than 1.0 log-unit) has been discovered when using several sequential disinfectant combinations at pH 6 and 8 and 22°C.

**Table 1-1 Studies of Inactivation of *Cryptosporidium parvum* Using Sequential Disinfection in a Batch Reactor**

| | | | Pretreatment | | Secondary Treatment | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Pretreatment/ Secondary | temp °C | pH | $C_o$ (mg/L) | time (min) | $C_o$ (mg/L) | time (min) | *Additive Effect (log-unit) | Observed kill (log-unit) | ‡Synergy (log-unit) |
| [1]*Chlorine dioxide/ free chlorine* | 22 | 8 | 1.3 | 120 | 1.6 | 120 | 1.3 | 3.0 | 1.7 |
| [1]*Chlorine dioxide/ free chlorine* | 22 | 6 | 1.2 | 119 | 1.8 | 120 | 1.0 | 2.2 | 1.2 |
| [1]*Chlorine dioxide/ free chlorine* | 22 | 11 | 1.3 | 119 | 2.1 | 120 | 1.6 | 2.2 | 0.6 |
| [1]*Chlorine dioxide/ monochloramine* | 22 | 8 | 1.5 | 120 | 2.8 | 180 | 1.5 | 2.8 | 1.3 |
| [1]*Chlorine dioxide/ monochloramine* | 22 | 6 | 1.2 | 120 | 2.1 | 120 | 1.0 | 2.4 | 1.4 |
| [1]*Chlorine dioxide/ monochloramine* | 22 | 11 | 1.3 | 120 | 2.0 | 120 | 1.6 | 2.1 | 0.5 |
| [2]*Ozone/ chlorine dioxide* | 22 | 8 | 0.8 | 4.4 | 2.0 | 60 | 2.2 | 3.4 | 1.2 |
| [1]*Ozone/ free chlorine* | 22 | 6 | 0.8 | 3.7 | 2.0 | 265 | 2.0 | >2.9 | >0.9 |
| [3]*Ozone/ monochloramine* | 22 | 8 | 0.5 | 2.4 | 2.0 | 265 | NR | NR | 1.5 |
| [4]*Ozone/ monochloramine* | 22 | 8 | 1.4 | 4.5 | 2.0 | 240 | 1.4 | 2.1 | 0.7 |
| [1]*Free chlorine/ monochloramine* | 22 | 8 | 4.0 | 15 | 4.0 | 240 | 0.0 | 0.6 | 0.6 |

[1]Liyanage *et al.* 1997a, [2]Liyanage *et al.* 1996, [3]Finch *et al.* 1997, [4]Finch and Black 1994
*Expected inactivation from addition of single oxidants
‡Synergy = Observed kill – Additive effect
NR: not reported
Note: all references used animal infectivity (CD-1 neonatal mice) and logistic dose response model

### *Temperature Effects*

Water temperature can significantly affect the disinfection rate of chemical disinfectants. Microorganism inactivation rate generally increases with increased temperatures. According to Bryant *et al.* (1992), the SWTR Ct criteria for achieving 99.9% inactivation of *G. lamblia* decreases from 116 mg·min/L to 58 mg·min/L for 5 and 15°C, respectively for free chlorine at pH 6.0. For ozone at pH 6 to 9, the Ct values decrease from 2.9 to 0.95 mg·min/L at 5 and 15°C respectively and for chlorine dioxide at pH 6 to 9, Ct values decrease from 63 to 19 mg·min/L at 5 and 15°C respectively

(Bryant *et al.* 1992). The van Hoff-Arrhenius theory states that temperature partly determines the rate at which a disinfectant diffuses through the microorganism surface and the rate of reaction with the substrate (Metcalf and Eddy, 1991). It is generally accepted that increasing the water temperature by 10°C increases the rate of reaction of disinfectants by a factor of 2 or 3 (Langlais *et al.* 1991). A study by Wickramanayake *et al.* (1984) showed that in order to achieve 99% inactivation of *Giardia muris* using chlorine dioxide at pH 7 and a temperature of 5 and 25°C, a Ct of 11.2 and 2.1 mg·min/L were required. For the inactivation of *C. parvum*, similar temperature effects have been observed. Table 1-2 summarizes the results found in a comparison study of the inactivation of *C. parvum* using ozone or chlorine dioxide at 22 and 1°C (Finch and Li 1999). The results of this study suggest that increasing the temperature from 1 to 22°C can significantly increase the inactivation of *C. parvum*.

**Table 1-2 Inactivation of *Cryptosporidium parvum* Using Ozone or Chlorine Dioxide at 1°C and 22°C in a Batch Reactor**

| Treatment | Temp. °C | pH | Ct (mg·min/L) | Observed log inactivation (log-unit) |
|---|---|---|---|---|
| Ozone | 1 | 6.9 | 7.2 to 15 | 0.7 to 1.3 |
| Ozone | 22 | 6.9 | 8.5 to 6.0 | 3.4 to 3.7 |
| Chlorine dioxide | 1 | 6.9 | 120 to 135 | 0.5 to 0.6 |
| Chlorine dioxide | 22 | 6.9 | 120 | 1.8 to 2.1 |

Note: all references used animal infectivity (CD-1 neonatal mice) and logistic dose response model
Source: Finch *et al.* 1999

### *Natural Water Effect*

Natural waters contain many water quality parameters that may affect the decay rate and demand of disinfectants. It is unclear, however, whether differences in water quality parameters can impact the kinetic rate of disinfection of *C. parvum* oocysts.

Important water quality parameters include temperature, pH, total hardness, conductivity, turbidity and true colour. Temperature effects have been discussed above. A higher pH water (especially above pH 8.0) causes ozone to decompose faster, however, the changes in disinfecting efficiency with variations in pH are small. Farooq *et al.*

(1977) showed that using a constant residual ozone concentration at various pH values resulted in similar degrees of inactivation. With free chlorine, as discussed previously, increasing pH results in an increased Ct requirement for the inactivation of microorganisms. Bryant *et al.* (1992) shows that the Ct criteria for 99.9% inactivation of *G. lamblia* increases from 165 to 500 mg·min/L for pH 6 and 9, respectively.

The inhibiting effect of turbidity depends on the type and to a lesser degree the level. Water containing high concentrations of natural organic materials (NOM's) have a high disinfectant chemical demand. This is because organic materials consume large quantities of chemicals and demand for the oxidant must be satisfied before microorganism reduction can take place (Langlais *et al.* 1991). Colour in water is commonly associated with naturally occurring humic materials. A high colour index may indicate the presence of a large amount of humic materials, which can also increase the disinfectant demand (Bryant *et al.* 1992). A higher level of turbidity can also affect disinfectant efficiencies. A study by Wallis *et al.* (1990) indicated a decreased inactivation of *Giardia* spp. at turbidities greater than 1.1 NTU when compared to low turbidity water. This may be due to the protection of *Giardia* spp. cysts by clumps of debris.

# CHAPTER 2

# MATERIALS AND METHODS

## INTRODUCTION

The experiments that were conducted in this research used laboratory methods and material preparations that have been developed over the years for other microorganism reduction research. The parasitology methods that were followed in this project are currently accepted methods for microorganism reduction experiments (Gyürék *et al.* 1999). A microorganism reduction protocol was developed for use in microorganism reduction studies (Haas *et al.* 1993). These guidelines, however, were further developed in later comprehensive microorganism reduction research due to improvements of some technologies (Finch *et al.* 1997, Finch *et al.* 1994). The microorganism reduction methods used in the latter studies were followed in this project.

Due to the nature of the experiments, it was desirable to eliminate as many sources of error as possible from the experiments in order to obtain reproducible results. This required that developed methods and procedures were followed accurately under controlled laboratory conditions. Reproducible water was used for all laboratory water experiments to reduce sources of error due to batch variation in other types of water. Animal infectivity was used as a measure of effectiveness of disinfectants for *C. parvum* inactivation since in-vitro methods under estimate the degree of inactivation.

## PARASITOLOGY METHODS

### Production and Purification of *Cryptosporidium parvum* oocysts

The strain of *C. parvum* oocysts that was used in this study was obtained from Dr. Harley Moon (National Animal Disease Control center) and is known as the Iowa strain.

The oocysts were produced in neonatal Holstein calves by dosing the calves with 2 x $10^7$ oocysts. Feces were collected and passed through 75, 150 and 400 mesh sieves by agitating and washing the sieves with 0.01 percent (v/v) Tween 20. The sieved feces were collected in centrifuge tubes and centrifuged for 15 min at 3500 rpm in order to collect the particulate matter. Oocysts isolated from calf feces were purified in sucrose and cesium chloride gradient centrifugation steps (Kilani and Sekla 1987; Belosevic *et al.* 1997). Stock suspensions of purified oocysts were stored at 4°C in deionized water with antibiotics (100 µg/mL streptomycin, 100 µg/mL gentamicin, 100 U/mL penicillin) and 0.01% Tween. For each experiment, oocysts from the stock suspension were washed and concentrated by centrifugation (14,500 x *g*, 10 min), and counted using a hemocytometer. Oocysts older than 60 days were not used in experiments.

## Sample Collection and Concentration

Centrifuge tubes containing exposed *C. parvum* oocysts were centrifuged at 27,000 x *g* for 10 min in order to concentrate oocysts. The supernatant was aspirated and the cell pellet re-suspended in deionized water. Oocysts were counted in quadruplicate using a hemocytometer and appropriate dilutions of the oocysts made in deionized water for infectivity analysis.

## Infectivity in Neonatal CD-1 Mice

A neonatal mouse model was used to evaluate infectivity of *C. parvum* (Ernest *et al.* 1986). Breeding pairs of outbred CD-1 mice were obtained from the Charles River Breeding Laboratories (St. Constant, Quebec, Canada). Breeding pairs of outbred neonatal CD-1 mice were housed in cages with covers fitted with a 0.22 µm filter in a specific pathogen-free (P-2 level) animal facility. Mice were inoculated intragastrically 5 days after birth with a known number of oocysts suspended in 50 µL of deionized water. Intragastric inoculation was preformed using a ball-point neonate feeding needle (24 gauge syringe, Popper and Sons Inc.) attached to a tuberculin syringe.

The infectivity of the oocysts was determined 7 days after infection. The mice were killed by cervical dislocation and the large intestine was removed and placed in 10 mL of Milli-Q® water. The intestine was homogenized for 45 to 60s in a Sorvall Omni-Mixer and the homogenate placed in a 15 mL polypropylene testube. The suspension was centrifuged at 2000 x *g* for 15 min. The supernatant was then removed, the pellet re-suspended in 10 mL of deionized water containing 0.01% Tween 20, and centrifuged at 2000 x *g* for 15 minutes. After centrifugation, the supernatant was discarded and 20 µL of the viscous pellet removed and place into a 6 mL polystyrene flow cytometer test tube fitted with a 35 µm sieve (Becton Dickinson). The intestinal homogenate was forced through the sieve by adding 400 µL of 1% BSA in PBS. Samples were allowed to incubate for 15 minutes at room temperature, in order to block non-specific absorption of the monoclonal antibody. One hundred µL of a 1:400 dilution of fluorescein labeled anti-*C. parvum* oocyst monoclonal antibody (ImmuCell), diluted in 1% BSA, was subsequently added to each sample and incubated at 37°C for 30 minutes. The resulting suspension was examined for the presence of parasites using flow cytometry (Neuman *et al.* 2000). All flow cytometric analysis was done at a high flow rate using PBS as the sheath fluid. Fifty-thousand events were collected for each intestinal homogenate sample. Mice were scored as infected with *C. parvum* when the number of events segregating into a defined fluorescence region was $\geq$ 1.25%. Flow cytometric results were confirmed using conventional microscopy methods at regular intervals.

## Interpretation of Infectivity Data

The viability of treated *C. parvum* oocysts was determined using animal infectivity in neonatal CD-1 mice. The first step of the infectivity analysis involved establishing a suitable dose-response model for each batch of oocysts that is designed to give the infectious dose for a median response in the mice ($ID_{50}$). The second step required that after the experiment was performed, oocysts were recovered from the reactor and mice were inoculated with appropriate serial dilution to bracket the $ID_{50}$. Five mice were used randomly for each dilution. This reduces experimental error as genetic differences in various litters could confound the results. Five mice have been

used in past studies and have shown to give adequate results (Finch *et al.* 1997; Finch *et al.* 1993). The survival ratio (log-units) of *C. parvum* after disinfection was estimated from:

$$\log\frac{N}{N_o} = \log\left(\frac{n}{n_o}\right) \tag{2.1}$$

where n is the estimated infectious dose per animal after disinfection from the dose response model and $n_o$ is the infectious number of oocysts given to each animal as estimated from the dose-response model.

The estimated infectious dose (n) was determined from a logistic dose-response model, that was developed for each batch of oocysts. The logit mean response developed by Neter *et al.* (1989), is given by:

$$\Pi = \ln\frac{P}{1-P} = \beta_o + \beta_1 X \tag{2.2}$$

where, P is the proportion of the cohort infected for a given inoculum X (log-units) and β is the logit response model parameter. The model parameters are estimated using the statistical method of maximum likelihood shown later.

Dose response trials were conducted each experimental week, independent of microorganism reduction trials. The viability of stock oocysts has been shown to decrease with respect to time (Belosevic *et al.* 1997). Any loss in infectivity of a stock of oocysts over time is accounted for by the dose response model, which gives the reduction of infectivity of experimental results.

## Parameter Estimation

The model parameters were estimated using maximum likelihood. Maximum likelihood provides parameter estimates with many desirable statistical properties and

also allows the use of censored data. The parameters $\beta_o$ and $\beta_1$ are the values that maximize the natural logarithm of the likelihood function (ln L). These are estimated using the solver function in Excel 97/98, to maximize the $\log_e$ likelihood function (L) for binary data given by (Brand *et al.* 1973):

$$\ln L = \sum_{i=1}^{a} Y_i(\beta_o + \beta_1 X_i) - \sum_{i=1}^{a} \ln[1 + \exp(\beta_o + \beta_1 X_i)] \qquad (2.3)$$

where, $Y_i = 0, 1$; and i=1 to a (the number of mice used in the dose response for a particular batch of oocysts).

## DISINFECTION METHODS

### Reactor Vessels

The reactor vessels were 250 mL erlenmyer flasks that were made oxidant demand free (ODF). The reactor contents were mixed continuously using an ODF teflon coated magnetic stir bar and a magnetic stir plate. The agitation speed was set for rapid mixing without forming a vortex in the solution. Room temperature experiments (22 ± 1°C) were conducted on the lab bench with the reactor covered in aluminum foil to minimize chemical decomposition. For experiments conducted at 1 and 10°C the reactors were placed in a covered, water bath set at the appropriate temperature.

Suspensions of oocysts were prepared at the beginning of each experimental week as needed and stored in the dark at 4°C in 5 mL vials. A graduated cylinder was used to measure 200 mL oxidant demand free, 0.05M phosphate buffer. Phosphate buffer at pH 6.0 was used for free chlorine trials and pH 8.0 for monochloramine trials. Approximately 100 mL phosphate buffer was added to a reactor. The suspension of oocysts were vortexed and transferred to the reactor using a micropipet with an ODF pipet tip. The vial that contained the oocysts was then rinsed into the reactor using the 0.05M phosphate buffer to ensure all of the oocysts were added. The remaining

phosphate buffer was then added to the reactor and the reactor contents were agitated continuously.

## Chlorine Dioxide

Chlorine dioxide stock solution was generated using a chlorine dioxide generator provided by CDG Technology, Inc. The system uses a patented process to generate chlorine dioxide gas at a fixed concentration. Chlorine gas in nitrogen was passed through a packed bed of sodium chlorite where the chlorine reacts with sodium chlorite to produce chlorine dioxide. The flow rate was adjusted so that most of the chlorine gas was consumed in the reaction column. The process gas was then bubbled through oxidant demand free (ODF) water in a covered 500 mL gas absorption flask, for approximately 15 minutes. Since temperature and ultraviolet light affect solution stability, the chlorine dioxide stock solution was stored in airtight amber borosilicate bottles with Teflon lined septa at 4°C. A fresh stock of chlorine dioxide solution was prepared each experimental week. The concentration of the stock solution was checked using ultraviolet spectrophotometry at 360 nm using an absorptivity of 1250 $M^{-1}cm^{-1}$ (Gordon *et al.* 1992).

## Ozone

Ozone stock solution was prepared by passing oxygen gas through a water cooled corona discharge generator (Model T-816, Welsbach Ozone System Corporation, Sunnyvale CA). The generated ozone was bubbled through deionized water from an Elga system (Fisher Scientific Inc.) operating at a resistivity of at least 18 Mohm/cm, in a 500 mL gas absorption flask for at least 20 minutes. The ozone concentration in the carrier gas is approximately 4.8% ozone (wt/wt) at ambient temperature and pressure, giving an ozone stock solution concentration ranging from 18 to 20 mg/L (Labatiuk *et al.* 1991). When using the ozone in higher demand waters, the stock solution concentration was increased to approximately 40 mg/L by lowering the temperature of the solution to 4°C. The gas absorption flask containing Elga Ultra Pure laboratory water was refrigerated for

a few hours to reduce the temperature of the contents to 4°C. The flask was stored in an ice water bath when it was used on the lab bench to maintain the low temperature of the contents.

## Free Chlorine and Monochloramine

Chlorine stock solutions were prepared daily as needed using a 1:100 dilution of sodium hypochlorite 6% available chlorine solution with ODF water to give approximately 150 mg/L chlorine solution. The stock solution was stored in dark, refrigerated conditions.

Monochloramine was prepared daily as needed using sodium hypochlorite and stock ammonium chloride solution (1000 mg/L). The sodium chlorite and ammonium chloride solutions were diluted using ODF water to give 300 mg/L chlorine solution and 100 mg/L $NH_4Cl$-N. The two solutions were added together and mixed for 30 min in the dark to produce a monochloramine stock solution of approximately 150 mg/L as $Cl_2$. The resultant solution was then checked for free chlorine and monochloramine using the DPD procedure (Eaton *et al.* 1995) and could be used if only monochloramine was detected. Monochloramine stock solution was also stored in dark, refrigerated conditions.

## Residual Oxidant Measurement

### *Chlorine Dioxide*

The concentration of chlorine dioxide stock solution was measured at the beginning of each experimental trial day and a calculated volume was added to the reactor contents using a calibrated pipet. The concentration of chlorine dioxide in the aqueous solution was measured continuously during an experimental trial using an ultraviolet spectrophotometer at 360 nm using a molar absorption coefficient of 1250 $M^{-1}cm^{-1}$ (Gordon *et al.* 1992). For continuous monitoring of chlorine dioxide residual a

diode-array spectrophotometer (Hewlett-Packard Model 8452A) with a 10 mm light path, 35 μL flow through cell was operated in a closed loop using a peristaltic pump with a flow rate of 8 mL/min.

At the end of the contact time residual chlorine dioxide was neutralized by purging with nitrogen gas for 15 minutes at a flow rate of approximately 17 mL/min. According to the results found for an unpublished American Water Works Association Research Foundation (AWWARF) sponsored project nitrogen purging neutralizes residual chlorine dioxide in approximately 5 minutes. Initial chlorine dioxide residual was the observed concentration in the solution immediately after adding the chlorine dioxide. Final residual was calculated from the change in absorbance over the total contact time. To date there is no information on the effects of nitrogen gas on *C. parvum* and for this study it was assumed that effects due to nitrogen purging were negligible compared to the inactivation achieved by chemical treatment.

### *Ozone*

The concentration of the ozone stock solution was measured three times before adding it to the reactor and three times immediately after. The applied ozone dose was calculated from the overall average of the six concentrations. The concentration of ozone was measured continuously using ultraviolet spectrophotometry at 260 nm and using a molar absorption coefficient of 3300 $M^{-1}cm^{-1}$ (Hart *et al.* 1983). For continuous monitoring of ozone residual the diode-array spectrophotometer used above was operated as described. Ozone residual was neutralized using sodium formate and the final ozone concentration was calculated from the change in absorbance after the addition of the sodium formate. Initial ozone residual was the observed concentration in the solution immediately after adding ozone.

The indigo colorimetric procedure was used in parallel with the spectrophotometer to confirm ozone concentration measurements. The Swiss standard method for the determination of ozone in water (indigo method), as developed by Bader *et al.* (1982) was followed. An indigo stock solution (1 mmol/L pure indigo trisulfonate)

was prepared, along with a diluted form of the solution (indigo reagent). Several vials were prepared with 5 to10 mL of the indigo reagent. One of these was a blank and diluted with 10 mL of experimental water. After ozone was applied to the reactor contents, the vials were diluted with 10 mL samples taken from the reactor at specific times during the contact period. The absorbance was then measured on a spectrophotometer set at a wavelength of 600 nm and the ozone concentration was calculated using the equation:

$$C_o = \frac{\Delta A \cdot (V_s + V_i)}{f \cdot V_s} \tag{2.4}$$

where, A is the difference in absorbance between sample and blank, $V_s$ is the volume of the sample added, $V_i$ is the volume of the indigo reagent and f is a factor of 0.42 corresponding to an absorption coefficient for aqueous ozone at 260 nm, 2900 $mol^{-1}cm^{-1}$ (Bader *et al.* 1982).

### *Free Chlorine and Monochloramine*

The concentration of the chlorine and monochloramine stock solutions were measured using the DPD colorimetric procedure (Eaton *et al.* 1995). This procedure involves using free or total chlorine Hach packets to measure free chlorine or monochloramine residual, respectively. The contents of the Hach packets are mixed with a 5:1 dilution of sample with oxidant demand free water in oxidant demand free vials. The chlorine residual was measured on a spectrophotometer at a wavelength of 515 nm providing a light path of 10 mm. Chlorine and monochloramine residuals were neutralized using sodium sulfite.

## Oxidant Demand Free Materials

Oxidant demand free (ODF) water was prepared by bubbling ozone through Elga Ultra Pure laboratory water, for at least 30 min and then boiling for at least 10 min. After

boiling, the water was cooled to room temperature under a clean air hood and later transported and stored in an amber 4L bottle on the lab bench.

Oxidant demand free glassware was prepared by exposing acid washed glassware to laboratory water with an ozone residual of at least 20 mg/L overnight and then drying for at least 2 days in a drying oven at 80°C. All glassware openings were covered with aluminum foil to prevent dust from entering.

The pH 6.0 and 8.0, 0.05 M phosphate buffer were prepared using Elga Ultra Pure water and dissolving the appropriate amounts of potassium dihydrogen orthophosphate and disodium hydrogen orthophosphate to provide the required pH. The buffer was then made oxidant demand free by bubbling ozone through the solution for at least 30 min and boiling for at least 10 min. The buffer was cooled to room temperature and transported to a 4L amber bottle and stored on the lab bench.

## DISINFECTION KINETICS

### Chick-Watson Model

Inactivation of *C. parvum* using free chlorine or monochloramine alone or sequentially in laboratory water was predicted using the Chick-Watson model (Equation 1.9). As the Chick-Watson model was developed for aqueous solutions at room temperature (approximately 22°C) a temperature correction factor (θ) was applied to the model to account for the difference in inactivation rate of chemicals at lower temperatures. If the Ct product is expressed as $C_{avg}t$ (average of the initial and final concentrations of the disinfectant x contact time) the Chick Watson model can be rewritten as follows:

$$\log\frac{N}{N_o} = -K_{22}\theta^{(T-22)}C_{avg}t \qquad (2.5)$$

where, $K_{22}$ is the rate of inactivation at 22°C and T is the water temperature.

## Incomplete Gamma Hom Model

The I.g.H model (Equation 1.11) was used in this project to predict the log inactivation of *C. parvum* using ozone and free chlorine in natural water. In Excel 97/98 the incomplete gamma function can be formulated as follows:

$$\text{Gammadist((nk't),m,true) x exp(Gammaln(m))} \tag{2.6}$$

## Model Parameter Estimation

The parameters k and θ for the Chick Watson (n=1) model with temperature correction were estimated for the disinfection of *C. parvum* oocysts using free chlorine or monochloramine alone in lab water. Model parameters k and θ (n=1) were also estimated for the secondary chemicals used in chlorine dioxide/free chlorine or chlorine dioxide/monochloramine sequential inactivation. Each set of parameters were calculated separately using the solver function of Excel 97/98 to maximize the $\log_e$ likelihood function for normally distributed errors (Seber and Wild 1989):

$$\ln L = -n \ln \sigma - \frac{1}{2} \sum_{i=1}^{n} \left( \frac{y_i - \hat{y}_i(x_i, \beta)}{\sigma} \right)^2 \tag{2.7}$$

where, $n$ = the number of disinfection trials, $\sigma$ = standard deviation of the regression error estimated along with the model parameters, $y_i$ = observed log reduction in infectivity of oocysts (survival ratio in log-units) for trial i, $\hat{y}_i(x_i, \beta)$ = the Chick-Watson or I.g.H model predicted survival ratio for trial $i$, $x_i$ = experimental settings for trial i ($C_o$, k' and t) and $\beta$ = parameters k, m and n for the I.g.H model and k for the Chick-Watson model (Gyürék *et al.* 1999).

Upper and lower 90% confidence limits were calculated for model parameter estimates using the likelihood ratio test (Seber and Wild 1989):

$$\ln L_{1-\alpha}(\beta) \geq \ln L_{\max}(\hat{\beta}) - \frac{1}{2} \chi^2_{p,\alpha} \tag{2.8}$$

where, ln L(β) is the ln likelihood and $L_{max}(\hat{\beta})$ its maximum. Excel 97/98 was used to compute the limits by varying one parameter at a time with the other two parameters fixed to their optimal estimates.

All trials that were conducted in this project followed the methods explained. These are widely used and well-developed microorganism reduction research methods. Using consistent methodologies is important for comparing the results of microorganism reduction studies.

# CHAPTER 3

# SYNERGISTIC EFFECT OF SEQUENTIAL EXPOSURE OF *CRYPTOSPORIDIUM PARVUM* OOCYSTS TO CHLORINE DIOXIDE AND CHLORINE SPECIES

## INTRODUCTION

Sequential disinfection experiments were conducted using laboratory water at 1, 10 and 22°C. The results of these experiments are analyzed and discussed in this section. All trials were randomized so that independent observations were obtained. This eliminates the problem of serial correlation of the data, which is necessary for proper statistical analysis of data (Box *et al.* 1978). Experiments were run in duplicate to ensure that results were reproducible and duplicate trials were randomized to use different batches of mice on different days. Controls were run for each day's trials and animal infectivity (using CD-1 neonatal mice and a logistic dose response model) was used for each experimental trial.

## EXPERIMENTAL DESIGN

Sequential and single chemical trials were conducted using laboratory waters. Reactors were seeded with oocysts and received either both primary and secondary chemical treatments, sequentially, or secondary chemical treatment alone. The primary chemical applied was chlorine dioxide and the secondary chemical was free chlorine or monochloramine.

For the most part, different concentration and contact time combinations were conducted as individual trials. However, free chlorine and monochloramine treatment required long contact times (up to 980 min) and for some of the sequential chemical trials samples were taken at two different contact times from the same reactor.

## Sequential Disinfection

A preparation of approximately 40 million oocysts was transferred to a 250 mL reactor containing 200 mL 0.05 M phosphate buffer and the reactor contents were agitated continuously. Reactors were placed in water baths set at either 1°C or 10°C for cold temperature trials or on the lab bench for room temperature trials.

After the reactor contents temperature had stabilized a control sample was taken and stored in the dark at 4°C. A calibrated volume of the chlorine dioxide stock solution was then added to the reactor and the concentration of chlorine dioxide in the reactor was monitored continuously over the required contact time using ultraviolet spectrophotometry at 360 nm. At the end of the contact time, residual chlorine dioxide was neutralized by purging with nitrogen gas for 15 min.

Following nitrogen purging a sample was taken from the reactor and stored in the dark at 4°C. The appropriate volume of free chlorine or monochloramine stock solution was then added to the reactor. A sample for the initial chlorine residual measurement was taken after approximately five minutes. The residual was determined using DPD colorimetric procedures described in Chapter 2. At the end of the first contact time (240 min or 480 min), residual free chlorine or monochloramine was measured and a sample was collected and stored at 4°C. At the end of the final contact time (approximately 960 min) residual chlorine was measured and then neutralized with 0.1 N sodium sulfite and a sample was taken.

All samples were then submitted for animal infectivity. Cohorts of 5 neonatal CD-1 mice were infected with a predetermined dose of oocysts from the disinfection samples and later scored either positive or negative for oocysts. The cohort size, number of oocysts in each inoculum and number of infected mice from the disinfection trials are provided in Tables A.2a and A.2b in Appendix A.

## Free Chlorine and Monochloramine

A suspension of 5 million oocysts was transferred to a reactor containing 200 mL, 0.05M phosphate buffer and the reactor contents were agitated continuously in a water bath or on the lab bench. After the reactor contents temperature had stabilized a control sample was taken and stored in the dark at 4°C. A known volume of free chlorine or monochloramine was then added to the reactor and an initial chlorine residual was determined as before. At the end of the intermediate and final contact times, residual free chlorine or monochloramine was measured and a sample was taken. At the end of the final contact time chlorine residual was neutralized with 0.1 N sodium sulfite and the samples were submitted for animal infectivity.

## RESULTS

Raw data for sequential disinfection experiments at 1, 10 and 22°C in laboratory waters are included in Appendix A. The infectivity data for the dose response trials are in Tables A.1a and A.1b in Appendix A. The infectivity data for the microorganism reduction trials are in Tables A.2a and A.2b in Appendix A. Both sets of data include the cohort size, inoculum and number of infected mice. The raw data for the chlorine dioxide/free chlorine, chlorine dioxide/monochloramine and single treatment trials are in Appendix B in Tables B.1, B.2 and B.3, respectively.

## Dose Response Model Parameter Estimates

Two batches of oocysts (34 and 35) were used in the experiments conducted for this project. Dose response trials were conducted each week. Cohorts of 5 and 10 mice were used for dose response trials as shown in Tables A.1a and A.1b in Appendix A. The logistic dose response model parameters were estimated using maximum likelihood as described in Chapter 2. The model parameters for each batch of oocysts have been summarized in Table 3-1. According to the table, the 50% infective dose ($ID_{50}$) was estimated to be 58 oocysts for batch 34 and 82 oocysts for batch 35. Also, the 90%

confidence intervals for the estimated model parameters were small indicating that the models are accurate.

**Table 3-1 Logistic Dose Response Model Parameters for *Cryptosporidium parvum* Infectivity of Neonatal CD-1 Mice**

| | Oocyst Batch Number | |
|---|---|---|
| | 34 | 35 |
| Relevant trial numbers | 747 to 816 | 817 to 826 |
| $\beta_0$ | -5.4 | -9.9 |
| 90% $\beta_0$ limits | -5.7, -5.1 | -10.2, -9.6 |
| $\beta_1$ | 3.1 | 5.2 |
| 90% $\beta_1$ limits | 2.9, 3.2 | 5.0, 5.3 |
| $ID_{50}$ | 58 | 82 |
| Natural logarithm of the Likelihood (Ln) | -96.2 | -98.2 |
| Pearson Chi-Square | 2.7 | 2.7 |
| No. of mice used to develop logit equation | 220 | 279 |

## Controls

A control sample was collected from a reactor at the beginning of each experimental trial and before any of the chemicals were applied to the reactor contents. A summary of the observed log inactivation (referred to as log kill) of *C. parvum* for the control samples is shown in Table 3-2. The log inactivation for trials conducted at 1, 10 and 22°C were essentially equal to zero, as expected. The overall average of the log inactivation of the untreated oocysts was –0.07 log-units or, basically, zero log-units. All control data vary about zero and can be considered noise. The standard deviation of the control data was 0.4 log-units and the data ranged from –0.6 to 0.5 log-units. This suggests that there were variations in the dose response model but this was expected due to the inherent variability in animal model systems.

**Table 3-2 Observed reductions in animal infectivity for untreated (5 min control) *Cryptosporidium parvum* oocysts at 1, 10 and 22°C and pH 6.0 and pH 8.0**

| Trial No. | Temperature (°C) | pH* | Observed kill‡ (log-units) |
|---|---|---|---|
| 759-760 | 1 | 6 | -0.01 |
| 766-767 | 1 | 6 | -0.51 |
| 776-777 | 1 | 6 | 0.20 |
| 825-826 | 1 | 6 | -0.39 |
| 792-793 | 1 | 8 | 0.39 |
| 802-803 | 1 | 8 | 0.39 |
| 810-811 | 1 | 8 | 0.39 |
| 815-816 | 1 | 8 | -0.61 |
| 820-821 | 1 | 8 | -0.18 |
| 761 | 10 | 6 | 0.07 |
| 768-769 | 10 | 6 | -0.51 |
| 778-779 | 10 | 6 | -0.16 |
| 794-795 | 10 | 8 | 0.49 |
| 797 | 10 | 8 | -0.61 |
| 804-805 | 10 | 8 | -0.19 |
| 812-813 | 10 | 8 | -0.01 |
| 757-758 | 22 | 6 | 0.44 |
| 763 | 22 | 6 | -0.51 |
| 774-775 | 22 | 6 | 0.34 |
| 780-781 | 22 | 6 | 0.34 |
| 790-791 | 22 | 8 | -0.19 |
| 796 | 22 | 8 | -0.61 |
| 800-801 | 22 | 8 | 0.39 |
| 808-809 | 22 | 8 | -0.19 |
| 818-819 | 22 | 8 | -0.39 |

*Free chlorine trials at pH 6 and monochloramine trials at pH 8.
‡Kill refers to inactivation of *C. parvum*

## Free Chlorine and Monochloramine Kinetic Model

The effects of free chlorine and monochloramine on the inactivation of *C. parvum* at different levels of disinfectant $C_{avg}t$ and varying water temperatures were studied. Single treatment trials were conducted to estimate Chick-Watson model parameters for free chlorine and monochloramine inactivation of *C. parvum* at 1, 10 and 22°C. The

model parameter estimates were calculated using the results from the single treatment experiments conducted in this study along with a more complete, unpublished data set developed for an American Water Works Association Research Foundation (AWWARF) sponsored project. The single treatment experiments conducted for the AWWARF project used equivalent methods to those conducted in this study but the results have not been included in this thesis. The large data set obtained by combining the two sets of results allowed for a more statistically accurate estimate of model parameters. The proposed Chick-Watson model with temperature correction has been adapted from the AWWARF report and the model parameters are provided in Table 3-3. The method of maximum likelihood, discussed in Chapter 2, was used in the AWWARF report to calculate the parameter estimates. The developed models were reported to give an accurate prediction of the log inactivation of *C. parvum* oocysts under the conditions tested.

The results for the free chlorine and monochloramine treatment trials conducted in this research project are provided in Tables 3-4 and 3-5, respectively. The results from the single treatment trials indicated that at 22°C and a $C_{avg}t$ greater than 2000 mg·min/L more than 1.0 log-units inactivation of *C. parvum* was possible for free chlorine. At 22°C 1.0 log-units inactivation was observed when the monochloramine $C_{avg}t$ was greater than 4000 mg·min/L. At 1 and 10°C, for both chlorine species, no greater than 0.4 log-units inactivation was observed over a range of $C_{avg}t$ values. Also included in these tables are the predicted log inactivations of *C. parvum* using the model parameter estimates from Table 3-3. A comparison of the results of this study to the predicted inactivations showed that the mean difference was –0.1 log-units and the standard error of the mean was 0.2 log-units. The standard error is reasonable considering the inherent variability in the animal model used. The model seemed to adequately describe the inactivation of *C. parvum* using free chlorine or monochloramine at temperatures from 1 to 22°C.

**Table 3-3 Summary of Chick-Watson Model Parameters for Inactivation of *Cryptosporidium parvum* Using Free Chlorine or Monochloramine**

| Disinfectant | Free Chlorine | Monochloramine |
|---|---|---|
| Temperature (°C) | 1 to 22 | 1 to 22 |
| pH | 6 | 8 |
| Number of data points | 19 | 25 |
| Temperature coeff. θ | 1.08 | 1.12 |
| K (22°C) | 0.00031 | 0.00024 |
| Error, σ | 0.25 | 0.28 |
| Model constraints for disinfection initial residual $C_o$ (mg/L) and Contact time t (min) | $0.9 \leq C_o \leq 14.2$<br>$60 \leq t \leq 1032$ | $0.9 \leq C_o \leq 15.2$<br>$5 \leq t \leq 980$ |

- Adapted from an AWWARF sponsored report using unpublished data with permission.

**Table 3-4 Summary of *Cryptosporidium parvum* Inactivation Using Free Chlorine in 0.05M Oxidant Demand-free Phosphate Buffer at pH 6.0 and 1°C, 10°C and 22°C**

| Trial No. (1) | | Temp. (°C) (2) | $C_o$ (mg/L) (3) | $C_r$ (mg/L) (4) | t (min) (5) | $C_{avg}t$ (mg·min/L) (6) | Observed $I_r$ (log-units)† (7) | Predicted kill (log-units)* (8) |
|---|---|---|---|---|---|---|---|---|
| *826.1* | *826.1* | 1 | 2.7 | 2.1 | 960 | 2309 | 0.0 | 0.1 |
| *776.1* | *776.2* | 1 | 5.4 | 4.9 | 960 | 4973 | 0.1 | 0.3 |
| *824.1* | *824.2* | 10 | 1.1 | 0.8 | 990 | 936 | 0.0 | 0.1 |
| *823.1* | *823.2* | 10 | 3.9 | 3.5 | 990 | 3643 | 0.4 | 0.4 |
| *780.1* | *780.2* | 22 | 2.7 | 2.3 | 995 | 2517 | >1.29 | 0.8 |
| *774.1* | *774.2* | 22 | 4.1 | 3.6 | 970 | 3735 | >1.29 | 1.2 |

†observed inactivation

*Predicted inactivation estimated from Chick-Watson model (Table 3-3)

**Table 3-5 Summary of *Cryptosporidium parvum* Inactivation Using Monochloramine in 0.05M Oxidant Demand-free Phosphate Buffer at pH 8.0 and 1°C, 10°C and 22°C**

| Trial No. (1) | | Temp. (°C) (2) | $C_o$ (mg/L) (3) | $C_r$ (mg/L) (4) | t (min) (5) | $C_{avg}t$ (mg·min/L) (6) | Observed $I_r$ (log-units)† (7) | Predicted kill (log-units)* (8) |
|---|---|---|---|---|---|---|---|---|
| *816.1* | *816.2* | 1 | 3.0 | 3.1 | 465 | 1428 | 0.01 | 0.03 |
| *815.1* | *815.2* | 1 | 4.6 | 4.6 | 470 | 2153 | 0.3 | 0.1 |
| *816.1* | *816.3* | 1 | 3.0 | 3.0 | 960 | 2890 | 0.0 | 0.1 |
| *815.1* | *815.3* | 1 | 4.6 | 4.5 | 960 | 4363 | 0.2 | 0.1 |
| *819.1* | *819.2* | 10 | 1.5 | 1.4 | 980 | 1411 | 0.0 | 0.1 |
| *806.1* | *806.2* | 10 | 4.5 | 4.4 | 480 | 2131 | 0.7 | 0.1 |
| *806.1* | *806.3* | 10 | 4.5 | 4.4 | 960 | 4253 | 0.7 | 0.3 |
| *797.1* | *797.2* | 10 | 4.7 | 4.4 | 960 | 4363 | 0.2 | 0.3 |
| *814.1* | *814.2* | 22 | 1.5 | 1.5 | 460 | 681 | 0.3 | 0.2 |
| *814.1* | *814.3* | 22 | 1.5 | 1.3 | 975 | 1346 | 0.3 | 0.3 |
| *796.1* | *796.2* | 22 | 4.6 | 4.2 | 960 | 4248 | 1.1 | 1.0 |

†observed inactivation

*Predicted inactivation estimated from Chick-Watson model (Table 3-3)

## Sequential Treatment Kinetic Model

A Chick-Watson model with temperature correction was developed for sequential inactivation using chlorine dioxide followed by free chlorine or monochloramine. The model parameter estimates are provided in Table 3-6. Sequential trials were grouped according to applied chlorine dioxide pretreatment levels and the method of maximum likelihood, described in Chapter 2, was used to calculate the parameter estimates. The details of these calculations are provided in Appendix C. The developed models predicted the log inactivation of *C. parvum* by free chlorine or monochloramine following pretreatment with high or low levels of chlorine dioxide. By adding the predicted value to the observed log inactivation after chlorine dioxide pretreatment, the gross predicted log inactivation was determined. The estimate of the standard deviation of the free chlorine and monochloramine models is also provided in Table 3-6. Plots of the observed log inactivation versus the predicted log inactivation for chlorine dioxide followed by free chlorine or monochloramine are shown in Figures C.1 to C.4 in Appendix C. All but one of the data points in these plots were within ± 1.0 log-units of the model prediction. The variation of the data in Figures C.1 to C.4 suggests that the

proposed model does not describe the observed data adequately. This is most likely due to the inherent variation in animal dose response models. The proposed model can be used for comparison purposes only, as more trials need to be conducted to obtain a model that describes the observed data accurately.

**Table 3-6 Summary of Chick-Watson Model Parameters for Sequential Inactivation of *Cryptosporidium parvum* Using Chlorine Dioxide Followed by Free Chlorine or Monochloramine**

| Secondary Disinfectant | Free Chlorine | | Monochloramine | |
|---|---|---|---|---|
| Temperature (°C) | 1 to 22 | | 1 to 22 | |
| pH | 6 | | 8 | |
| Chlorine dioxide pretreatment levels (log-units) | 0.5 | 1.4 | 0.6 | 1.3 |
| Number of data points | 12 | 26 | 18 | 21 |
| Temperature coeff. $\theta$ | 1.015 | 1.159 | 1.039 | 1.009 |
| K (22°C) | 0.00087 | 0.00077 | 0.00047 | 0.00026 |
| Error, $\sigma$ | 0.33 | 0.40 | 0.40 | 0.29 |
| Model constraints for secondary disinfection<br>Initial residual $C_o$ (mg/L)<br>Contact time t (min) | $1.1 \leq C_o \leq 4.8$<br>$230 \leq t \leq 1020$ | $0.8 \leq C_o \leq 4.3$<br>$225 \leq t \leq 970$ | $1.1 \leq C_o \leq 4.9$<br>$200 \leq t \leq 980$ | $1.7 \leq C_o \leq 3.8$<br>$430 \leq t \leq 985$ |

## Sequential Chemical Treatment

The standard deviation of the control data was approximately 0.4 log-units, which describes the variation in the dose response model used. Previous studies, that used the same animal model as this study, have shown that the animal model is accurate within ±0.5 log-units (Finch *et al.* 1993; Finch *et al*, 1997). The standard error of the estimate of the mean inactivation was approximately 0.2 log-units for the single treatment trials and 0.3 log-units for the sequential treatment trials. This describes both the variation in the animal model and the disinfection experiments. Therefore, in the sequential treatment trials where the inactivation beyond the simple sum of the individual chemicals was substantially greater than 0.5 log-units, a synergistic effect was signaled.

The factors studied for sequential treatment using chlorine dioxide followed by free chlorine and monochloramine included levels of chlorine dioxide pretreatment, the chlorine species $C_{avg}t$ product (average of initial and final chlorine concentration multiplied by contact time) and water temperature. Chlorine dioxide pretreatment levels were targeted at either 0.6 or 1.4 log-units inactivation of *C. parvum* oocysts. Chlorine species $C_{avg}t$ products ranged from 200 to 5000 mg·min/L and values in this range were studied at each chlorine dioxide pretreatment level. Temperature effects were studied at 1, 10 and 22°C and chlorine dioxide pretreatment was designed to obtain the same levels of inactivation at each temperature by adjusting the chlorine dioxide dose and contact time. The results of the experimental trials under these conditions were analysed and compared to model predictions.

### *Chlorine Dioxide Followed by Free Chlorine*

The trials for chlorine dioxide followed by free chlorine at pH 6.0 and 1°C were conducted using only a high level of chlorine dioxide pretreatment. The results for these trials are shown in Table 3-7. This table also includes the calculated synergy (log-units) and gross predicted inactivation (log-units). The synergy was calculated by subtracting the observed inactivation of *C. parvum* after chlorine dioxide pretreatment and the predicted inactivation due to free chlorine used singly (Table 3-3) from the observed inactivation after sequential treatment. The gross predicted inactivation was determined by adding the observed inactivation after chlorine dioxide pretreatment to the predicted inactivation due to free chlorine used sequentially (Table 3-6). The predicted inactivations of the sequential treatment tended to have similar results to the observed inactivations. The observed inactivations were within ± 0.7 log-units of the predicted, which is acceptable due to the variability in the animal model used. The high level chlorine dioxide pretreatment required that a chlorine dioxide dose of approximately 2.6 mg/L was added to the reactor contents and that the contact time was 240 min. This gave an average inactivation of 1.3 log-units. For the secondary free chlorine treatment, different levels of free chlorine Ct products were applied. Some replicates were also conducted. The $C_{avg}t$ of the secondary disinfectant was used to interpret the level of

inactivation of *C. parvum* oocysts. The response of the survival ratio of *C. parvum* after chlorine dioxide pretreatment versus the free chlorine $C_{avg}t$ at 1°C is shown in Figure 3-1. The standard deviation (error bar) of the average log inactivation due to chlorine dioxide pretreatment at 1°C was 0.3 log-units and is marked on the y-axis on the figure. The Chick-Watson model predicted survival curve for free chlorine used singly (Table 3-3) is also provided on the figure. Censored data (less than or greater than the measurement limit for the particular trial) were not included in the figure as duplicate trials were conducted to obtain more accurate, non-censored values. The results showed that even when the $C_{avg}t$ product was quite high (greater than 3000 mg·min/L) the log inactivation by free chlorine after chlorine dioxide pretreatment was negligible and synergy was not observed. Due to these findings at high level chlorine dioxide pretreatment, low level chlorine dioxide pretreatment trials were not conducted.

The results for the chlorine dioxide followed by free chlorine trials at 10°C and pH 6.0, along with the level of synergy and overall predicted log inactivation are provided in Table 3-8. The survival ratio (log-units) of *C. parvum* for different free chlorine Ct products after high and low level chlorine dioxide pretreatments are shown in Figure 3-2. The Chick-Watson model predicted survival ratio for free chlorine is also shown on the figure. The censored result after high level chlorine dioxide pretreatment was not included in the figure as the condition for this data point was duplicated, which provided a non-censored result. The observed inactivations of the sequential treatments were within ± 0.6 log-units of the predicted inactivations at the high pretreatment level but were different by 0.9 log-units at the low pretreatment level. The model prediction did not seem adequate at this temperature after low level pretreatment. For high level chlorine dioxide pretreatment, a chlorine dioxide dose of 1.8 mg/L for a contact time of 180 minutes resulted in an average chlorine dioxide inactivation of 1.4 log-units. At the low level chlorine dioxide pretreatment, a chlorine dioxide dose of 1.4 mg/L for 90 minutes resulted in a chlorine dioxide inactivation that ranged from 0.1 to 0.7 log-units and gave an average inactivation of 0.4 log-units. The standard deviations for the high and low level chlorine dioxide inactivation were 0.3 and 0.4 log-units respectively, and are included on the figure. Several levels of free chlorine Ct products were applied at each pretreatment level and some replicates were conducted. It appeared that under both

chlorine dioxide pretreatment levels, when $C_{avg}t$ products were above 1000 mg·min/L the survival ratio decreased roughly linearly with increasing $C_{avg}t$. This linear decrease appeared to be more substantial for free chlorine inactivation after low level chlorine dioxide pretreatment compared to high level pretreatment. Also, a low level of chlorine dioxide pretreatment seemed favorable for obtaining a synergistic effect. Synergy of about 0.8 log-units was observed for low level chlorine dioxide pretreatment after a free chlorine $C_{avg}t$ of 2500 mg·min/L. For the high level chlorine dioxide pretreatment condition the same level of synergy was not observed until a free chlorine $C_{avg}t$ of 4000 mg·min/L was reached. In addition, under both chlorine dioxide pretreatment levels, there was a trend toward an increase in potential synergy as the free chlorine Ct increased. The level of additional inactivation due to synergy increased from 0 to 0.7 log-units over a free chlorine $C_{avg}t$ of 500 to 4000 mg·min/L at the high chlorine dioxide pretreatment level and from 0 to 0.8 log-units over a free chlorine $C_{avg}t$ of 500 to 1000 mg·min/L at the low pretreatment level.

The room temperature (22°C) experimental results for chlorine dioxide followed by free chlorine are provided in Table 3-9. The survival curves for the experimental results and free chlorine predictions are shown in Figure 3-3. Most of the censored data were not included in the figure as duplicate trials were conducted that provided non-censored results. Two levels of chlorine dioxide pretreatment were used in these experiments. The dose for chlorine dioxide pretreatment was lower than that applied at 1°C and 10°C. At the high level pretreatment, a chlorine dioxide dose of 1.4 mg/L chlorine dioxide for 90 min gave approximately 1.3 log-units inactivation. At the low level pretreatment, a chlorine dioxide dose of 1.0 mg/L for 45 min gave an average inactivation ratio of 0.6 log-units. Error bars for the standard deviation of chlorine dioxide log inactivation for both high and low level pretreatments are included in Figure 3-3. The observed inactivation seemed to follow the predicted level fairly well (within ± 0.6 log-units).

High log inactivation and synergy were observed at a high free chlorine $C_{avg}t$ product of 4300 mg·min/L and low level chlorine dioxide pretreatment. An overall inactivation of 4.4 log-units was observed with a synergy of 2.6 log-units. About 1.0 log-

unit of synergy was observed after a high level of chlorine dioxide pretreatment and a free chlorine $C_{avg}t$ of 1500 mg·min/L. For low level chlorine dioxide pretreatment a free chlorine $C_{avg}t$ of about 2000 mg·min/L resulted in an additional inactivation of approximately 1.0 log-unit. The level of potential synergy increased with the free chlorine Ct product and appeared to increase more rapidly after a low level of chlorine dioxide pretreatment compared to a high level. For a high level of chlorine dioxide pretreatment, a free chlorine Ct product of about 2500 mg·min/L resulted in a synergy of about 1.2 log-units and for a low level pretreatment the same free chlorine Ct resulted in a synergy of 1.5 log-units. It also appeared that the overall inactivation was the same for both high and low levels of pretreatment once the free chlorine $C_{avg}t$ reached 2500 mg·min/L. According to Figure 3-3, beyond approximately 2500 mg·min/L the same overall inactivation was obtained after high or low level pretreatment. For this reason, a higher level of additional inactivation due to synergy was observed after this free chlorine $C_{avg}t$ at the low chlorine dioxide pretreatment level compared to the high pretreatment level.

**Table 3-7 Summary of *Cryptosporidium parvum* Inactivation Using Chlorine Dioxide Followed by Free chlorine in 0.05M Oxidant Demand-free Phosphate Buffer at pH 6.0 and 1°C**

| Trial No. | Primary disinfectant (chlorine dioxide) | | | | Secondary disinfectant (Free chlorine) | | | | | Total | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | $C_o$ (mg/L) | $C_r$ (mg/L) | t (min) | Obs. $I_{r1}$ (log-units) | $C_o$ (mg/L) | $C_r$ (mg/L) | t (min) | $C_{avg}t$ (mg·min/L) | $I_{r2}$ † (log-units) | Obs. $I_r$ (log-units) | Synergy‡ (log-units) | Predicted kill' (log-units) |
| (1) | (2) | (3) | (4) | (5) | (6) | (7) | (8) | (9) | (10) | (11) | (12) | (13) |
| *760.1 760.2* | 2.5 | 2.5 | 240 | 1.1 | 0.8 | 0.2 | 457 | 229 | 0.0 | 1.7 | 0.6 | 1.1 |
| *760.1 760.3* | 2.5 | 2.5 | 240 | 1.1 | 0.8 | 0.2 | 936 | 468 | 0.0 | 1.7 | 0.6 | 1.1 |
| *767.1 767.2* | 2.7 | 2.2 | 240 | 1.4 | 1.4 | 0.7 | 450 | 470 | 0.0 | 1.1 | -0.3 | 1.4 |
| *759.1 759.2* | 2.4 | 1.9 | 240 | 0.8 | 2.9 | 2.8 | 243 | 693 | 0.0 | <1.09 | <0.3 | 0.8 |
| *766.1 766.2* | 2.7 | 2.0 | 240 | 1.4 | 3.0 | 2.9 | 240 | 710 | 0.0 | 0.8 | -0.6 | 1.5 |
| *767.1 767.3* | 2.7 | 2.2 | 240 | 1.4 | 1.4 | 0.7 | 960 | 994 | 0.1 | <1.09 | <-0.4 | 1.4 |
| *777.1 777.2* | 2.6 | 2.1 | 240 | 1.5 | 4.2 | 4.0 | 486 | 1993 | 0.1 | 1.1 | -0.5 | 1.6 |
| *759.1 759.3* | 2.4 | 1.9 | 240 | 0.8 | 2.9 | 2.5 | 900 | 2430 | 0.1 | <1.79 | <0.9 | 0.9 |
| *766.1 766.3* | 2.7 | 2.0 | 240 | 1.4 | 3.0 | 2.5 | 960 | 2659 | 0.2 | 1.1 | -0.5 | 1.6 |
| *777.1 777.3* | 2.6 | 2.1 | 240 | 1.5 | 4.2 | 3.9 | 960 | 3888 | 0.2 | 1.1 | -0.6 | 1.7 |

Note: † Free chlorine inactivation ($I_{r2}$) was estimated by the Chick-Watson model (Table 3-3);

‡ Synergy = Observed $I_r - (I_{r_1} + I_{r_2})$;

'Predicted inactivation was estimated by Chick-Watson model (Table 3-6)

predicted kill = model prediction (Table 3-6) + observed $I_{r1}$

kill refers to inactivation of *C. parvum*

< denotes less than measurement limit for particular trial

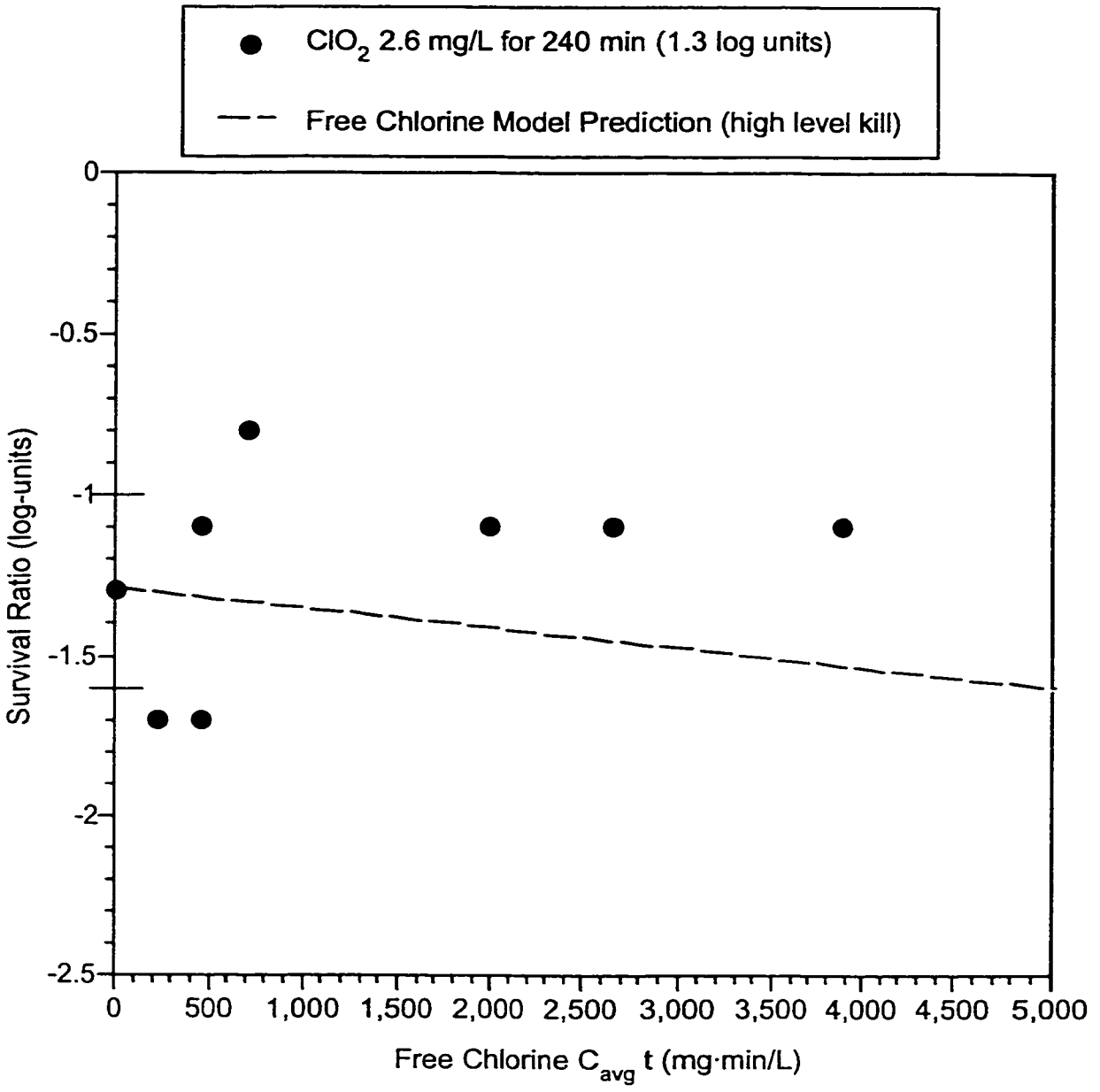


**Figure 3-1 Survival of *Cryptosporidium parvum* Oocysts as a Function of Free Chlorine $C_{avg}t$ after Chlorine dioxide Pretreatment in 0.05M Oxidant Demand Free Phosphate Buffer at pH 6.0 and 1°C**

Note: Survival determined using animal (CD-1 neonatal mice) infectivity and logit dose response model
Each data point is the result of a single trial
Free chlorine predicted survival curve estimated from Chick-Watson model (Table 3-3)

**Table 3-8 Summary of *Cryptosporidium parvum* Inactivation Using Chlorine Dioxide Followed by Free chlorine in 0.05M Oxidant Demand-free Phosphate Buffer at pH 6.0 and 10°C**

| Trial No. | | Primary disinfectant (chlorine dioxide) | | | | Secondary disinfectant (Free chlorine) | | | | | Total | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | $C_o$ (mg/L) | $C_r$ (mg/L) | t (min) | Obs. $I_{r1}$ (log-units) | $C_o$ (mg/L) | $C_r$ (mg/L) | t (min) | $C_{avg}t$ (mg·min/L) | $I_{r2}$ † (log-units) | Obs. $I_r$ (log-units) | Synergy‡ (log-units) | Predicted kill (log-units) (13) |
| (1) | | (2) | (3) | (4) | (5) | (6) | (7) | (8) | (9) | (10) | (11) | (12) | |
| *768.1* | *768.2* | 1.8 | 1.6 | 180 | 1.5 | 1.2 | 1.1 | 225 | 263 | 0.0 | 1.4 | -0.1 | 1.5 |
| *761.1* | *761.2* | 1.7 | 1.6 | 180 | 1.1 | 2.8 | 2.6 | 225 | 603 | 0.1 | 1.4 | 0.2 | 1.2 |
| *769.1* | *769.2* | 1.8 | 1.4 | 180 | 1.7 | 3.5 | 3.4 | 210 | 720 | 0.1 | 1.7 | -0.1 | 1.8 |
| *768.1* | *768.3* | 1.8 | 1.6 | 180 | 1.5 | 1.2 | 0.8 | 970 | 965 | 0.1 | <1.58 | <-0.02 | 1.6 |
| *779.1* | *779.2* | 2.0 | 1.4 | 180 | 1.2 | 4.3 | 4.1 | 514 | 2149 | 0.3 | 1.3 | -0.2 | 1.5 |
| *761.1* | *761.3* | 1.7 | 1.6 | 180 | 1.1 | 2.8 | 2.4 | 920 | 2383 | 0.3 | 1.8 | 0.4 | 1.5 |
| *769.1* | *769.3* | 1.8 | 1.4 | 180 | 1.7 | 3.5 | 3.2 | 960 | 3221 | 0.4 | 1.8 | -0.3 | 2.2 |
| *779.1* | *779.3* | 2.0 | 1.4 | 180 | 1.2 | 4.3 | 4.0 | 960 | 3994 | 0.5 | 2.4 | 0.7 | 1.8 |
| *771.1* | *771.2* | 1.3 | 1.2 | 90 | 0.7 | 1.4 | 0.9 | 426 | 488 | 0.1 | 0.4 | -0.4 | 0.8 |
| *770.1* | *770.2* | 1.3 | 1.2 | 90 | 0.1 | 4.3 | 4.2 | 230 | 975 | 0.1 | 1.0 | 0.8 | 0.3 |
| *771.1* | *771.3* | 1.3 | 1.2 | 90 | 0.7 | 1.4 | 0.8 | 960 | 1051 | 0.1 | 1.7 | 0.9 | 0.9 |
| *770.1* | *770.3* | 1.3 | 1.2 | 90 | 0.1 | 4.3 | 4.0 | 960 | 4013 | 0.5 | <1.49 | <0.9 | 0.7 |

Note: † Free chlorine inactivation ($I_{r2}$) was estimated by the Chick-Watson model (Table 3-3);

‡ Synergy = Observed $I_r - (I_{r_1} + I_{r_2})$;

'Predicted inactivation was estimated by Chick-Watson model (Table 3-6)

predicted kill = model prediction (Table 3-6) + observed $I_{r1}$

kill refers to inactivation of *C. parvum*

'<' denotes less than measurement limit for particular trial

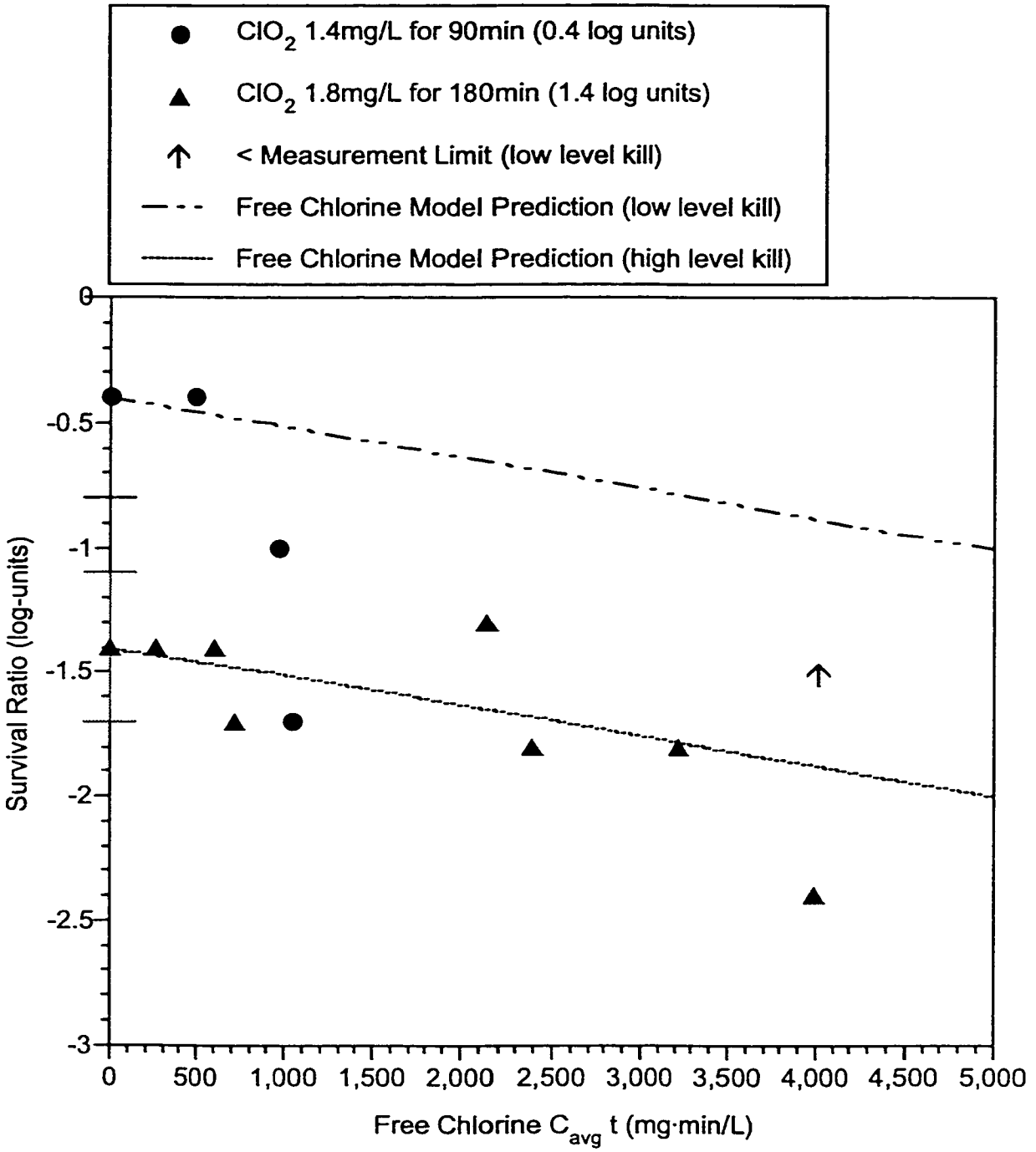


**Figure 3-2 Survival of *Cryptosporidium parvum* Oocysts as a Function of Free Chlorine $C_{avg}t$ after Chlorine dioxide Pretreatment in 0.05M Oxidant Demand Free Phosphate Buffer at pH 6.0 at 10°C**

Note: Arrow denotes less than measurement limit for particular trial
Survival determined using animal (CD-1 neonatal mice) infectivity and logit dose response model
Each free chlorine Ct data point is the result of a single trial
Free chlorine predicted survival curve estimated from Chick-Watson model (Table 3-3)

**Table 3-9 Summary of *Cryptosporidium parvum* Inactivation Using Chlorine Dioxide Followed by Free chlorine in 0.05M Oxidant Demand-free Phosphate Buffer at pH 6.0 and 22°C**

| Trial No. | | Primary disinfectant (chlorine dioxide) | | | | Secondary disinfectant (Free chlorine) | | | | | Total | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | $C_o$ (mg/L) | $C_r$ (mg/L) | t (min) | Obs. $I_{r1}$ (log-units) | $C_o$ (mg/L) | $C_r$ (mg/L) | t (min) | $C_{avg}t$ (mg·min/L) | $I_{r2}$ † (log-units) | Obs. $I_r$ (log-units) | Synergy‡ (log-units) | Predicted kill (log-units) (13) |
| (1) | | (2) | (3) | (4) | (5) | (6) | (7) | (8) | (9) | (10) | (11) | (12) | |
| *765.1* | *765.2* | 1.4 | 1.4 | 90 | 1.4 | 1.0 | 0.6 | 255 | 203 | 0.1 | 1.6 | 0.1 | 1.6 |
| *765.1* | *765.3* | 1.4 | 1.4 | 90 | 1.4 | 1.0 | 0.4 | 920 | 621 | 0.2 | 1.5 | -0.1 | 2.0 |
| *763.1* | *763.2* | 1.4 | 1.2 | 90 | 0.8 | 3.1 | 2.8 | 220 | 642 | 0.2 | 1.5 | 0.5 | 1.3 |
| *764.1* | *764.2* | 1.4 | 1.2 | 90 | 1.4 | 2.7 | 2.4 | 270 | 683 | 0.2 | 2.1 | 0.5 | 2.0 |
| *775.1* | *775.2* | 1.4 | 1.2 | 90 | 1.4 | 4.1 | 3.7 | 495 | 1931 | 0.6 | >3.69 | >1.7 | 3.0 |
| *764.1* | *764.3* | 1.4 | 1.2 | 90 | 1.4 | 2.7 | 2.2 | 950 | 2309 | 0.7 | 3.1 | 1.0 | 3.3 |
| *763.1* | *763.3* | 1.4 | 1.2 | 90 | 0.8 | 3.1 | 2.4 | 960 | 2602 | 0.8 | 3.4 | 1.8 | 2.9 |
| *775.1* | *775.3* | 1.4 | 1.2 | 90 | 1.4 | 4.1 | 3.5 | 960 | 3658 | 1.1 | 3.9 | 1.4 | 4.4 |
| *758.1* | *758.2* | 1.0 | 0.8 | 45 | 0.7 | 1.1 | 1.0 | 240 | 252 | 0.1 | 0.5 | -0.3 | 0.9 |
| *825.1* | *825.2* | 0.9 | 0.7 | 45 | 0.6 | 2.4 | 1.7 | 240 | 497 | 0.2 | <1.09 | <0.3 | 1.0 |
| *757.1* | *757.2* | 1.0 | 0.8 | 45 | 0.7 | 2.8 | 2.5 | 240 | 636 | 0.2 | 1.1 | 0.2 | 1.3 |
| *758.1* | *758.3* | 1.0 | 0.8 | 45 | 0.7 | 1.1 | 0.7 | 950 | 855 | 0.3 | <1.49 | <0.5 | 1.4 |
| *825.1* | *825.3* | 0.9 | 0.7 | 45 | 0.6 | 2.4 | 1.6 | 960 | 1934 | 0.6 | <1.79 | <0.6 | 2.2 |
| *781.1* | *781.2* | 1.0 | 0.8 | 45 | 0.5 | 4.8 | 4.1 | 460 | 2031 | 0.6 | 2.0 | 0.9 | 2.2 |
| *757.1* | *757.3* | 1.0 | 0.8 | 45 | 0.7 | 2.8 | 2.1 | 940 | 2303 | 0.7 | 2.8 | 1.4 | 2.6 |
| *781.1* | *781.3* | 1.0 | 0.8 | 45 | 0.5 | 4.8 | 4.0 | 985 | 4300 | 1.3 | 4.4 | 2.6 | 4.0 |

Note: † Free chlorine inactivation ($I_{r2}$) was estimated by the Chick-Watson model (Table 3-3);

‡ Synergy = Observed $I_r - ( I_{r_1} + I_{r_2} )$;

'Predicted inactivation was estimated by Chick-Watson model (Table 3-6)

predicted kill = model prediction (Table 3-6) + observed $I_{r1}$

kill refers to inactivation of *C. parvum*

'<' denotes less than measurement limit and '>' denotes greater than measurement limit for particular trial

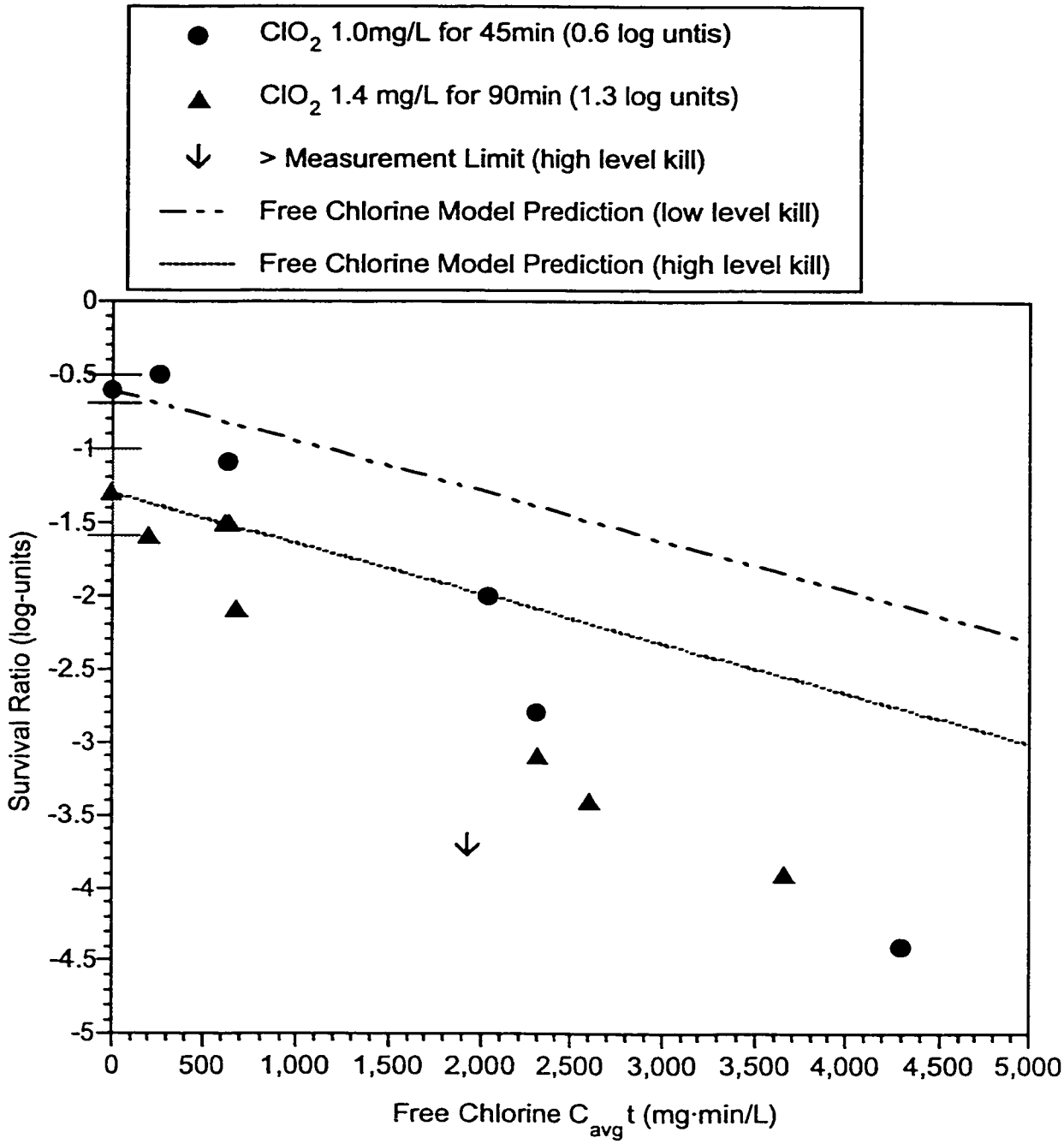


**Figure 3-3 Survival of *Cryptosporidium parvum* Oocysts as a Function of Free Chlorine $C_{avg}t$ after Chlorine dioxide Pretreatment in 0.05M Oxidant Demand Free Phosphate Buffer at pH 6.0 at 22°C**

Note: Arrow denotes greater than measurement limit for particular trial
Survival determined using animal (CD-1 neonatal mice) infectivity and logit dose response model
Each data point is the result of a single trial
Free chlorine predicted survival ratio estimated from Chick-Watson model (Table 3-3)

### *Chlorine Dioxide Followed by Monochloramine*

Results of the chlorine dioxide followed by monochloramine trials conducted at 1°C are summarized in Table 3-10. The survival ratios of *C. parvum* versus $C_{avg}t$ of monochloramine after applying chlorine dioxide at high and low levels are shown in Figure 3-4. The Chick-Watson model predicted survival curve for monochloramine, as estimated from the model parameters in Table 3-3, is included on the figure. The calculated synergy and predicted inactivation of *C. parvum* for the sequential treatment are included in Table 3-10. The synergy was calculated by subtracting the observed inactivation of *C. parvum* after chlorine dioxide pretreatment and the predicted inactivation due to monochloramine used singly (Table 3-3) from the observed inactivation after sequential treatment. The gross predicted inactivation was determined by adding the observed inactivation after chlorine dioxide pretreatment to the predicted inactivation due to monochloramine used sequentially (Table 3-6). Some censored data were not included in the figure as duplicates of these trials were conducted providing non-censored results. The observed inactivation of *C. parvum* is within ± 0.5 log-units of the predicted inactivation, which is acceptable due to the inherent variability in the animal response model. Two chlorine dioxide pretreatment levels were applied prior to monochloramine treatment. For the high level pretreatment a chlorine dioxide dose of 2.6 mg/L for 240 min contact time gave an average inactivation of 1.2 log-units, not including trial number 793. After high level chlorine dioxide pretreatment and a $C_{avg}t$ of around 3000 mg·min/L, the results for trial number 793 reported a 1.0 log-unit of synergy. This trial, however, provided a lower than expected inactivation for chlorine dioxide pretreatment (0.5 log-units compared to 1.4 log-units) and was inconsistent with other trials conducted under the same conditions. For this reason, trial 793 should not be considered as an accurate measurement. For the low level pretreatment, a chlorine dioxide dose of 1.9 mg/L for 120 min gave an average inactivation of 0.4 log-units. The standard deviations for the chlorine dioxide log inactivations at both high and low pretreatment levels are included in Figure 3-4. The results showed that the *C. parvum* survival ratio decreased slightly as the $C_{avg}t$ product increased. The survival ratio decreased at a similar rate at the high and low pretreatment levels. Also, the level of

potential synergy observed appeared to be negligible until a very high monochloramine $C_{avg}t$, of around 4000 mg·min/L, was reached for both high and low chlorine dioxide pretreatment levels.

The results for the trials conducted using chlorine dioxide followed by monochloramine at 10°C are provided in Table 3-11 and the survival curves for this sequential treatment, including some censored data, are provided in Figure 3-5. Also, included in this figure is the Chick-Watson model predicted survival curve for monochloramine used singly. The data points for trial number 804 have not been included in the figure or in the data anlaysis as they are considered to be outliers. High level chlorine dioxide pretreatment was targeted at 1.4 log-units. For trial 804 the observed inactivation by chlorine dioxide was only 0.4 log-units. This resulted in a low observed total log inactivation of *C. parvum* when compared to data from the other trials. Therefore, without including the data from trial 804, for the high level chlorine dioxide pretreatment, a chlorine dioxide dose of 1.8 mg/L for 180 min gave an average inactivation of 1.2 log-units. For the low level condition a chlorine dioxide dose of 1.4 mg/L for 90 min resulted in an average of 0.7 log-units inactivation. The standard deviation for the average chlorine dioxide pretreatment at high and low levels were 0.15 and 0.12 log-units, respectively and have been included in Figure 3.5. The difference between the overall observed and predicted inactivations was no greater than 0.6 log-units after both high and low levels of pretreatment. For low level chlorine dioxide pretreatment, there was only a slight increase in inactivation as monochloramine $C_{avg}t$ increased. The observed inactivation increased from 0.8 to 1.1 log-units over a monochloramine $C_{avg}t$ of 700 to 2000 mg·min/L. For high level chlorine dioxide pretreatment there seemed to be a more substantial increase in inactivation than the low level condition after a monochloramine $C_{avg}t$ of approximately 3000 mg·min/L. Basically no synergy was observed for the low level chlorine dioxide pretreatment condition. About 0.8 log-units of synergy was observed after a monochloramine $C_{avg}t$ of 3000 mg·min/L at the high pretreatment level.

The results for the experiments conducted at 22°C using chlorine dioxide followed by monochloramine are shown in Table 3-12. The survival ratio curves for this

sequential treatment and the survival ratio curve for the Chick-Watson model predicted for monochloramine (Table 3-3) are summarized in Figure 3-6. For the high level pretreatment the difference between the overall predicted and observed inactivations is no more than 0.6 log-units. At the low level pretreatment level the difference is as high as 0.9 log-units. The model does not seem to be an adequate prediction for the low level pretreatment condition at 22°C. For the high level chlorine dioxide pretreatment, a chlorine dioxide dose of 1.4 mg/L for 90 min gave an average log inactivation of 1.4 log-units. For low level pretreatment a chlorine dioxide dose of 1.0 mg/L for 45 min was targeted to give approximately 0.6 log-units inactivation. The observed log inactivation after this low level pretreatment varied from 0 to 0.7 log-units providing an average of 0.2 log-units. The standard deviations for the average inactivations after high and low chlorine dioxide pretreatment have been included in Figure 3-6. Potential synergistic effects were observed at lower monochloramine $C_{avg}t$ levels after low chlorine dioxide pretreatment than was previously observed at 1 and 10°C. After low chlorine dioxide pretreatment and a monochloramine $C_{avg}t$ of 600 mg·min/L, about 0.9 log-units of synergy was observed. It appeared that at the low chlorine dioxide pretreatment level, after a monochloramine $C_{avg}t$ of about 1200 mg·min/L, the degree of synergy declined. The same was not found, however, after high level chlorine dioxide pretreatment. After high level chlorine dioxide pretreatment it appeared that no additional inactivation due to synergy was observed for monochloramine $C_{avg}t$'s ranging from 700 to 4500 mg·min/L. In fact, the observed inactivation for monochloramine after chlorine dioxide pretreatment seemed to follow the model predicted curve for monochloramine used singly. Also, for high chlorine dioxide pretreatment the level of inactivation seemed to increase, but only slightly, as monochloramine $C_{avg}t$ increased. At a $C_{avg}t$ of 700 mg·min/L approximately 1.4 log-unit inactivation was observed and at a $C_{avg}t$ of 3000 mg·min/L only about 2.1 log-units of inactivation was observed.

According to Figure 3-6, after high level chlorine dioxide pretreatment there appeared to be a lag, where there was no inactivation due to monochloramine until approximately 700 mg·min/L. This lag was not observed at the low chlorine dioxide pretreatment level. There was a more substantial initial monochloramine inactivation

observed after the low level pretreatment condition compared to the high level pretreatment conditions (0.4 log-units compared to 0 log-units at 700 mg·min/L).

**Table 3-10 Summary of *Cryptosporidium parvum* Inactivation Using Chlorine dioxide Followed by Monochloramine in 0.05M Oxidant Demand-free Phosphate Buffer at pH 8.0 and 1°C**

| Trial No. | | Primary disinfectant (chlorine dioxide) | | | | Secondary disinfectant (Monochloramine) | | | | | Total | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| (1) | | $C_o$ (mg/L) (2) | $C_r$ (mg/L) (3) | t (min) (4) | Obs. $I_{r1}$ (log-units) (5) | $C_o$ (mg/L) (6) | $C_r$ (mg/L) (7) | t (min.) (8) | $C_{avg}t$ (mg·min/L) (9) | $I_{r2}$ † (log-units) (10) | Obs. $I_r$ (log-units) (11) | Synergy‡ (log-units) (12) | Predicted kill (log-units) (13) |
| *802.1* | *802.2* | 2.6 | 2.1 | 240 | 1.4 | 4.7 | 4.6 | 470 | 2195 | 0.05 | 1.9 | 0.4 | 1.9 |
| *792.1* | *792.2* | 2.6 | 2.0 | 240 | 1.2 | 4.8 | 4.7 | 495 | 2351 | 0.05 | 1.8 | 0.5 | 1.7 |
| *803.1* | *803.2* | 2.6 | 2.5 | 240 | 1.4 | 3.2 | 3.0 | 960 | 2995 | 0.07 | 2.0 | 0.5 | 2.0 |
| *793.1* | *793.2* | 2.6 | 2.1 | 240 | 0.5 | 3.2 | 3.1 | 960 | 3024 | 0.07 | 1.6 | 1.0 | 1.1 |
| *802.1* | *802.3* | 2.6 | 2.1 | 240 | 1.4 | 4.7 | 4.4 | 985 | 4487 | 0.10 | <1.8 | <0.3 | 2.4 |
| *792.1* | *792.3* | 2.6 | 2.0 | 240 | 1.2 | 4.8 | 4.6 | 965 | 4536 | 0.10 | 2.2 | 0.9 | 2.1 |
| *820.1* | *820.2* | 1.7 | 1.7 | 120 | 0.5 | 4.6 | 4.6 | 480 | 2198 | 0.05 | 0.8 | 0.2 | 1.0 |
| *810.1* | *810.2* | 1.9 | 1.7 | 120 | 0.5 | 4.9 | 4.8 | 465 | 2248 | 0.05 | <0.8 | <0.2 | 1.0 |
| *821.1* | *821.2* | 2.0 | 1.9 | 120 | 0 | 3.0 | 3.0 | 960 | 2851 | 0.06 | 0.8 | 0.7 | 0.6 |
| *811.1* | *811.2* | 1.9 | 1.8 | 120 | 0.5 | 3.3 | 3.0 | 980 | 3063 | 0.07 | <1.1 | <0.5 | 1.1 |
| *820.1* | *820.3* | 1.7 | 1.7 | 120 | 0.5 | 4.6 | 4.5 | 960 | 4378 | 0.10 | <1.1 | <0.5 | 1.4 |
| *810.1* | *810.3* | 1.9 | 1.7 | 120 | 0.5 | 4.9 | 4.6 | 965 | 4555 | 0.10 | 1.5 | 0.9 | 1.5 |

Note: † Monochloramine inactivation ($I_{r2}$) was estimated by the Chick-Watson model (Table 3-3);

‡ Synergy = Observed $I_r - (I_{r_1} + I_{r_2})$;

'Predicted inactivation was estimated by Chick-Watson model (Table 3-6)

predicted kill = model prediction (Table 3-6) + observed $I_{r1}$

kill refers to inactivation of *C. parvum*

'<' denotes less than measurement limit and '>' denotes greater than measurement limit for particular trial

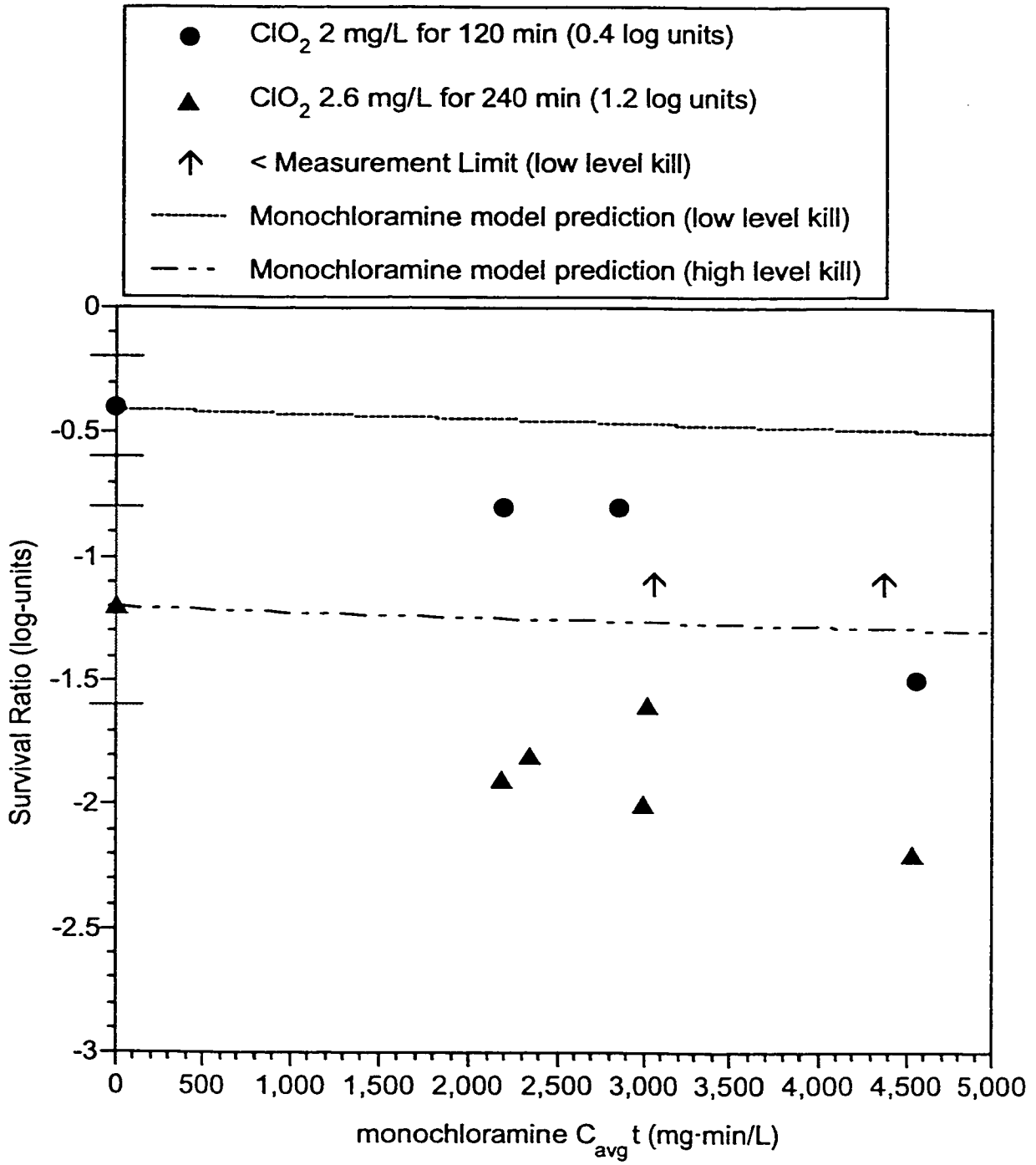


**Figure 3-4 Survival of *Cryptosporidium parvum* Oocysts as a Function of Monochloramine $C_{avg}t$ after Chlorine dioxide Pretreatment in 0.05M Oxidant Demand Free Phosphate Buffer at pH 8.0 at 1°C**

Note: Survival determined using animal (CD-1 neonatal mice) infectivity and logit dose response model
Each data point is the result of a single trial
Monochloramine predicted survival ratio estimated from Chick-Watson model (Table 3-3)
Arrows denote less than measurement limit for particular trial

**Table 3-11 Summary of *Cryptosporidium parvum* Inactivation Using Chlorine dioxide Followed by Monochloramine in 0.05M Oxidant Demand-free Phosphate Buffer at pH 8.0 and 10°C**

| Trial No. | | Primary disinfectant (chlorine dioxide) | | | | Secondary disinfectant (Monochloramine) | | | | | Total | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| (1) | | $C_o$ (mg/L) (2) | $C_r$ (mg/L) (3) | t (min) (4) | Obs. $I_{r1}$ (log-units) (5) | $C_o$ (mg/L) (6) | $C_r$ (mg/L) (7) | t (min) (8) | $C_{avg}t$ (mg·min/L) (9) | $I_{r2}$ † (log-units) (10) | Obs. $I_r$ (log-units) (11) | Synergy‡ (log-units) (12) | Predicted kill (log-units) (13) |
| *805.1* | *805.2* | 1.7 | 1.1 | 180 | 1.1 | 3.1 | 3.0 | 480 | 1464 | 0.1 | 1.6 | 0.4 | 1.5 |
| *804.1* | *804.2* | 1.5 | 0.8 | 180 | 0.4 | 4.5 | 4.5 | 480 | 2158 | 0.1 | 0.5 | 0.0 | 0.9 |
| *794.1* | *794.2* | 1.8 | 1.2 | 180 | 1.4 | 4.6 | 4.6 | 480 | 2218 | 0.1 | 1.7 | 0.2 | 1.9 |
| *805.1* | *805.3* | 1.7 | 1.1 | 180 | 1.1 | 3.1 | 2.9 | 960 | 2880 | 0.2 | <1.5 | <0.2 | 1.8 |
| *795.1* | *795.2* | 1.8 | 1.5 | 180 | 1.1 | 3.2 | 3.1 | 960 | 2995 | 0.2 | 2.1 | 0.8 | 1.8 |
| *804.1* | *804.3* | 1.5 | 0.8 | 180 | 0.4 | 4.5 | 4.4 | 960 | 4291 | 0.3 | 0.8 | 0.1 | 1.4 |
| *794.1* | *794.3* | 1.8 | 1.2 | 180 | 1.4 | 4.6 | 4.4 | 965 | 4367 | 0.3 | 2.4 | 0.7 | 2.5 |
| *813.1* | *813.2* | 1.5 | 1.4 | 90 | 0.8 | 1.5 | 1.5 | 450 | 684 | 0.04 | 0.8 | -0.1 | 1.0 |
| *813.1* | *813.3* | 1.5 | 1.4 | 90 | 0.8 | 1.5 | 1.2 | 960 | 1296 | 0.1 | <1.1 | <0.2 | 1.1 |
| *812.1* | *812.2* | 1.4 | 1.2 | 90 | 0.6 | 4.8 | 4.8 | 460 | 2197 | 0.1 | 1.1 | 0.4 | 1.1 |
| *812.1* | *812.3* | 1.4 | 1.2 | 90 | 0.6 | 4.8 | 4.7 | 975 | 4607 | 0.3 | <1.1 | <0.2 | 1.7 |

Note: † Monochloramine inactivation ($I_{r2}$) was estimated by the Chick-Watson model (Table 3-3);

‡ Synergy = Observed $I_r - (I_{r_1} + I_{r_2})$;

'Predicted inactivation was estimated by Chick-Watson model (Table 3-6)

predicted kill = model prediction (Table 3-6) + observed $I_{r1}$

kill refers to inactivation of *C. parvum*

'<' denotes less than measurement limit for particular trial

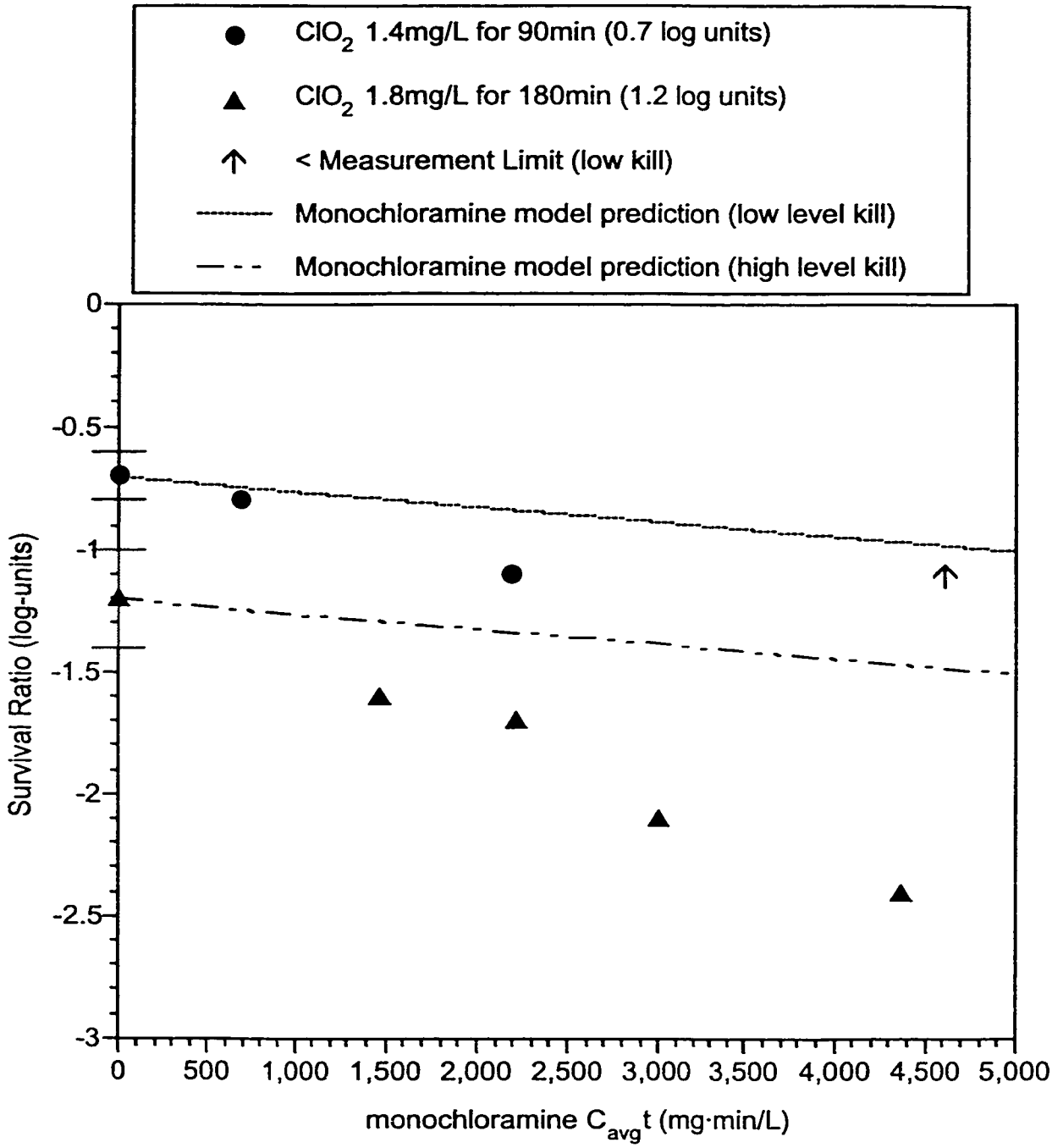


**Figure 3-5 Survival of *Cryptosporidium parvum* Oocysts as a Function of Monochloramine $C_{avg}t$ after Chlorine dioxide Pretreatment in 0.05M Oxidant Demand Free Phosphate Buffer at pH 8.0 at 10°C**

Note: Survival determined using animal (CD-1 neonatal mice) infectivity and logit dose response model
Each data point is the result of a single trial
Monochloramine survival ratio estimated from Chick-Watson model (Table 3-3)
Arrow denote less than measurement limit for particular trial

**Table 3-12 Summary of *Cryptosporidium parvum* Inactivation Using Chlorine dioxide Followed by Monochloramine in 0.05M Oxidant Demand-free Phosphate Buffer at pH 8.0 and 22°C**

| Trial No. | | Primary disinfectant (chlorine dioxide) | | | | Secondary disinfectant (Monochloramine) | | | | | Total | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| (1) | | $C_o$ (mg/L) (2) | $C_r$ (mg/L) (3) | t (min) (4) | Obs. $I_{r1}$ (log-units) (5) | $C_o$ (mg/L) (6) | $C_r$ (mg/L) (7) | t (min) (8) | $C_{avg}t$ (mg·min/L) (9) | $I_{r2}$ † (log-units) (10) | Obs. $I_r$ (log-units) (11) | Synergy‡ (log-units) (12) | Predicted kill (log-units) (13) |
| *801.1* | *801.2* | 1.5 | 1.2 | 90 | 1.4 | 1.7 | 1.6 | 450 | 752 | 0.2 | 1.4 | -0.2 | 1.6 |
| *791.1* | *791.2* | 1.3 | 1.2 | 90 | 1.4 | 3.2 | 3.0 | 430 | 1337 | 0.3 | 1.7 | 0.0 | 1.8 |
| *800.1* | *800.2* | 1.5 | 1.2 | 90 | 1.2 | 3.2 | 3.0 | 460 | 1428 | 0.3 | 2.2 | 0.7 | 1.6 |
| *801.1* | *801.3* | 1.5 | 1.2 | 90 | 1.4 | 1.7 | 1.5 | 960 | 1546 | 0.4 | 1.5 | -0.3 | 1.9 |
| *790.1* | *790.2* | 1.3 | 1.0 | 90 | 1.5 | 4.8 | 4.6 | 435 | 2036 | 0.5 | 2.1 | 0.1 | 2.0 |
| *791.1* | *791.3* | 1.3 | 1.2 | 90 | 1.4 | 3.2 | 2.9 | 920 | 2778 | 0.7 | <2.9 | <0.8 | 2.2 |
| *800.1* | *800.3* | 1.5 | 1.2 | 90 | 1.2 | 3.2 | 2.9 | 960 | 2923 | 0.7 | 2.1 | 0.2 | 2.0 |
| *790.1* | *790.3* | 1.3 | 1.0 | 90 | 1.5 | 4.8 | 4.4 | 930 | 4264 | 1.0 | 2.6 | 0.1 | 2.7 |
| *818.1* | *818.2* | 1.0 | 0.8 | 45 | 0.0 | 1.1 | 1.1 | 240 | 262 | 0.1 | <0.5 | <0.4 | 0.1 |
| *809.1* | *809.2* | 1.0 | 0.9 | 45 | 0.0 | 1.5 | 1.3 | 455 | 632 | 0.2 | 1.1 | 0.9 | 0.2 |
| *822.1* | *822.2* | 1.1 | 1.0 | 45 | 0.7 | 4.6 | 4.5 | 200 | 903 | 0.2 | <1.1 | <0.2 | 1.0 |
| *818.1* | *818.3* | 1.0 | 0.8 | 45 | 0.0 | 1.1 | 1.0 | 980 | 1019 | 0.2 | <1.1 | <0.9 | 0.3 |
| *809.1* | *809.3* | 1.0 | 0.9 | 45 | 0.0 | 1.5 | 0.9 | 965 | 1144 | 0.3 | 1.1 | 0.8 | 0.3 |
| *822.1* | *822.3* | 1.1 | 1.0 | 45 | 0.7 | 4.6 | 4.1 | 975 | 4197 | 1.0 | <2.1 | <0.4 | 1.9 |

Note: † Monochloramine inactivation ($I_{r2}$) was estimated by the Chick-Watson model (Table 3-3);

‡ Synergy = Observed $I_r - (I_{r_1} + I_{r_2})$;

'Predicted kill was estimated by Chick-Watson model (Table 3-6)

predicted kill = model prediction (Table 3-6) + observed $I_{r1}$

kill refers to inactivation of *C. parvum*

'<' denotes less than measurement limit for particular trial

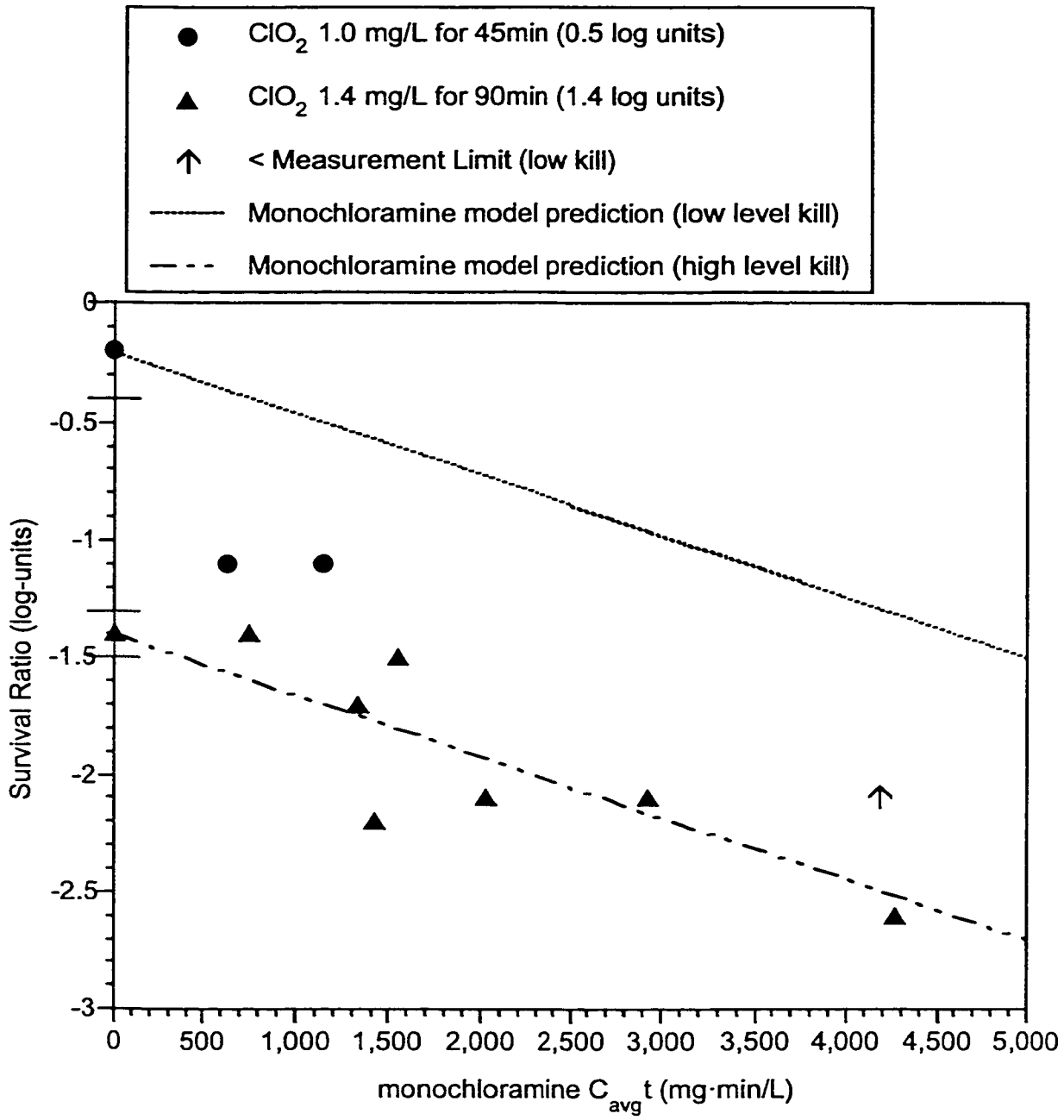


**Figure 3-6 Survival of *Cryptosporidium parvum* Oocysts as a Function of Monochloramine $C_{avg}t$ after Chlorine dioxide Pretreatment in 0.05M Oxidant Demand Free Phosphate Buffer at pH 8.0 at 22°C**

Note: Survival determined using animal (CD-1 neonatal mice) infectivity and logit dose response model
Each monochloramine Ct data point is the result of a single trial
Monochloramine predicted kill estimated from Chick-Watson model (Table 3-3)
Arrows denote less than measurement limit for particular trial

## DISCUSSION

A comparison of the survival ratio of *C. parvum* for free chlorine and monochloramine treatments at 1, 10 and 22°C after a high level of chlorine dioxide pretreatment is shown in Figure 3-7. A comparison of the survival curves for free chlorine and monochloramine at these temperatures after a low level of pretreatment is shown in Figure 3-8. Water temperature has a significant effect on the inactivation of *C. parvum*. In general, at higher temperatures a greater inactivation is observed. This may be attributed to changes in the rates of the inactivation mechanism at colder temperatures. The processes that could be affected are the rates of diffusion (by free chlorine and monochloramine) and rates of reaction.

The required chlorine dioxide dose and contact time to achieve an equivalent log inactivation of *C. parvum* were higher at lower temperatures. To achieve 1.4 log-units inactivation at 22°C a chlorine dioxide dose of 1.4 mg/L for 90 min was required (Table 3-9), while at 1°C 2.6 mg/L for 240 min contact time was required (Table 3-7). These chlorine dioxide dose and contact times were applied at both pH 6 and 8 and equivalent inactivations were observed at the different pH's. Thus, in order to obtain the same level of chlorine dioxide inactivation at 1°C as at 22°C approximately five times the Ct was required. A similar finding was reported in a study by Li *et al.* (1998). This study looked at the effects of chlorine dioxide on *C. parvum* at 1 and 22°C and pH 6.0 and discovered that the chlorine dioxide inactivation at 1°C was one fourth that at 22°C.

The results in Table 3-4 and 3-5 show that for single disinfection, using free chlorine, at pH 6 or monochloramine, at pH 8, a high temperature and a high $C_{avg}t$ were essential for achieving inactivation. A higher $C_{avg}t$ was required for a monochloramine inactivation of 1.0 log-units at 22°C compared to free chlorine inactivation (4000 mg·min/L compared to less than 2500 mg·min/L). This may be due to the fact that monochloramine is generally not as effective in microorganism reduction as free chlorine. A study conducted in a batch reactor at 22°C, reported that monochloramine at

pH 8 had no detectable effect on the infectivity of *C. parvum* oocysts, however, free chlorine at pH 6 had a low but detectable reduction in infectivity (Finch *et al.* 1997).

For chlorine dioxide followed by free chlorine higher overall inactivations after equivalent chlorine dioxide pretreatment and free chlorine $C_{avg}t$ conditions were observed at higher temperatures than lower temperatures. According to Figures 3-1 and 3-3, at 1°C, after high level chlorine dioxide pretreatment and a free chlorine $C_{avg}t$ of 3000 mg·min/L only 1.1 log-units inactivation was observed, while at 22°C under the same conditions 3.7 log-units inactivation was observed. It was also found that at 22°C, a $C_{avg}t$ of free chlorine of approximately 1500 mg·min/L and 2000 mg·min/L was required to obtain greater than 1.0 log-units of synergy after high and low levels of chlorine dioxide pretreatment, respectively (Figure 3-3). It appeared, however, that as free chlorine $C_{avg}t$ increased a lower chlorine dioxide pretreatment provided a greater synergistic effect for free chlorine inactivation. After a low level pretreatment there was a more substantial increase in the level of inactivation of *C. parvum* by free chlorine over a smaller $C_{avg}t$. The same was found for trials conducted at 10°C. After low chlorine dioxide pretreatment and a free chlorine $C_{avg}t$ of 2500 mg·min/L approximately 0.8 log-units of synergy was observed, while at the high level pretreatment condition basically no synergy was observed (Figure 3-2). This observation suggests that a low level of chlorine dioxide preconditioning provided conditions in oocysts that optimize the synergistic effects of free chlorine at both 22 and 10°C. There may be an optimum dose at which chlorine dioxide should be applied to enable optimum diffusion of free chlorine through the membrane of the oocyst. Applying a dose that exceeds the optimum may increase the overall inactivation of *C. parvum* but not enhance synergistic effects. According to Figure 3-1, the inactivation of *C. parvum* at 1°C with chlorine dioxide followed by free chlorine showed no additional inactivation due to synergy under the conditions tested for free chlorine $C_{avg}t$ as high as 4000 mg·min/L. It may, therefore, be impractical to apply this sequential chemical combination at water treatment plants where the source water temperature is low. This is discussed further in Chapter 5.

When chlorine dioxide was followed by monochloramine at pH 8, higher overall inactivation was observed at higher temperatures for the same treatment conditions but

temperature differences appeared to have a more significant effect on free chlorine as a secondary chemical treatment than monochloramine. This effect is shown in Figures 3-7 and 3-8. After high chlorine dioxide pretreatment and a monochloramine $C_{avg}t$ of 3000 mg·min/L at 1°C, 1.6 log-units inactivation was observed and at 22°C, 2.1 log-units inactivation was observed (0.6 log-units difference). This difference is smaller than that found for free chlorine secondary treatment under the same treatment conditions (2.6 log-units difference was observed). This difference was most likely due to the difference in chemical nature of monochloramine compared to free chlorine and the way the chemicals react at different temperatures.

For the chlorine dioxide/monochloramine sequential trials, it appeared that additional inactivation due to synergy was achievable at 1 and 10°C after high monochloramine $C_{avg}t$ values and at 22°C after lower $C_{avg}t$ values. The results for the chlorine dioxide/monochloramine trials at 1, 10 and 22°C, however, appeared to be quite varied. According to Figure 3-4, at 1°C there was about 1.0 log-units of synergy after high and low chlorine dioxide pretreatment and a $C_{avg}t$ of 4000 mg·min/L and the level of chlorine dioxide pretreatment did not appear to effect the overall level of inactivation. At 10°C no synergy was observed for monochloramine $C_{avg}t$ from 600 to 4600 mg·min/L after low level chlorine dioxide pretreatment (Figure 3-5). There was, however, 0.8 log-units of synergy observed after high level pretreatment and a $C_{avg}t$ of 3000 mg·min/L. The opposite was observed at 22°C, according to Figure 3-6. At this temperature there seemed to be additional inactivation due to synergy after low chlorine dioxide pretreatment and monochloramine $C_{avg}t$'s ranging from 600 to 1200 mg·min/L but no synergy was observed after high pretreatment for monochloramine $C_{avg}t$'s ranging from 700 to 4500 mg·min/L. In general, the results tended to suggest that applying chlorine dioxide followed by monochloramine had a positive effect on the inactivation of *C. parvum* at 1, 10 and 22°C but the level of chlorine dioxide pretreatment effected the level of inactivation differently at all three temperatures. This difference is not well understood, but the estimate for the Chick-Watson model parameters for monochloramine used sequentially, in Table 3-6, suggest that monochloramine may be sensitive to the level of chlorine dioxide pretreatment. The Chick-Watson model parameter $K_{22}$ describes the rate of inactivation at 22°C. The estimated value of $K_{22}$ for

monochloramine is higher at the low chlorine dioxide pretreatment level than the high pretreatment level. This difference suggests, that according to the model prediction, a higher level of inactivation due to monochloramine may occur after a low level chlorine dioxide pretreatment compared to a high level of pretreatment.

At 22°C, it appeared that the degree of synergy for the chlorine dioxide/monochloramine trials was independent of monochloramine $C_{avg}t$. According to the results in Table 3-12, 0.9 log-units of synergy was observed at the low chlorine dioxide pretreatment level and a monochloramine $C_{avg}t$ of 600 to 1200 mg·min/L. After 1200 mg·min/L, however, less than 0.4 log-units of additional inactivation was observed. Thus, in contrast to the chlorine dioxide/free chlorine results, the synergism between chlorine dioxide and monochloramine did not increase with increasing monochloramine Ct at 22°C after 1200 mg·min/L. This may be a result of the difference in chemical nature of monochloramine compared to free chlorine and the way the chemicals react with the membrane of the oocyst. There may be a threshold concentration that exits for monochloramine when used sequentially, but not for free chlorine.

The inactivation due to free chlorine as a secondary treatment was greater than that of monochloramine under the same chlorine dioxide pretreatment conditions. According to Figures 3-7 and 3-8, after equivalent chlorine dioxide pretreatment at 1 and 10°C, the log inactivation due to free chlorine and monochloramine treatments were similar. However, at 22°C the inactivation level by free chlorine as a secondary disinfectant was much better than that of monochloramine. At 22°C, gross inactivation by chlorine dioxide followed by free chlorine reached 3.0 log-units when the free chlorine $C_{avg}t$ product was about 2500 mg·min/L. For monochloramine secondary treatment, the gross inactivation was less than 3.0 log-units even when the monochloramine $C_{avg}t$ reach about 4400 mg·min/L. In addition, it appeared that the potential for synergy was greater for chlorine dioxide/free chlorine treatments than chlorine dioxide/monochloramine. According to Tables 3-3 and 3-6, the value of the Chick-Watson model parameter, $K_{22}$, for free chlorine increased from 0.00031, when used singly, to 0.00087 and 0.00077, when used sequentially. This increase was more substantial than that observed for monochloramine indicating a more significant effect on

*C. parvum* inactivation when using free chlorine sequentially compared to monochloramine. This is probably due to the fact that in general free chlorine is a more effective microorganism reducer than monochloramine.

The survival of *C. parvum* oocysts after free chlorine or monochloramine secondary treatment declined approximately linearly with increased $C_{avg}t$. It appeared, however, that there was a lag, where no inactivation occurred, for chlorine dioxide/free chlorine sequential treatment after both high and low levels of pretreatment until a free chlorine $C_{avg}t$ of approximately 600 mg·min/L was reached. This lag is shown in Figures 3-7 and 3-8 and was not as apparent in the chlorine dioxide/monochloramine sequential reaction. These differences could be due to differences in the chemicals nature and the way the chemicals react. Free chlorine may require a higher $C_{avg}t$ than monochloramine to initially inactivate the oocyst but after the initial requirement is overcome it seems to be more effective than monochloramine.

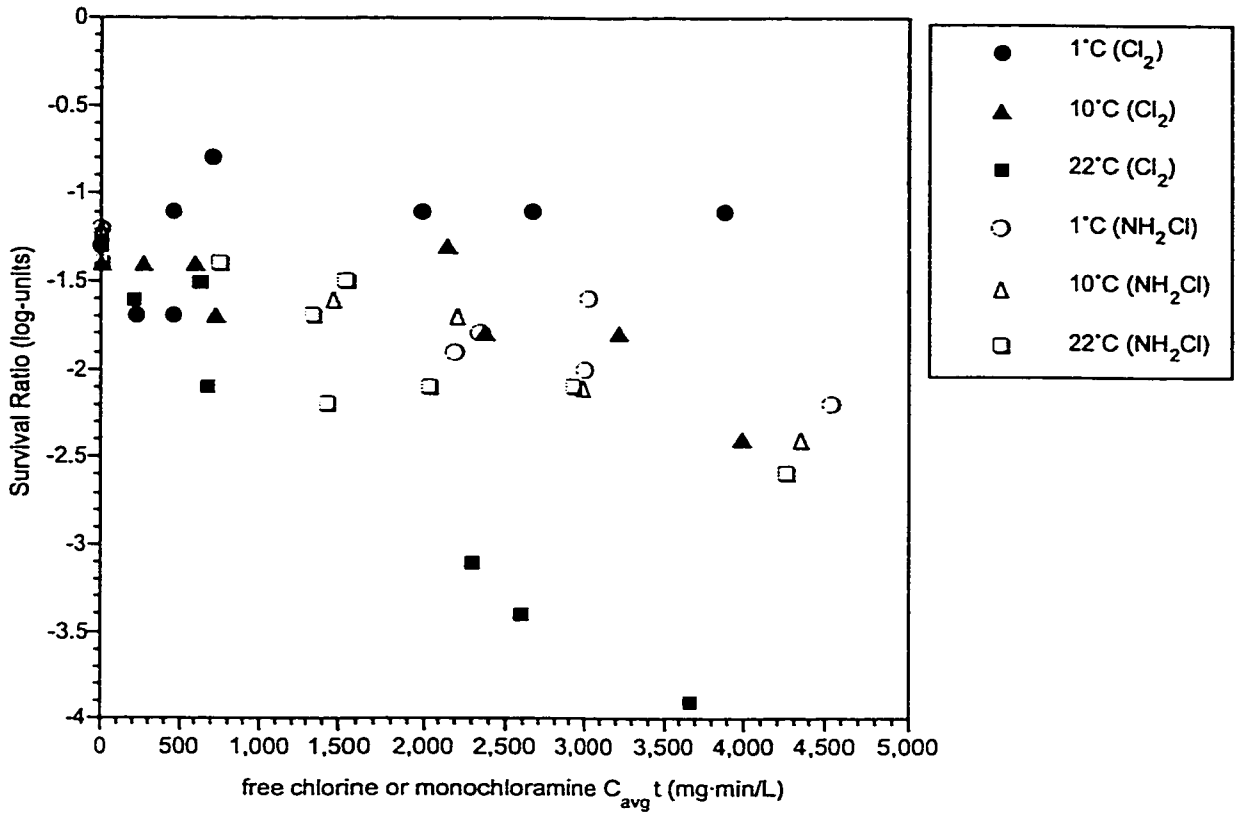


**Figure 3-7 Survival of *Cryptosporidium parvum* oocyst as a function of free chlorine and monochloramine $C_{avg}t$ after high level chlorine dioxide pretreatment in 0.05M oxidant demand-free phosphate buffer at pH 6.0 (free chlorine) and pH 8.0 (monochloramine) at 1°C, 10°C and 22°C.**

Note: Survival determined using animal (CD-1 neonatal mice) infectivity and logit dose response model
Each chlorine CT data point is the result of a single trial

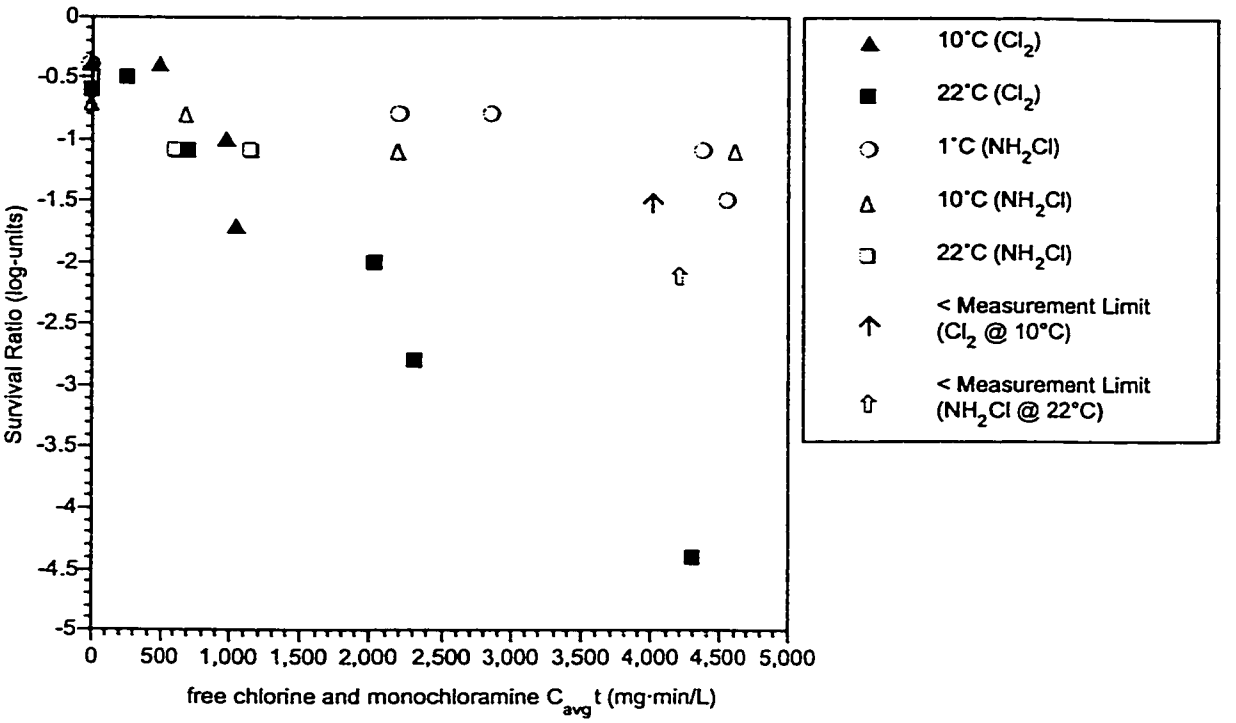


**Figure 3-8 Survival of *Cryptosporidium parvum* oocyst as a function of free chlorine and monochloramine $C_{avg}t$ after low level chlorine dioxide pretreatment in 0.05M oxidant demand-free phosphate buffer at pH 6.0 (free chlorine) and pH 8.0 (monochloramine) at 1°C, 10°C and 22°C.**

Note: Survival determined using animal (CD-1 neonatal mice) infectivity and logit dose response model
Each chlorine CT data point is the result of a single trial
Arrows denote less than measurement limit data

# CHAPTER 4

# NATURAL WATER EFFECTS OF SEQUENTIAL EXPOSURE OF *CRYPTOSPORIDIUM* OOCYSTS TO OZONE AND CHLORINE SPECIES

## INTRODUCTION

The second part of this project studied the effects of sequential disinfection on *Cryptosporidium parvum* in natural water. The sequential disinfection experiments were conducted using ozone followed by free chlorine at 22°C in natural water. All trials were randomized so that independent observations were obtained. In order to reduce experimental error, experiments were run in duplicate, duplicate trials were randomized and controls were run for each day's trials. Each concentration and contact time combination was conducted as an individual trial. Animal infectivity using CD-1 neonatal mice and a logistic dose response model were used for each experimental trial.

## HISTORICAL WATER QUALITY

The test water used in these trials was a sample of North Saskatchewan River (NSR) water collected in October, 1999 from the overflow of the lime softening clarifier at the EPCOR Water Services, Inc. Rossdale water treatment plant. The raw water had also undergone coagulation/flocculation prior to softening.

Historical source water quality data was gathered from the water treatment utility. According to this data the North Saskatchewan River (NSR) has a temperature of 10°C to 15°C in the fall months. The water pH fluctuates very little, 8.0 to 8.4 units, throughout the year and is an average of 8.0 units in the month of October. NSR water total hardness is an average of 160 mg/L in October and the total alkalinity is approximately 150 mg/L. Conductivity is also stable with an average of 480 μS/cm in the month of October. NSR

turbidity is subject to extreme variation but from September to March raw water turbidity is low (between 2-6 NTU). True color measurements mirror the turbidity with a measure of 5 units from September to February. Based on this information, the water used in this study can be classed as a high quality natural water source that was modified during coagulation, flocculation and lime softening.

## TEST WATER CONDITIONS

The NSR water, that was collected after modification, was stored in the refrigerator at 4°C until use. On the experimental trial day, approximately 500 mL of the water was brought to room temperature (22°C ± 1°C) and filtered through a coarse paper filter (Whatman 41) to remove solids. The solids are products of the lime softening process and are not natural to the NSR water. The pH of the modified water was measured to be approximately 8.5 units. In some of the trials that were conducted, the pH of the paper filtered water sample was adjusted to 6.0 by addition of hydrochloric acid.

## EXPERIMENTAL DESIGN

Three identical reactors were prepared each day using 250 mL erlenmyer flasks containing a Teflon-coated magnetic stir bar for agitation. These reactors were run in parallel for each trial. Each reactor was seeded with oocysts. Reactor #1 was seeded with 50 million oocysts and received both primary and secondary chemical treatments, sequentially. Reactor #2 was seeded with 25 million oocysts and received only the secondary chemical. Reactor #3 was seeded with 25 million oocysts, served as the positive control and received no chemical treatment. This combination of reaction flasks enabled determination of the inactivation due to the primary treatment alone, the secondary treatment alone, the total sequential treatment and the synergistic effect. All trials were conducted at room temperature (22 ± 1°C), with constant stirring of the reactor contents and with the reactor covered in aluminum foil to minimize UV exposure and chemical decomposition.

Samples were collected at the end of each chemical contact time and at the end of each experimental day all samples were submitted for animal infectivity analysis. A cohort of 10 or 15 neonatal CD-1 mice were infected with a predetermined dose of oocysts from each of the treated samples.

## Sequential Treatment (Reactor #1)

In trials 1 to 4, the sample in reactor # 1 was pre-ozonated by addition of ozone stock solution of approximately 20 mg/L. The ozone was then left to decay completely, prior to addition of oocysts to the sample flask. The purpose of the pre-ozonation was to overcome the high ozone demand of the test water and to enable the necessary ozone residual to be achieved during the disinfection step. After the reactor was seeded with oocysts a second volume of ozone stock solution of 20 mg/L was applied.

In trials 5 to 14, this pre-ozonation step was not done. Rather, the ozone demand was overcome by the addition of approximately 40 mg/L ozone stock solution to the oocyst suspension in the reactor. The concentrated ozone stock was prepared by cooling the ozone preparation wash bottle and contents in an ice bath to 4°C.

For all of the trials, the concentration of ozone in the reactor was monitored continuously using ultraviolet spectrophotometry at 240 nm and the Indigo method. At the end of the contact time, residual ozone was neutralized using 1.0 M sodium formate and a sample was collected and submitted for infectivity analysis.

In trials 1 to 14, following ozone treatment, a measured volume of the secondary chemical (free chlorine) stock solution was added to the reactor. Initial and final free chlorine residuals were measured using the DPD colorimetric procedures described previously. At the end of the secondary treatment contact time, residual free chlorine was neutralized with 0.1 N sodium sulfite and a second sample was collected for infectivity analysis.

### Single Chemical Treatment and Control

At the same time free chlorine stock solution was added to reactor #1, an equivalent concentration was added to reactor #2. An initial and final residual measurement was taken using the DPD method and at the end of the contact time, residual free chlorine was neutralized with 0.1 N sodium sulfite. A sample was collected and submitted for animal infectivity.

Reactor #3 received no chemical treatment. After five minutes 1M sodium formate was added to the reactor and a sample was collected and stored at 4°C. After the required chlorine contact time (240 or 1000 min) 0.1 N sodium sulfite was added to the reactor and a sample was collected and submitted for animal infectivity analysis.

## RESULTS

All of the raw data for the modified natural water microorganism reduction experiments are included in Table B.4 in Appendix B. One batch of oocysts (Batch 35) was used for the trials conducted using natural water. The estimates for the logisitic dose response model parameters for this batch are provided in Table 3.1 and the dose response trial infectivity data are in Table A.1b in Appendix A. The microorganism reduction infectivity data are in Table A2.b.

### Positive Controls

The inactivations observed in the positive control reactor flask are summarized in Table 4-1. Inactivation in the controls ranged from less than –0.4 to 0.7 log-units for exposure times up to 1000 min. The majority of the control inactivation results were between 0 and 0.3 log-units. The average inactivations of *C. parvum* when exposed to modified NSR water for exposure times of 5 min and 240 to 1000 min at 22°C were 0.03 (or zero) and 0.15 log-units, respectively. The difference in inactivation between the two time intervals was negligible. The overall average inactivation was 0.10 log-units, which

suggests that there was essentially zero log inactivation of oocysts, when exposed to modified natural water for up to 1000 min. The standard deviation of the control data was 0.3 log-units. This suggests that there was variation in the animal infectivity model.

**Table 4-1 Observed reductions in animal infectivity for untreated (control) *Cryptosporidium parvum* oocysts in modified North Saskatchewan River water at 22°C**

| | | Exposure times | |
|---|---|---|---|
| | | (5 min) | (240 to 1000 min) |
| Trial No. | pH | Observed kill (log-units) | Observed kill (log-units) |
| *1* | 8.5 | <-0.18 | 0.01 |
| *2* | 8.5 | 0.16 | 0.01 |
| *3* | 8.5 | 0.16 | 0.32 |
| *4* | 8.5 | ND | 0.15 |
| *5* | 8.5 | -0.40 | 0.27 |
| *6* | 8.5 | 0.27 | <-0.4 |
| *7* | 8.5 | 0.00 | -0.48 |
| *8* | 8.5 | -0.14 | 0.05 |
| *9* | 6.0 | 0.19 | <ML |
| *10* | 6.0 | <ML | -0.14 |
| *11* | 6.0 | ND | 0.19 |
| *12* | 6.0 | ND | 0.71 |
| *13* | 6.0 | ND | 0.52 |
| *14* | 6.0 | ND | ND |

ND: data not available
ML: measurement limit for particular data point
'<' refers to less than measurement limit for particular trial
kill refers to inactivation of *C. parvum*

## Single Disinfection

### *Ozone Inactivation*

The results of the inactivation of *C. parvum* oocysts after ozone pretreatment (reactor #1) are provided in Table 4-2, along with the Incomplete gamma Hom (I.g.H) model predictions of inactivation (based on laboratory water trials) for equivalent ozone doses, ozone decay rates and contact times.

Ozone residual was measured using UV spectrophotometry and the indigo method. The results for each method were compared and the initial and final ozone concentrations and decay rates for each trial were calculated. The spreadsheets detailing these comparisons, for each trial, are in Appendix D. These methods showed similar results, with the exception of consistently higher (approximately 0.3 mg/L) initial (within 20 seconds) ozone residual measured by the indigo method. After about 1 min, however, the residual measurements were the same. As the difference was small an average of the two measurements was used to estimate the initial ozone residual.

High level ozone pretreatment was targeted at 2.0 log-units inactivation of *C. parvum*. To obtain this level of inactivation, trials conducted at pH 8.5 required an ozone dose of approximately 2.5 mg/L for 5 min contact time and trials at pH 6.0 required approximately 1.5 mg/L ozone dose for 5 min. A low level ozone pretreatment aimed for 0.7 log-units inactivation of oocysts. To obtain this level of inactivation an ozone dose of approximately 1.0 mg/L was applied to both the pH 8.5 and pH 6.0 waters for 2 min. The ozone decay rate was greater at the higher pH.

The I.g.H model predicted inactivations (log-units) are compared to the observed ozone inactivation (log-units) in Figure 4-1. The I.g.H model predictions are conservative when experimental data points are above the perfect fit I.g.H model line. According to the figure, the I.g.H. model provided good predictions of inactivation of ozone treated *C. parvum* oocysts in the modified NSR water at both pH 8.5 and pH 6.0, at 22°C. However, the pH 8.5 data varied slightly and the pH 6.0 data tended to fit the model better.

The survival ratio of *C. parvum* after ozone treatment in modified NSR water at 22°C, pH 8.5 and pH 6.0 and the best fit lines for the pH 8.5 (pre-ozonated and not pre-ozonated) and pH 6.0 results are shown in Figure 4-2. The slope of the line fit through the pH 6.0 results was slightly greater than that fit through the pH 8.5 (not pre-ozonated) results. The survival ratio was consistently lower at pH 6.0 compared to pH 8.5 at equivalent ozone $C_{avg}t$ values. The results of the trials conducted in pre-ozonated water at pH 8.5 showed a rapid decreased in survival ratio of *C. parvum* (from 1.0 to 2.1 log-

units) over a small $C_{avg}t$ interval (3.2 to 4.0 mg·min/L). This decrease was more substantial than that observed for the modified NSR water that was not pre-ozonated at pH 6.0 or pH 8.5 over the same $C_{avg}t$ interval. At an ozone $C_{avg}t$ of 3.2 mg·min/L the pre-ozonated NSR water at pH 8.5 had a similar survival ratio to that of the not pre-ozonated NSR water at pH 8.5 and at 4 mg·min/L the results of the pre-ozonated NSR water were similar to those observed at pH 6.0.

**Table 4-2 Summary of *Cryptosporidium parvum* inactivation after ozone treatment in modified North Saskatchewan River water at 22°C**

| Trial No. | pH | Applied Dose mg/L | $C_o$ mg/L | $C_\tau$ mg/L | t min | k' $min^{-1}$ | Predicted kill+ log-units | Observed kill log-units | Control log-units |
|---|---|---|---|---|---|---|---|---|---|
| *1 | 8.5 | 2.06 | 1.8 | 0.5 | 4 | 0.34 | 1.9 | 2.16 | -0.18 |
| *2 | 8.5 | 2.10 | 1.5 | 0.1 | 4 | 0.62 | 1.3 | 1.16 | 0.16 |
| *3 | 8.5 | 1.96 | 1.5 | 0.2 | 4 | 0.49 | 1.5 | 1.01 | 0.16 |
| *4 | 8.5 | 1.94 | 1.6 | 0.4 | 4 | 0.37 | 1.7 | 2.16 | 0.00 |
| 5 | 8.5 | 1.20 | 0.8 | 0.2 | 2 | 0.57 | 0.7 | 0.16 | -0.40 |
| 6 | 8.5 | 1.22 | 0.9 | 0.5 | 2 | 0.34 | 0.8 | 0.82 | 0.27 |
| 7 | 8.5 | 2.45 | 2.2 | 0.1 | 5 | 0.56 | 1.9 | 1.68 | 0.00 |
| 8 | 8.5 | 2.46 | 2.2 | 0.5 | 5 | 0.31 | 2.5 | 1.76 | -0.14 |
| 9 | 6.1 | 1.48 | 1.3 | 0.7 | 5 | 0.18 | 2.0 | 2.01 | 0.19 |
| 10 | 6.0 | 1.50 | 1.4 | 0.8 | 5 | 0.12 | 2.3 | 2.35 | -0.14 |
| 11 | 6.1 | 0.96 | 0.8 | 0.6 | 2 | 0.19 | 0.9 | 0.82 | 0.00 |
| 12 | 6.0 | 1.47 | 1.2 | 0.7 | 5 | 0.10 | 2.1 | 2.35 | 0.00 |
| 13 | 6.0 | 0.98 | 0.8 | 0.6 | 2 | 0.12 | 0.8 | 0.82 | 0.00 |
| 14 | 6.0 | 1.48 | 1.4 | 1.0 | 5 | 0.08 | 2.4 | 2.16 | 0.00 |

Kill refers to inactivation of *C. parvum*

* pre-ozonated NSR water

** Net observed kill = (observed kill – control)

+Ozone inactivation ratios predicted using the incomplete gamma Hom model with the following model parameters: k = 0.68 $min^{-1}$, m=0.70 and n=0.73. (Gyürék et al. 1999)

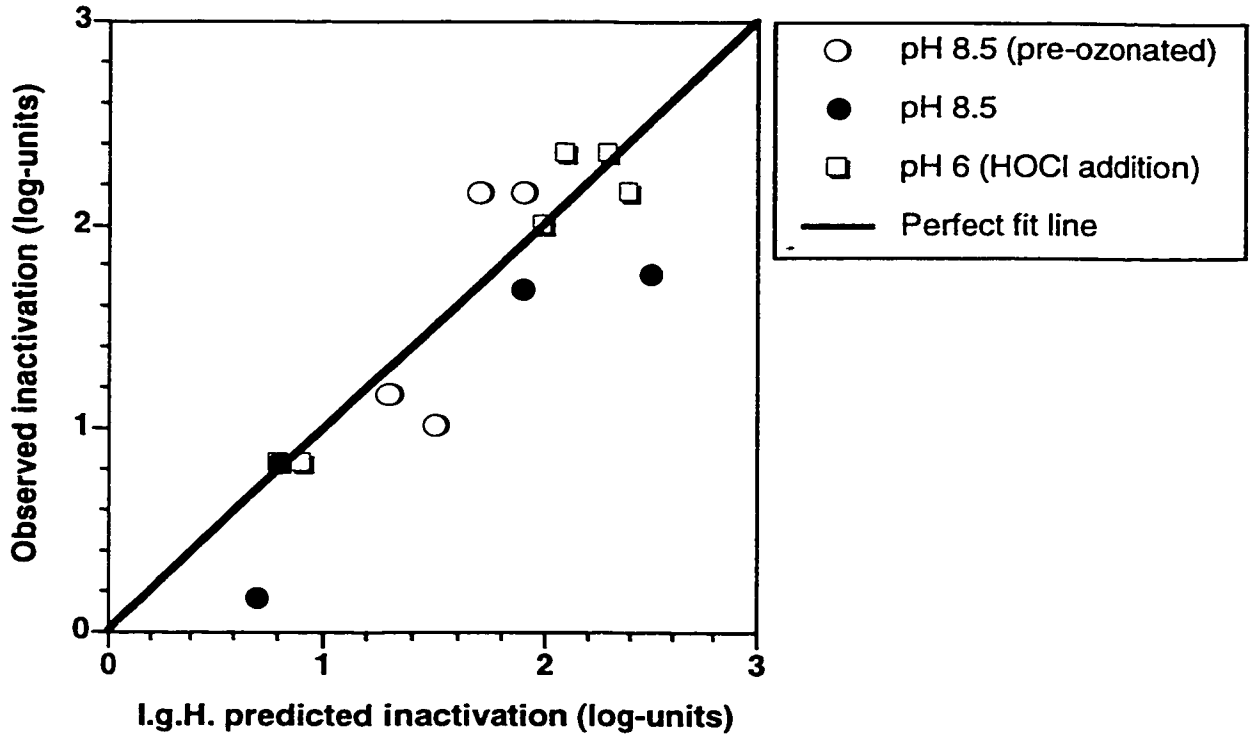


**Figure 4-1 Incomplete gamma Hom predicted inactivation ratios versus observed reduction in infectivity for ozone treated *Cryptosporidium parvum* oocysts in modified North Saskatchewan River water at 22°C**

Note: Each data point represents a single trial
Survival determined using animal infectivity and logistic dose response model

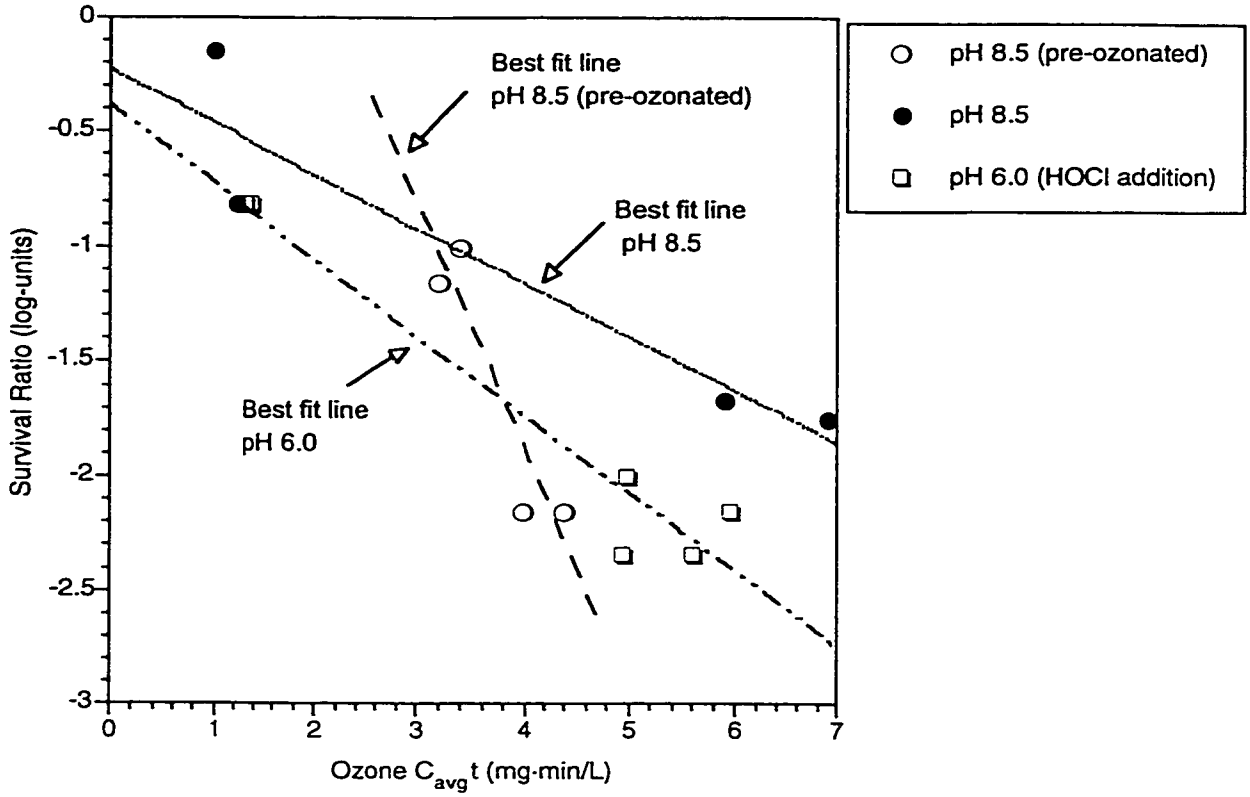


**Figure 4-2 Survival ratio of *Cryptosporidium parvum* oocysts as a function of ozone $C_{avg}t$ in North Saskatchewan River water at 22°C**

Note: Each data point represents a single trial
Survival determined using animal infectivity and logistic dose response model

### *Free Chlorine Inactivation*

The results of the free chlorine treatment alone (reactor #2) and I.g.H. lab water model predictions for equivalent free chlorine treatment conditions are presented in Table 4-3. The I.g.H model predicted inactivation is compared to the net observed free chlorine inactivation (log-units) shown in Figure 4-3. There seemed to be two data points that were particularly high and did not fit the I.g.H model prediction curve at pH 8.5, where hypochlorite predominates. The observations were 1.0 and 0.7 log-units more than what was expected, according to the I.g.H model prediction. The observations at pH 6.0 were closer to the predictions of the I.g.H model and the model gave a conservative estimate for all of the observed inactivations at pH 6.0. One result at pH 6.0, however, was substantially higher (by 0.7 log-units) than what was predicted using the I.g.H model. All other results were within ±0.5 log-units of the model prediction.

Free chlorine $C_{avg}t$ was targeted at greater than 1000 mg·min/L for a high level of treatment and approximately 500 mg·min/L for low level treatment. The survival ratio of *C. parvum* in NSR water at 22°C after free chlorine treatment is shown in Figure 4-4. Varying results were obtained for free chlorine treatment at pH 8.5 but the majority of the observed inactivations were 0.4 log-units or less. It appeared that at pH 8.5 the level of inactivation increased after a free chlorine $C_{avg}t$ of approximately 500 mg·min/L until about 1500 mg·min/L. After 1500 mg·min/L the level of inactivation decreased again to less than 0.4 log-units. When the pH was adjusted to pH 6.0, the net observed inactivation increased linearly with increasing $C_{avg}t$ values. Observed inactivation of *C. parvum* increased from about 0.6 to 1.4 log-units as free chlorine $C_{avg}t$ increased from 400 to 2000 mg·min/L. For low level free chlorine $C_{avg}t$ (less than 600 mg·min/L) the observed inactivation of *C. parvum* was low, approximately 0.7 log-units but after a $C_{avg}t$ of approximately 2000 mg·min/L the observed inactivation was quite high (1.4 log-units). The free chlorine decay rates were similar for both pH 6.0 and pH 8.5 waters. The average decay rate was 0.0016 $min^{-1}$ with a standard deviation of 0.0008.

**Table 4-3 Summary of *Cryptosporidium parvum* inactivation using free chlorine in modified North Saskatchewan River water at 22°C**

| Trial No. | pH | Applied Dose mg/L | $C_o$ mg/L | $C_r$ mg/L | t min | $C_{avg}t$ mg·min/L | k' $min.^{-1}$ | Predicted kill[+] log-units | Observed kill log-units | Control log-units |
|---|---|---|---|---|---|---|---|---|---|---|
| *1 | 8.5 | 4.1 | 3.4 | 1.0 | 960 | 1771 | 0.0011 | 0.1 | -0.18 | 0.01 |
| *2 | 8.5 | 3.6 | 1.9 | 0.1 | 960 | 455 | 0.0029 | 0.1 | 0.35 | 0.01 |
| *3 | 8.5 | 1.5 | 0.4 | 0.0 | 1000 | [++]100 | ND | 0.0 | 0.01 | 0.32 |
| *4 | 8.5 | 1.5 | 0.6 | 0.0 | 960 | [++]150 | ND | 0.0 | 0.16 | 0.15 |
| 5 | 8.5 | 5.2 | 3.6 | 1.6 | 940 | 2287 | 0.0009 | 0.1 | 0.01 | 0.27 |
| 6 | 8.5 | 3.2 | 2.7 | 1.6 | 970 | 2024 | 0.0009 | 0.1 | 0.30 | <-0.40 |
| 7 | 8.5 | 4.1 | 3.2 | 0.6 | 960 | 1370 | 0.0017 | 0.1 | 1.16 | -0.48 |
| 8 | 8.5 | 4.1 | 3.2 | 2.1 | 240 | 625 | 0.0004 | 0.1 | 0.82 | 0.05 |
| 9 | 6.1 | 4.1 | 2.3 | 0.5 | 995 | 1109 | 0.0015 | 0.5 | 0.82 | 0.15 |
| 10 | 6.0 | 4.1 | 2.4 | 0.7 | 995 | 1303 | 0.0012 | 0.5 | 0.82 | -0.14 |
| 11 | 6.1 | 4.2 | 3.0 | 1.0 | 1095 | 1890 | 0.0010 | 0.7 | 1.4 | 0.2 |
| 12 | 6.0 | 3.6 | 2.4 | 1.3 | 230 | 404 | 0.0025 | 0.3 | 0.6 | 0.7 |
| 13 | 6.0 | 3.6 | 2.3 | 1.4 | 235 | 413 | 0.0021 | 0.3 | 0.8 | 0.5 |
| 14 | 6.0 | 3.6 | 2.4 | 1.3 | 240 | 430 | 0.0025 | 0.3 | 0.5 | 0.2 |

Kill refers to inactivation of *C. parvum*

* pre-ozonated NSR water

** net observed kill = (observed kill – control)

[+] Free chlorine inactivation ratios predicted using the following Incomplete gamma Hom model parameters: k = 0.011$min^{-1}$, m=0.50 and n=0.80 (Gyürék *et al.* 1997)

[++]The actual value is less than that shown here because the final measured concentration was zero.

ND: data not available

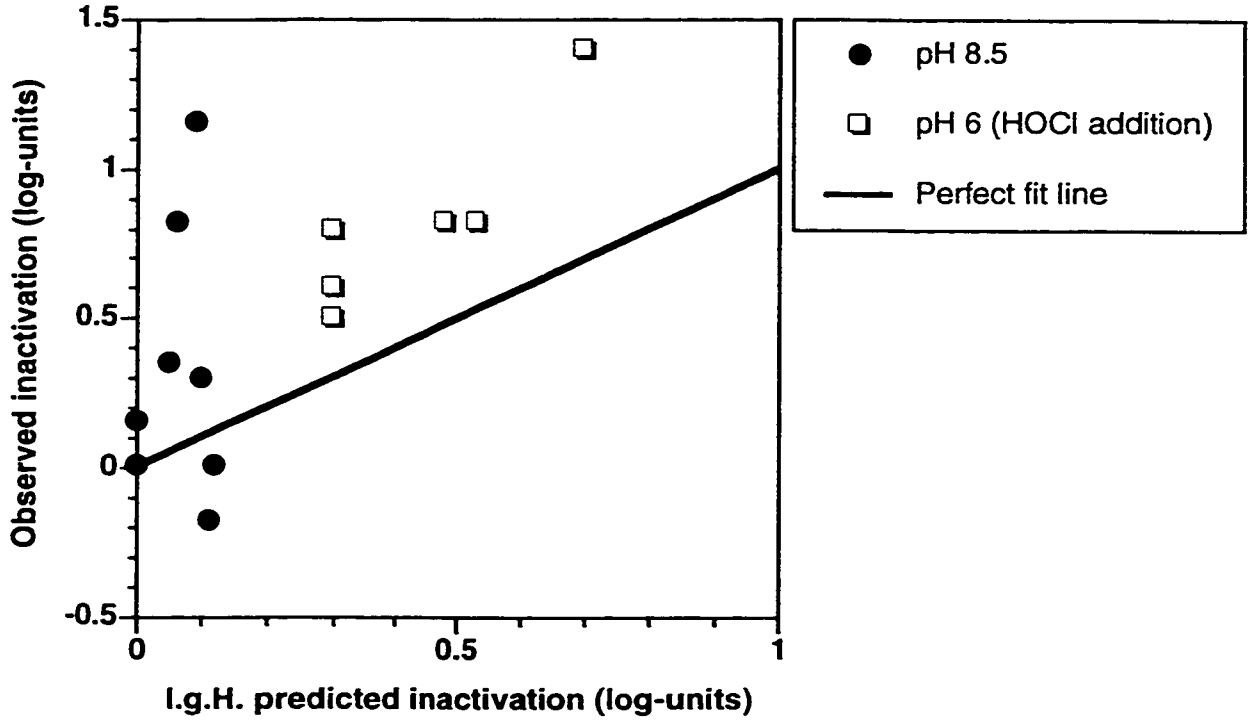


**Figure 4-3 Incomplete gamma Hom predicted inactivation ratios versus observed reduction in infectivity for free chlorine treated *Cryptosporidium parvum* oocysts in modified North Saskatchewan River water at 22°C**

Note: Each data point represents a single trial
Survival determined using animal infectivity and logistic dose response model

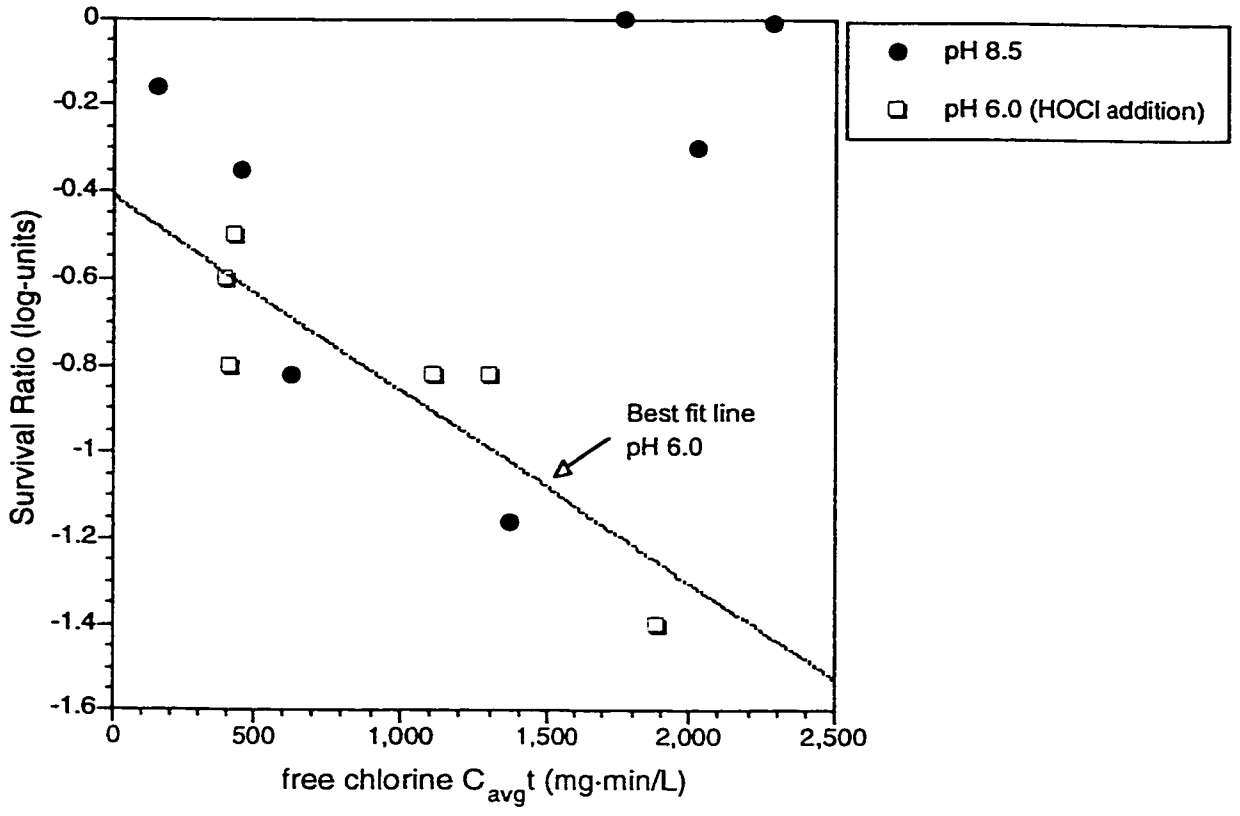


**Figure 4-4 Survival ratio of Cryptosporidium parvum oocysts as a function of ozone $C_{avg}t$ in North Saskatchewan River water at 22°C**

Note: Each data point represents a single trial
Survival determined using animal infectivity and logistic dose response model

## Sequential Treatment

The factors studied for sequential treatment using ozone followed by free chlorine included a high and low level of ozone pre-treatment, a high and low level of free chlorine $C_{avg}t$ product, and a high and low water pH. Ozone was applied to give an expected inactivation of 2.0 or 0.7 log-units. The free chlorine $C_{avg}t$ product was targeted for either a low level of approximately 500 mg·min/L or a high level of greater than 1000 mg·min/L and the pH was the actual modified NSR water pH 8.5 or adjusted pH 6.0.

The results of the ozone followed by free chlorine treatment are summarized in Table 4-4. The observed synergy effect and I.g.H model predictions for free chlorine and ozone are also included on this table. The effect of *C. parvum* oocysts exposed to free chlorine after ozone pretreatment in the modified NSR water at 22°C, pH 8.5 and pH 6.0 is shown in Figure 4-5. Best fit lines were fit to the data obtained for high and low level pretreatment at pH 6.0 and 8.5. The slopes of the best fit lines for modified NSR water at pH 6.0 were similar for both high and low level pretreatment. The slope of the best fit lines at pH 8.5 for high and low level pretreatment were also similar. In general, at pH 6.0 and pH 8.5 the level of inactivation increased linearly with increasing free chlorine $C_{avg}t$ but the increase in inactivation was greater for the modified NSR water at pH 6.0 compared to pH 8.5 over an equivalent $C_{avg}t$ interval. High levels of inactivation were observed after high level ozone pretreatment and high level free chlorine $C_{avg}t$. For the pH 8.5 modified natural water an average of 2.7 log-units inactivation of *C. parvum* was observed. For modified NSR water adjusted to pH 6.0 an average of 3.3 log-units reduction was observed.

According to Table 4-4, additional inactivation due to synergy was not observed for high or low ozone pretreatment levels and different free chlorine $C_{avg}t$ levels when modified NSR water was at pH 8.5 or pH 6.0. After high level ozone pretreatment and a free chlorine $C_{avg}t$ of 1100 mg·min/L, only greater than 0.5 log-units of additional inactivation due to synergy was observed.

**Table 4-4 Summary of synergistic effect of inactivation of *Cryptosporidium parvum* using ozone followed by free chlorine in modified North Saskatchewan River water at 22°C**

| | | Ozone pretreatment | | Free chlorine treatment | | | Total | | |
|---|---|---|---|---|---|---|---|---|---|
| Trial No. | pH | Predicted kill[+] log-units | Net Observed log-units | $C_{avg}t$ mg·min/L | Predicted kill[+] log-units | Net Observed log-units | Additive kill log-units | Total kill log-units | [‡]Synergy log-units |
| *1 | 8.5 | 1.9 | 2.16 | 1771 | 0.1 | -0.18 | 1.98 | 2.82 | 0.84 |
| *2 | 8.5 | 1.3 | 1.16 | 455 | 0.1 | 0.35 | 1.51 | 1.82 | 0.31 |
| *3 | 8.5 | 1.5 | 1.01 | [++]100 | 0.0 | 0.01 | 1.02 | 1.59 | 0.57 |
| *4 | 8.5 | 1.7 | 2.16 | [++]150 | 0.0 | 0.16 | 2.32 | 1.82 | -0.50 |
| 5 | 8.5 | 0.7 | 0.16 | 2287 | 0.1 | 0.01 | 0.17 | 0.87 | 0.70 |
| 6 | 8.5 | 0.8 | 0.82 | 2024 | 0.1 | 0.30 | 1.12 | <0.82 | <-0.30 |
| 7 | 8.5 | 1.9 | 1.68 | 1370 | 0.1 | 1.16 | 2.84 | 2.16 | -0.68 |
| 8 | 8.5 | 2.5 | 1.76 | 625 | 0.1 | 0.82 | 2.58 | 2.3 | -0.28 |
| 9 | 6.1 | 2.0 | 2.01 | 1109 | 0.5 | 0.82 | 2.83 | >3.35 | >0.52 |
| 10 | 6.0 | 2.3 | 2.35 | 1303 | 0.5 | 0.82 | 3.17 | 3.35 | 0.18 |
| 11 | 6.1 | 0.9 | 0.82 | 1890 | 0.7 | 1.4 | 2.17 | 2.35 | 0.18 |
| 12 | 6.0 | 2.1 | 2.35 | 404 | 0.3 | 0.6 | 2.94 | 2.82 | -0.12 |
| 13 | 6.0 | 0.8 | 0.82 | 413 | 0.3 | 0.8 | 1.64 | 1.16 | -0.48 |
| 14 | 6.0 | 2.4 | 2.16 | 430 | 0.3 | 0.5 | 2.65 | 2.82 | 0.17 |

*pre-ozonated NSR water

[‡]synergy = total kill – additive kill

[+] Ozone and free chlorine inactivation ratios predicted using the incomplete gamma Hom model for ozone (Gyürék *et al.* 1999) and free chlorine (Gyürék *et al.* 1997).

[++]The actual value is less than that shown here because the final measured concentration was zero.

ND: data not available

'<' refers to less than measurement limit and '>' refers to greater than measurement limit for particular trial

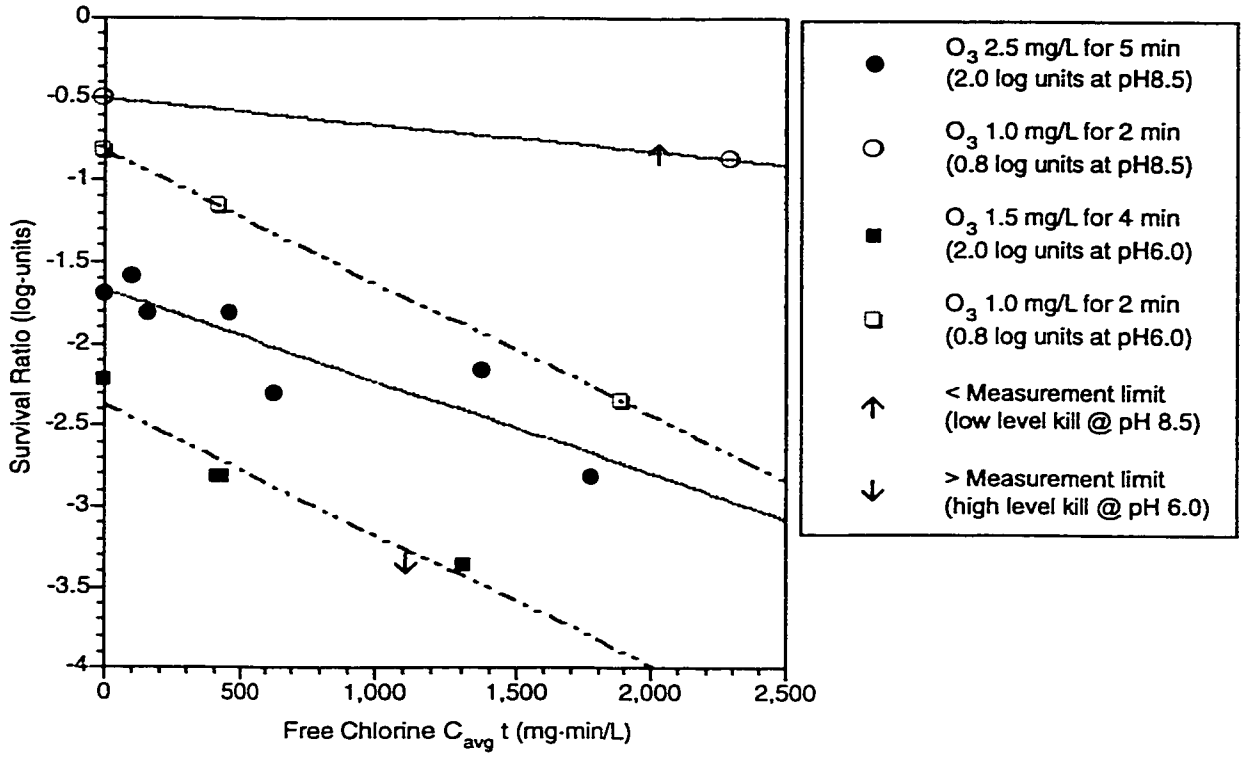


**Figure 4-5 Synergy effect of ozone followed by free chlorine on *Cryptosporidium parvum* oocysts inactivation in North Saskatchewan River water at 22°C**

Note: Direction of arrow denotes greater than or less than measurement limit for particular data point. Each data point represents a single trial.

## Discussion

Based on the results of the modified natural water trials, it appeared that modified NSR water does not affect the survival of oocysts. The results of the positive control trials indicated that exposure of the modified NSR water at 22°C had an insignificant effect on oocyst survival. All of the positive control results varied about zero within ±0.7 log-units. This range of variability is acceptable and most likely due to the inherent variability of the animal infectivity model used (Finch *et al.* 1993).

The modified NSR water did not appear to affect the ozone inactivation kinetics of *C. parvum*. The results of the natural water trials suggest that the I.g.H model developed for ozone inactivation using laboratory water at pH 6.0 to 8.0 (Gyürék et. al. 1999) adequately predicts the inactivation ratios for ozone treatment of *C. parvum* oocysts in modified NSR water at pH 6.0, pH 8.5 and 22°C. The observed inactivations at pH 6.0 tended to be fit by the model prediction quite well as shown in Figure 4-1. All of the observed results were within ± 0.3 log-units of the predicted. The ozone inactivations observed at pH 8.5 varied somewhat and did not fit the model prediction curve as well. This is most likely due to the fact that ozone decomposition rates increase with pH, especially above pH 8.0 (Bryant *et al.* 1992), which may imply that inactivation is more difficult to control at pH 8.5.

The I.g.H model developed for free chlorine in laboratory water did not predict the inactivation of *C. parvum* with free chlorine as well as the I.g.H model developed for ozone inactivation. At pH 6.0 and pH 8.5 the majority of the observed inactivations were within ± 0.5 log-units of the expected inactivation estimated from the model, shown in Figure 4-3. However, at pH 6.0 one observed free chlorine inactivation was 0.7 log-units more than predicted and at pH 8.5 two data points were 1.0 and 0.7 log-units more than what was predicted. The higher observed log inactivation compared to the model prediction at pH 6.0 was related to a high free chlorine $C_{avg}t$ (1900 mg·min/L). This suggests that it may be possible that constituents in the modified NSR water may enhance the inactivation of free chlorine after higher applied free chlorine doses at pH 6.0. More

research is required to verify this as only one trial was conducted at this free chlorine $C_{avg}t$. Also, much remains to be learned about the mechanisms of microorganism reduction in natural waters and how constituents in natural waters may effect the inactivation potentials of certain chemicals. The I.g.H model developed for free chlorine at pH 6.0 (Gyürék *et al.* 1997) was used to calculate infectivity reductions for pH 8.5 based on the fraction of total free chlorine as hypochlorous acid at this pH. Hypochlorous acid constitutes 0.79 and 0.28 of the total free chlorine at pH 7 and pH 8, respectively (Gyürék *et al.* 1997). For the pH 8.5 data, I.g.H model predictions for free chlorine at pH 6 were multiplied by 0.1. At pH 8.5 the variations in the model predictions compared to the observed inactivation could be explained by the fact that the model predictions were interpolated from a model developed for pH 6.0 water. Despite the variations noted at both pH 6.0 and 8.5 there is no clear evidence that the modified NSR water affects the free chlorine inactivation kinetics of *C. parvum*.

The results plotted in Figure 4-4 indicate that when the free chlorine $C_{avg}t$ was less than 500 mg·min/L or greater than 1500 mg·min/L, a greater level of inactivation of *C. parvum* could be achieved in modified NSR water at pH 6.0 than in water at pH 8.5. It also appeared that when the free chlorine $C_{avg}t$ was between 500 and 1500 mg·min/L the survival ratio of *C. parvum* was similar for NSR water at pH 6.0 and pH 8.5. At pH 6.0 a free chlorine $C_{avg}t$ of 2000 mg·min/L provided 1.4 log-units inactivation, while at pH 8.5 a $C_{avg}t$ of 2000 mg·min/L provided only 0.3 log-units inactivation. One explanation for the difference in the level of inactivation at pH 6.0 compared to pH 8.5 is that the free chlorine is present in the hypochlorous acid (HOCl) form at pH 6. At a higher pH, the free chlorine equilibrium shifts to favor the hypochlorite ion ($OCl^-$) form. Since HOCl is a much more effective germicide agent than $OCl^-$, greater free chlorine inactivation is attained at the lower pH. Also, pH can cause alterations to the surface of some organisms, increasing the permeability of the cyst wall to the disinfectant (Finch *et al.* 1994). The level of inactivation at pH 8.5 increased with free chlorine $C_{avg}t$ until about 1500 mg·min/L, after which point it dropped to less than 0.4 log-units. This sudden decrease in the level of inactivation may indicate that there is an optimum Ct for free chlorine inactivation of *C. parvum* at pH 8.5. After this optimum level is reached applying a higher free chlorine $C_{avg}t$ may have adverse effects on the inactivation of *C.*

*parvum*. The reason for this, however, is not clear and more trials should be conducted at pH 8.5 to verify this effect.

For NSR water that was not pre-ozonated, at pH 6.0 it was observed that a lower applied ozone dose for an equivalent contact time could achieve the same inactivation as that of the higher pH water. This is most likely due to the increase in decay rate of ozone at higher pH. As stated previously, increased rates of decomposition become significant at pH 8 or greater, making it difficult to maintain an ozone residual. (Bryant *et al.* 1992). At pH 8.5 an applied ozone dose of 2.0 mg/L reduced to approximately 1.5 mg/L almost immediately and the average decay rate was 0.5 $min^{-1}$. At pH 6.0 an applied dose of 1.5 mg/L reduced to only 1.4 mg/L after approximately 20 seconds and the average decay rate was 0.1 $min^{-1}$. The effects of ozone treatment on *C. parvum* inactivation for the pre-ozonated and not pre-ozonated trials can not be compared directly, as the treatments were different.

It appeared that the pH of the modified natural water had an effect on the level of synergy observed after sequential chemical treatment at 22°C and the overall observed inactivation. According to Table 4-4, at pH 8.5 the highest observed inactivation of *C. parvum* after ozone pretreatment and free chlorine treatment was 2.8 log-units. The total observed inactivations at pH 8.5 for both high and low levels of pretreatment, appeared to increase slightly as free chlorine $C_{avg}t$ increased at approximately the same rate. High ozone pretreatment followed by a high free chlorine $C_{avg}t$ gave an average of 2.4 log-units inactivation, while a low free chlorine $C_{avg}t$ gave an average of 1.6 log-units. This was expected and is probably due to the fact that free chlorine inactivation is generally greater at higher Ct's. Despite the slight increase over increased free chlorine $C_{avg}t$ values, there appeared to be no synergy at pH 8.5 at either the high or low ozone pretreatment levels followed by high or low levels of free chlorine $C_{avg}t$. At pH 6.0 the total observed inactivation reached greater than 3.4 log-units. The level of inactivation of *C. parvum* for the ozone/free chlorine sequential combination at pH 6.0 increased with increasing free chlorine $C_{avg}t$ and appeared to increase at the same rate for high and low level ozone pretreatment. It also appeared that at pH 6.0 there may be synergy after higher free chlorine $C_{avg}t$ values and high and low level ozone pretreatment. Greater than

0.5 log-units of synergy was observed after high level ozone pretreatment and a free chlorine $C_{avg}t$ of 1000 mg·min/L. More research is required to verify this, however, as only one trial showed this level of synergy. The level of inactivation was consistently greater after an equivalent pretreatment and an equivalent secondary $C_{avg}t$ value in modified NSR water at pH 6.0 compared to pH 8.5, as shown in Figure 4-5. The differences in the levels of inactivation at pH 8.5 and pH 6.0 were probably due to the higher ratio of total free chlorine as HOCl at the lower pH combined with a lower ozone decay rate at the lower pH.

There was rapid consumption of free chlorine and ozone by the modified NSR water at both pH 8.5 and pH 6.0. For free chlorine the decay rate was approximately 0.0016 $min^{-1}$ for the natural water trials compared to an average of 0.00016 $min^{-1}$ for the free chlorine trials conducted using lab waters (see Table B.1 and B.3, Appendix B). The results in Table 4-2 show that an applied free chlorine dose of 4.0 mg/L reduced to a free chlorine residual of approximately 3.0 mg/L after only 5 min in the modified NSR water. The modified NSR water also had a high ozone demand. First order ozone disappearance rate constant for pH 6.0 buffered laboratory water was reported to be approximately 0.03 $min^{-1}$ (Gyürék *et al.* 1999), whereas, the decay rate of the modified natural water used in this study at pH 6.0 was approximately 0.12 $min^{-1}$ (Table 4-2). The high demands that were observed were probably due to constituents in the modified NSR water having high ozone and chlorine demands.

For sequential treatment of *C. parvum* the level of ozone pretreatment seemed to affect the total observed inactivation. According to the results provided in Table 4-4, at pH 6.0 a significant inactivation of *C. parvum* after high level ozone pretreatment and low level free chlorine $C_{avg}t$ was observed (2.8 log-units). When a low level ozone pretreatment was followed by the same low level free chlorine $C_{avg}t$, only 1.2 log-units inactivation was observed. Also, the level of inactivation appeared to increase with free chlorine $C_{avg}t$ at approximately the same rate after both high and low level ozone pretreatment for modified NSR water at pH 6.0 and 8.5. This was most likely a result of the high potency of ozone on *C. parvum* oocysts that tends to increase with increasing

doses and contact times and the previously observed increase in inactivation with increasing free chlorine Ct's.

# CHAPTER 5

# GENERAL DISCUSSION AND CONCLUSIONS

## INTRODUCTION

Ideally, sequential microorganism reduction combinations should consist of a primary chemical that is a strong oxidizer and a secondary disinfectant that will both attack the microorganism more easily after primary chemical treatment and maintain a chemical residual in the distribution system. It is generally believed that bacterial kill through the use of ozone or chlorine dioxide occurs directly because of cell wall disintegration. Chlorine species, on the other hand, are believed to diffuse through the cell wall and alter the chemical arrangement and deactivate enzymes. When ozone or chlorine dioxide first weakens or alters the permeability of the cell wall it makes it easier for chlorine to diffuse through it. This results in a higher overall inactivation compared to single chemical inactivation and is known as a synergistic effect. A practical outcome of synergy from sequential treatment is that disinfection processes can be designed to use less chemicals to achieve the desired kill when compared to using the chemicals singly.

### Comparison of Findings to Previous Studies

The present research studied sequential treatment effects on *C. parvum* at different temperatures in laboratory water and in modified natural water. As microorganism reduction studies have used different viability assays, experimental methods and strains of oocysts, significant differences in the results have been found (Li *et al.* 1999). Studies that used similar methods should report comparable results. Several studies have been conducted that show similar findings to those obtained in this research.

In general, the present study found that for single chemical treatment free chlorine was more effective in inactivating *C. parvum* than monochloramine at 22°C and chemical treatment is more effective at higher water temperatures. In addition, for the chlorine

dioxide based sequential treatment, lower levels of inactivation of *C. parvum* were observed at 1°C and 10°C compared to 22°C. Potential synergistic effects were observed after a high Ct of the secondary chemical for chlorine dioxide based sequential treatment. The overall level of inactivation increased with $C_{avg}t$ at all three temperatures and was lower for chlorine dioxide/monochloramine treatment than chlorine dioxide/free chlorine treatment at 22°C. In addition, it appeared that a low chlorine dioxide pretreatment level was slightly more favorable for obtaining a synergistic effect.

A higher overall inactivation of *C. parvum* was observed for free chlorine treatment compared to monochloramine treatment at equivalent $C_{avg}t$'s and 22°C. The results in Tables 3-4 and 3-5 indicated that free chlorine could inactivate greater than 1.3 log-units of *C. parvum* at a $C_{avg}t$ of 2500 mg·min/L and monochloramine required a $C_{avg}t$ of 4000 mg·min/L to inactivate 1.1 log-units. At 1 and 10°C, however, the results in Tables 3-4 and 3-5 indicated that the inactivations provided by free chlorine or monochloramine at equivalent $C_{avg}t$'s were similar. A lower level of inactivation was observed at the lower water temperatures for both free chlorine and monochloramine trials, however, the difference in the level of inactivation at 10°C and 22°C was greater for the free chlorine trials. This suggests that free chlorine is more sensitive to temperature change than monochloramine and that although free chlorine was more effective than monochloramine at room temperatures it was not more effective at lower water temperatures. Limited research has been conducted comparing the effects of free chlorine and monochloramine on *C. parvum* oocysts at low water temperatures but it has been found that at 22°C, free chlorine is more effective than monochloramine at high Ct's (Finch *et al.* 1997), which agrees with the observed results found in this study. According to the U.S. EPA (1998), for water temperatures between 1°C and 10 °C the Ct values required for 99.9 percent inactivation of *G. lamblia* cysts by chloramines are much larger than the Ct values required for 99.9 percent inactivation using free chlorine at the same water temperature. The effects of free chlorine and monochloramine on *C. parvum* inactivation at low temperatures, therefore, were found to be quite different than the effects on *G. lamblia* inactivation at the same temperatures. Chemicals react with different microorganisms differently and this observation suggests that free chlorine may be less sensitive to temperature changes when used to inactivate *G. lamblia* compared to

*C. parvum*. This may be due to the fact *C. parvum* is more difficult to inactivate than *G. lamblia*.

Chlorine dioxide inactivation of *C. parvum* oocysts was studied in laboratory waters at 1°C and 22°C, and pH 6.0 using animal infectivity for evaluation of oocyst viability (Li and Finch 1998). Li and Finch (1998) found that an initial concentration of chlorine dioxide of 1.4 mg/L for 120 min resulted in an inactivation of 0.5 log-units at 1°C and about 2.0 log-units at 22°C. For the present study, an equivalent inactivation was obtained at 1°C under the same treatment conditions, but at pH 8.0 (see Table 3-10). These treatment conditions were not used at pH 6.0 but the inactivation of *C. parvum* at pH 8.0 was directly related to the reduction at pH 6.0. Experiments were conducted using an initial chlorine dioxide concentration of 2.6 mg/L for 240 min at both pH 6.0 and 8.0 and similar log inactivations were observed (Tables 3-7 and 3-10). According to Table 3-7, at 22°C, 1.5 mg/L for 90 min gave 1.4 log-units inactivation. By increasing the contact time to 120 min it is likely that 2.0 log-units inactivation would be achieved.

Previous work conducted on the synergistic effect of chemical treatments on *C. parvum* inactivation in laboratory waters reported that a synergistic effect was only observed when the secondary disinfectant was at its optimum pH (Finch *et al.* 2000). For chlorine this was pH 6 and for monochloramine this was pH 8. Also, in a study conducted by Finch *et al.* (1997) it was reported that free chlorine could inactivate *C. parvum* at a high Ct in lab water at pH 6 but not at pH 8. The present study used 0.05 M phosphate buffered water at pH 6 for chlorine dioxide/free chlorine trials and pH 8 for chlorine dioxide/monochloramine trials to optimize conditions for synergy. Synergistic effects were observed for both of these conditions.

A synergistic effect has been found in past studies at low temperature conditions. Most of the literature dealing with the effect of chemicals on protozoan cysts involves the effects on *Giardia* spp. cysts and limited research has been conducted investigating temperature effects on the inactivation of *C. parvum* oocysts. The inactivation of *Giardia muris* using sequential chemicals has been studied in pH 6 and pH 8 phosphate buffer at low temperatures (5°C) by Finch *et al.* (2000). This study used in vitro excystation to

measure the level of inactivation. The results suggested that 1.0 log-unit of inactivation more than what was expected from the single disinfectants was achievable at 5°C for several chemical combinations. These combinations included chlorine dioxide/free chlorine at pH 6 and 8, ozone/free chlorine at pH 6 and 8, ozone/monochloramine at pH 8, and free chlorine/monochloramine at pH 8. For these chemicals a primary treatment of 0.7 log-units inactivation was used followed by a secondary treatment also targeted for 0.7 log-units inactivation when used singly.

According to the results of the present study, temperature and the level of free chlorine $C_{avg}t$ have a significant effect on the inactivation of *C. parvum* oocysts when applying chlorine dioxide and free chlorine sequentially. According to Figure 3-1, no additional inactivation due to synergy was observed at 1°C with chlorine dioxide followed by free chlorine Ct as high as 4000 mg·min/L. The level of inactivation and potential synergy, however, tended to increase with temperature and free chlorine Ct. The results in Table 3-8 indicate that at 10°C, about 0.8 log-units of synergy was observed after low level chlorine dioxide pretreatment and free chlorine $C_{avg}t$ of 2500 mg·min/L and after high pretreatment and a $C_{avg}t$ of 4000 mg·min/L. According to Figure 3-3, at 22°C, about 1.0 log-units of synergy was observed at free chlorine $C_{avg}t$ values of approximately 1500 mg·min/L and 2000 mg·min/L after high and low levels of chlorine dioxide pretreatment, respectively. A study using chlorine dioxide sequentially with free chlorine for the inactivation of *C. parvum* reported similar findings. Corona-Vasquez *et al.* (1999) reported that an additional 1.0 log-unit inactivation of *C. parvum* after 1.0 log-unit inactivation with chlorine dioxide at pH 6.0, and 20°C and 4°C could be achieved using a free chlorine Ct of 1600 and 5200 mg·min/L, respectively. The study mentioned used a modified in-vitro method to assess the viability of treated oocysts and claimed that previous work had shown results that were consistent with animal infectivity data. It has been reported in many studies, however, that in-vitro excystation underestimates the kill of oocysts (Owens *et al.* 1994; Black *et al.* 1996).

The effects of chlorine dioxide followed by free chlorine on *C. parvum* inactivation at different temperatures, also relates to the effects found when using ozone followed by free chlorine. A study conducted by Finch *et al.* (Finch and Li 1999) showed

that when using ozone followed by free chlorine in pH 6 phosphate buffer at 1 and 22°C, the level of synergy was highly dependent on the water temperature and free chlorine Ct product. In general, it was found that more significant synergy was obtained at a higher temperature with higher applied free chlorine Ct. The same was true of chlorine dioxide/free chlorine sequential chemical treatment.

The results of the laboratory water trials using chlorine dioxide followed by monochloramine, provided in Tables 3-10 to 3-12, tended to suggest that additional inactivation due to synergy could be achieved at 1, 10 or 22°C. Some degree of synergy was observed at 1 and 10°C after a very high $C_{avg}t$ of approximately 4000 and 3000 mg·min/L, respectively. At 22°C after low chlorine dioxide pretreatment and a monochloramine $C_{avg}t$ of about 600 mg·min/L additional inactivation due to synergy was observed (Table 3-12). After this point, the synergy appeared to be independent of the monochloramine Ct and declined at the highest Ct value. This suggests that at 22°C chlorine dioxide followed by monochloramine could contribute to enhanced inactivation of *C. parvum* until a threshold monochloramine $C_{avg}t$ value was reached. Higher monochloramine $C_{avg}t$ values resulted in less synergy, even though there was increased inactivation from monochloramine alone. A study by Finch et al (1997) suggested that a threshold concentration might exist for monochloramine. It was found that a relatively small increment in monochloramine dose at pH 8.0 and 22°C was necessary to increase *C. parvum* inactivation from 0.5 to 1.0 log-units. After this threshold concentration, however, little additional inactivation of *C. parvum* by monochloramine was observed. Another study suggested this same threshold hypothesis when looking at ozone followed by monochloramine. A study using ozone followed by monochloramine at 20°C and pH 8 (Driedger *et al.* 1999) reported that there was a fairly rapid increase in inactivation of *C. parvum* after fairly small changes in monochloramine Ct. This study used a modified in-vitro excystation method to determine oocyst viability and the present study used animal infectivity.

Inactivation of *C. parvum* oocysts using free chlorine or monochloramine alone has been studied previously. It has been found that for both chemicals an initial lag phase, during which little inactivation occurs, is followed by a pseudo-first order decrease

in viability of *C. parvum* (Finch *et al.* 1994; Driedger et al 1999). When free chlorine or monochloramine were used as secondary chemicals in sequential inactivation, however, this lag phase tended to reduce or disappear. The study by Driedger *et al.* (1999) reported that when using ozone followed by monochloramine sequentially, the lag region disappeared. The chlorine dioxide/monochloramine treatment trials conducted in the present research also showed a disappearance in this lag region. Finch *et al.* (1997) reported that a lag region existed for free chlorine until a Ct greater than 2500 mg·min/L was reached. The chlorine dioxide/free chlorine disinfection trials displayed a lag region until a free chlorine Ct of about 600 mg·min/L was reached (Figure 3-7 and 3-8). After this there was a pseudo-first order increase in inactivation. A study by Corona-Vasquez (1999) using chlorine dioxide followed by free chlorine at pH 6.0, 4°C and 20°C for the inactivation of *C. parvum* showed a similar result to the present study. The study also showed that at lower temperatures there tended to be a more substantial lag phase. The same seemed to be true for the chlorine dioxide/free chlorine results found in this research. According to Figures 3.7 and 3.8, at 10°C the lag phase ended at about 700 mg·min/L, while at 22°C the lag phase ended around 300 mg·min/L. The disappearance or reduction of the lag phase for sequential chemical addition tends to support the hypothesis of the synergistic effect. This hypothesis states that oocyst wall permeability may decrease with increased pretreatment making it easier for the secondary chemical to diffuse through the cell wall and disrupt the microorganism life processes.

The lag phase for chlorine dioxide followed by free chlorine, also indicated that at lower free chlorine $C_{avg}t$ values after chlorine dioxide pretreatment, little or no inactivation of *C. parvum* occurred. This does not mean that the oxidant does not have an effect on the oocyst at lower Ct's. Changes in the oocyst wall may occur at very low doses of chlorine species without actual inactivation of the parasite. A study by Finch *et al.* (1997) reported increased inactivation of *C. parvum* when using free chlorine and monochloramine sequentially compared to using them singly. The findings of this study suggest that the higher than expected inactivation was observed after sequential treatment because lower doses of free chlorine may change the permeability of the oocyst wall making it easier for monochloramine to diffuse through the wall and attack the oocyst.

Previous studies have shown that a low chlorine dioxide pretreatment followed by free chlorine was favorable for a higher degree of synergy. Finch *et al.* (2000) used chlorine dioxide followed by free chlorine for the inactivation of *C. parvum* at pH 8 and 22°C. That study showed that the degree of synergy was greater at the low chlorine dioxide pretreatment level compared to the high pretreatment level. At pH 8 free chlorine is present in the hypochlorite ion form ($OCl^-$). The same was found in the present study using chlorine dioxide followed by free chlorine at pH 6 and was discussed in Chapter 3. At pH 6 free chlorine is present in the hypochlorous acid (HOCl) form, which is a more effective germicide agent than $OCl^-$. This suggests that there may be an optimum level of chlorine dioxide pretreatment at which maximum synergy can be observed between chlorine dioxide and free chlorine at pH 8 and pH 6, and after which additional pretreatment produces no benefits for synergy. Chlorine dioxide may selectively attack certain components of the membrane of the oocyst, permitting easier passage of free chlorine through the membrane. After the chlorine dioxide has reacted with the membrane to its fullest extent the addition of more chlorine dioxide may increase inactivation but not change the permeability further, thus not enhancing conditions for free chlorine treatment further.

## Comparison of Modified Natural Water to Laboratory Water

Results of *C. parvum* exposure to ozone in a modified natural water at pH 6.0 and 8.5 compared to the I.g.H. model developed for ozone in laboratory water and showed similar results to those found for laboratory water in previous studies. Finch *et al.* (1994) conducted a study on the effect of ozone on the inactivation of *C. parvum* at 7 and 22°C in pH 6.9, 0.05 M phosphate buffer. It was found that at 22°C an applied ozone dose of 1.5 mg/L for 5 min gave about 2.5 log-units inactivation. According to Table 4-2, the present study observed the same results, at pH 6.0 and 22°C. Gyürék *et al.* (1999) conducted a study that compared the effect of ozone on *C. parvum* oocysts in laboratory waters at 22°C and different pH levels. It was reported that at pH 6.0 an initial ozone concentration of 0.9 mg/L for 5 min gave an inactivation of 2.2 log-units and an initial residual of 1.9 mg/L for 5 min gave an inactivation of 3.2 log-units. These data, along

with a larger data set, were used to develop the I.g.H model for the inactivation kinetics of ozone at pH 6 to 8 in laboratory waters. A comparison to the I.g.H model prediction in Figure 4-1, showed that ozone inactivation of *C. parvum* in natural water and laboratory water at pH 6.0 and 22°C were similar. In the study conducted by Gyürék *et al.* (1999), at pH 8.0 an initial ozone residual of 1.5 mg/L for 4 min gave an inactivation of 1.1 log-units. For the modified natural water trials (Table 4-2) at pH 8.5, it was found that an initial ozone concentration of 1.5 mg/L for 4 min gave an inactivation of 1.0 log-unit. This was a similar result to that obtained in laboratory water at pH 8.0.

Several studies have reported that higher pH adversely affects the inactivation of *Giardia* spp. and *Cryptosporidium* spp. using ozone, which was also observed in the present study. A slightly lower applied ozone dose was required in the modified natural water (not pre-ozonated) at pH 6.0 than in the same water at pH 8.5 to achieve the same level of inactivation of *C. parvum* (Figure 4-2). At pH 8.5 more hydroxide ions are present, which react with ozone to form hydroxide radicals. Hydroxide radicals are more reactive than ozone, which suggests that applying ozone to higher pH waters, may have a greater effect on *C. parvum* inactivation. However at a high pH the rate of ozone decomposition increases. This increase in rate of decomposition may adversely affect the inactivation potential of hydroxide radicals on *C. parvum* at the high pH. Labitatiuk *et al.* (1992) studied the factors affecting *Giardia muris* inactivation using ozone in laboratory and natural waters. It was reported that when ozone contact times were more than 2 min, the level of pH was a significant factor. It was also reported that previous work had shown that *G. muris* cysts were more sensitive to ozone at pH 5 than pH 7. Finch *et al.* (1997) studied ozone inactivation of *C. parvum* in oxidant demand free phosphate buffer at pH 6 and pH 8. It was reported that after greater than 2 min of contact time at 22°C, the inactivation of *C. parvum* in pH 8 water was 33 percent that of the pH 6 trial.

The I.g.H model developed for free chlorine in lab water at pH 6, did not compare adequately to the results found in the modified natural water trials at pH 6 or pH 8.5, when free chlorine was applied alone, as shown in Figure 4-3 and as discussed in Chapter 4. In addition, previous studies report results similar to those found in this study for modified natural water trials conducted at pH 6.0 but not at pH 8.5. A report by Finch *et*

*al.* (1997) looked at *C. parvum* inactivation by free chlorine at pH 6 and pH 8 and 22°C. The results showed that a free chlorine Ct of 1000 mg·min/L could achieve 0.7 log-units inactivation at pH 6 and 0.1 log-units at pH 8.0. The study also reported that the level of inactivation increased with free chlorine Ct at pH 6.0 and was consistently low (less than 0.4 log-units) at pH 8.0. This agrees with the observed inactivations found in modified natural water at pH 6.0 only. The results plotted in Figure 4-4 showed that the level of inactivation of *C. parvum* increased with free chlorine Ct at pH 6.0. At pH 8.5, however, the level of inactivation appeared to increase until a free chlorine Ct of 1500 mg·min/L, after which point the level of inactivation dropped to less than 0.4 log-units. When the free chlorine $C_{avg}t$ was between approximately 500 and 1500 mg·min/L greater than expected inactivations were observed. At pH 8.5 two of the observed inactivations were 1.0 and 0.7 log-units greater than the model prediction at a free chlorine $C_{avg}t$ of 1400 and 600 mg·min/L, respectively. The reason for the difference between previous findings or model predictions and the observed inactivations at pH 8.5 is not clear but it is possible that constituents in the modified natural water enhanced the inactivation potential of free chlorine when a threshold free chlorine dose was applied. Since only two trials at pH 8.5 resulted in higher than expected inactivations, more work would be required to verify this.

Finch *et al.* (1999) conducted experiments treating *C. parvum* oocysts with the level of ozone inactivation set at 1.5 log-units followed by varying free chlorine Ct products at pH 6.0 and 22°C. It was reported that in order to achieve 1.0 log-unit of inactivation due to synergism, a free chlorine Ct of approximately 700 mg·min/L was required. The results from the natural water trials showed that an ozone pretreatment of 2.0 log-units followed by a free chlorine $C_{avg}t$ of 1300 mg·min/L at pH 6.0 did not result in evident synergy. The difference between the results of the previous study and the present one was most likely due to effects of constituents in the modified NSR water. However, since the results obtained for the ozone single treatment trials in modified NSR water at pH 6.0 are similar to model predictions estimated from lab water experiments, the reason for this discrepancy is not clear. A larger data set may be required to verify this as only six trials were conducted at pH 6 and only three of these used a free chlorine $C_{avg}t$ above 1000 mg·min/L. If this is a valid assumption there may be constituents that

exist in the modified natural water that impede or block free chlorine diffusion through the oocyst cell wall after ozone pretreatment, thus decreasing the synergistic potential of free chlorine.

For the sequential treatment trials in natural water the level of natural water pH and the level of treatments effected the overall level of inactivation. In general, a lower water pH and higher primary and secondary treatments resulted in a higher overall inactivation. A study conducted by Li *et al* (1999) using ozone followed by free chlorine for *C. parvum* inactivation in lab water at pH 6 and 22°C showed, that for a certain level of ozone pretreatment, as the free chlorine $C_{avg}t$ increased so did the level of inactivation. The results of the current study also showed an increase in inactivation with increasing free chlorine $C_{avg}t$ at pH 6.0 (Figure 4-5). This increase in inactivation with $C_{avg}t$ also occurred at pH 8.5 but the increase was less substantial. The increase in inactivation is due to free chlorine inactivation potential increasing with dose and contact time, as described earlier. No studies in the literature were found that looked at using ozone/free chlorine for *C. parvum* inactivation at different pH values. In sequential treatment, the pH effect could be explained by the way pH effects the ozone decay rate and free chlorine inactivation kinetics. As discussed previously, the difference in the slopes at the different pH levels may be due to the higher inactivation potential of free chlorine at pH 6.0 compared to pH 8.5 and faster decay rate of ozone at pH 8.5 compared to pH 6.0. Apart from this explanation, the effects of sequential treatment on *C. parvum* inactivation are not well understood and further investigation is required.

General results for the chlorine dioxide/free chlorine sequential laboratory water trials and the ozone/free chlorine sequential natural water trials can be compared. For both modified natural water and lab water trials as free chlorine Ct product increased so did the level of inactivation. This was true for free chlorine in both single and sequential disinfection. In order to obtain any synergy, free chlorine required a high Ct after pretreatment with a strong oxidant in laboratory and modified natural waters. According to Figure 3-3, synergy was observed in the chlorine dioxide/free chlorine trials at higher free chlorine $C_{avg}t$ values (1500 to 2000 mg·min/L) at pH 6 and 22°C. For the modified natural water trials, according to Figure 4-3, if synergy is achievable, a free chlorine $C_{avg}t$

greater than about 1500 mg·min/L would be required when ozone pretreatment is followed by free chlorine treatment at pH 6 and 22°C. The level of ozone pretreatment seemed to have a greater impact on the overall inactivation compared to chlorine dioxide. A high level of ozone pretreatment followed by a high level of free chlorine Ct gave a total kill of 3.4 log-units, while a low level of ozone pretreatment and a high level free chlorine Ct gave 2.2 log-units inactivation (1.2 log-units difference) at pH 6 (Table 4-4). According to the results in Table 3-9, a high level of chlorine dioxide pretreatment and a free chlorine Ct of 3000 mg·min/L gave 3.1 log units inactivation and a low level of chlorine dioxide pretreatment and the same free chlorine Ct gave 2.8 log-units inactivation (0.3 log-units difference). This is probably because ozone is more effective against *C. parvum* than chlorine dioxide (Peeters *et al.* 1989) and requires a much smaller increase in dose and contact time to provide a greater effect on the inactivation of *C. parvum* than chlorine dioxide. This may also be explained by the hypothesis explained earlier that there is a threshold amount of pretreatment by the primary oxidant past which additional pretreatment provides no benefits for synergy. This threshold may have been met and surpassed under the chlorine dioxide conditions used in this study but not under the ozone treatments applied.

## Practical Uses

The critical aspects of designing a disinfection program involve methods required to achieve overall disinfection goals. These goals require providing a barrier at the water treatment plant to prevent the introduction of waterborne pathogens into the distribution system and prevent subsequent growth of microbes in the distribution system.

For chlorine dioxide treatment, a maximum oxidant residual concentration of 1.0 mg/L is required in the finished water (U.S. Environmental Protection Agency 1998). According to results of this study, the chlorine dioxide dose required for a significant synergistic effect at low temperatures is too high to meet the disinfection by product limit without residual removal after treatment. To meet this requirement, a maximum chlorine dioxide dose of 1.4 mg/L can be applied to the water. The results in Table 3-9, show that this concentration was found to only be effective at room temperature after a relatively

long contact time (greater than 90 min). Applying 1.4 mg/L chlorine dioxide for 90 min (equivalent to the high chlorine dioxide pretreatment condition used in this study) at 22°C provided 1.4 log-units inactivation of *C. parvum* in a batch reactor.

Conventional treatment may have to provide 2 log-units reduction of *Cryptosporidium* spp. after effective particle removal, in order to meet the $10^{-4}$ annual risk of *Cryptosporidium* spp infection goal (Fayer *et al.* 1996). In finished water US EPA (1998) requires a free chlorine residual between 0.4 and 3.0 mg/L. Sequential disinfection in a batch reactor using a high level chlorine dioxide pretreatment followed by free chlorine at 22°C, provided 2.0 log-units removal of *C. parvum* when free chlorine $C_{avg}t$ was 700 mg·min/L, however, no additional inactivation due to synergy was observed at this Ct product (Table 3-9). As discussed, synergy was observed at 22°C after a high chlorine dioxide pretreatment and a free chlorine Ct of 1500 mg·min/L. The resulting inactivation under this condition was 3.0 log-units, which may be desirable to some water treatment utilities. The results in Table 3-7 show that at 1°C 2.0 log-units inactivation was not observed over a range of free chlorine $C_{avg}t$ values. At 10°C, 2.0 log-units inactivation was observed after a high level chlorine dioxide pretreatment and a free chlorine $C_{avg}t$ of 2500 mg·min/L (Table 3-8). No synergy was observed at this temperature until a free chlorine $C_{avg}t$ of about 4000 mg·min/L was reached.

At 22°C, when a high level chlorine dioxide pretreatment was followed by a monochloramine $C_{avg}t$ of about 1400 mg·min/L, 2.0 log-units inactivation of *C. parvum* was observed (Table 3-12). A synergy of 0.7 log-units was observed at this monochloramine treatment level. This suggests that the chlorine dioxide/monochloramine combination at this temperature may lead to higher than expected inactivations of *C. parvum*. At 1 and 10°C after high level chlorine dioxide pretreatment, a monochloramine $C_{avg}t$ of about 3500 and 3000 mg·min/L, respectively, were required to obtain 2.0 log-units inactivation (Tables 3-10 and 3-11). Synergy was observed at 10°C and a monochloramine $C_{avg}t$ of 3000 mg·min/L but was not observed at 1°C until a $C_{avg}t$ of 4000 mg·min/L was reached. These monochloramine $C_{avg}t$ values, however, are higher than can be achieved at most water treatment plants. Typical Ct values are around 1000 mg·min/L and lower at water treatment facilities.

Although ozone is highly reactive and dissipates in a short period of time it can still maintain a residual for microorganism reduction for a short period of time. Dissolved ozone residuals of approximately 0.4 to 0.5 mg/L for 4 to 6 min have been typical objectives for the microorganism reduction process (Bryant *et al.* 1992). According to the modified natural water experiments, it appeared that this residual could be achieved after high and low ozone pretreatment (Table 4-2). For ozone/free chlorine sequential disinfection at 22°C, 2 log-units inactivation of *C. parvum* was achieved when a high level of ozone pretreatment was followed by a low level of free chlorine Ct (Table 4-4). This condition, however, did not provide a synergistic effect and was, therefore, found to be ineffective in lowering required chemical doses. A high level of ozone pretreatment followed by a high level of free chlorine Ct (greater than 1000 mg·min/L) provided an overall inactivation greater than 3.4 log-units. This high log kill may be desirable to certain water treatment plants.

In general, the contact times required to obtain synergistic effects may be too long for many systems to readily adapt, especially at low temperatures. Actual applied Ct products of free chlorine or monochloramine for conventional treatment are generally 1000 mg·min/L or less. However, treatment plants that have longer residence times may benefit from chlorine dioxide/free chlorine, chlorine dioxide/monochloramine or ozone/free chlorine treatment at pH 6 and 22°C. Also, due to the fact that little or no synergy was obtained at low temperatures when using chlorine dioxide/free chlorine and chlorine dioxide/monochloramine at practical Ct's, treatment plants with colder source waters may not benefit from these sequential chemical combinations.

All of the experiments conducted for this study were conducted in batch reactors and the results relate to batch type systems. When considering conventional water treatment plants the actual contact times within the system must be evaluated to determine the allowable contact time for the Ct criteria. As discussed in Chapter 1, for continuous flow systems the allowable contact time is referred to as $t_{10}$. According to the US EPA criteria, the $t_{10}$ corresponds to a time of retention that can be achieved by 90 percent of the flow (Bryant *et al.* 1992). This criteria, however, has been found to be conservative in some cases. At treatment plants actual $t_{10}$ values will depend on such

things as basin configuration and basin and tankage inlet and outlet conditions. For example, unbaffled basins are estimated to have a $t_{10}$ of only 10 percent of the contact time, while baffled basins with an element of plug flow are estimated to have $t_{10}$ values of 30 to 70 percent of calculated hydraulic retention times (Bryant *et al.* 1992). Where the available $t_{10}$ is low, disinfectant concentration must be higher to meet the Ct criteria.

A major consideration in evaluating microorganism reduction alternatives is the effect on the formation of disinfection by-products. An important issue that must be considered is the reaction between chlorine dioxide residual and free chlorine to produce chlorate (Bryant *et al.* 1992). Sequential disinfection using chlorine dioxide followed by free chlorine will increase the chlorate concentration in the finished water. As discussed in Chapter 1, 50 to 70% chlorine dioxide is converted to chlorite. When the concentrations of chlorine and chlorite are low or when chlorine is in excess, chlorine oxidizes chlorite to chlorate. Under typical water treatment plant conditions, chlorite and free chlorine concentrations are expected to be low, however, application of chlorine dioxide followed by free chlorine will result in the oxidation of chlorite to chlorate. Chlorate is less toxic than chlorite but there is no known technology that can effectively remove chlorate ions from drinking water. Since free chlorine will react with chlorite in water to form chlorate when used after chlorine dioxide treatment it is important to remove the chlorite before free chlorine treatment. This could be achieved by using granular activated carbon absorption, powdered activated carbon, reverse osmosis membrane treatment or ferrous-iron reduction process (Gordon *et al.* 1995).

Under certain conditions, low concentrations of chlorine dioxide may be formed as a result of oxidation of chlorite by free chlorine giving a "kerosinelike" smell to the water. Monochloramine does not appear to react with chlorite, which avoids the potential odour problem and problem of chlorate formation (Liyanage *et al.* 1997b). Microorganism reduction with chlorine dioxide followed by monochloramine results in less formation of potentially toxic by-products and less odour problems. However, monochloramine is not as effective in inactivation as free chlorine and provides less additional kill due to synergy.

There are a number of advantages to using a combination of chlorine dioxide and free chlorine compared to traditional treatment processes or ozone. In the absence of bromine in raw waters at approximately neutral pH, pretreatment using chlorine dioxide followed by free chlorine has shown to reduce trihalomethane (THM) production (Rav-Acha *et al.* 1985). Some total organic halogens (TOX's) may be formed under these conditions though, so care must be taken (Liyanage *et al.* 1997b). Therefore, in addition to the synergistic effect observed in this project, sequential application of chlorine dioxide followed by free chlorine may result in lower THM and halogenated byproduct formation. Also, small systems that currently use chlorine can be retrofitted to include chlorine dioxide easily (Rav-Acha *et al.* 1985).

When ozone is used at water treatment plants it may produce harmful byproducts when bromide ion is present. If bromide ion is present in sufficient quantities and pH is sufficiently low, ozone can react with it to form halogenated by-products such as bromoform and dibromoacetic acid (Bryant *et al.* 1992). On the other hand, ozone pretreatment can remove iron and manganese, and taste and odour problems and reduce colour levels. Using ozone/free chlorine sequentially may not only decrease the dose required to obtain a certain level of inactivation but also improve certain water quality parameters.

## CONCLUSIONS

The new need for *Cryptosporidium* removal combined with the disinfection by-product rule results in great challenges for water treatment processes. Since chlorine has been shown to be a poor killer of *Cryptosporidium* oocysts new treatment methods and alternative microorganism reduction processes must be considered in order to meet new demands. Using sequential chemicals at treatment facilities may be a valuable alternative to conventional processes.

Alternative methods of microorganism reduction have been reported in literature and the inactivation of *C. parvum* has been studied extensively at room temperatures. Studies of sequential chemical exposure to *C. parvum* have been conducted, however,

there is very little information in literature on the effectiveness of sequential treatment at lower than room temperatures. This is of interest to more Northern communities, where treated source water rarely reaches these higher temperatures.

In addition, sequential inactivation experiments have been conducted fairly extensively for *C. parvum* in laboratory waters but no research has been reported to date in natural water. Several models have been developed for the inactivation kinetics of disinfectants using laboratory waters and it is important to determine if these models can accurately predict microorganism reduction kinetics in natural waters. This information is required in order to develop accurate microorganism reduction design criteria for full-scale treatment plants.

The results of the two studies conducted to determine the effects of sequential chemical treatment on *C. parvum* showed in general that:

- free chlorine was more effective in inactivating *C. parvum* oocysts than monochloramine at 22°C in laboratory water but not at 1 or 10°C;
- chlorine dioxide/free chlorine sequential treatment was more effective than chlorine dioxide/monochloramine sequential treatment at 22°C in laboratory water but not at 1 or 10°C;
- the level of inactivation increased with increased free chlorine Ct in lab water and modified natural water;
- the level of inactivation increased with monochloramine Ct in lab water;
- the level of inactivation and synergy increased with increased temperature for both chlorine dioxide/free chlorine and chlorine dioxide/monochloramine sequential treatment;
- the level of synergy was higher after low chlorine dioxide pretreatment compared to high chlorine dioxide pretreatment in lab water;
- the level of synergy increased with free chlorine Ct;

- there were no significant natural water effects on the inactivation of *C. parvum*;
- the I.g.H model developed for ozone inactivation in lab water adequately predicted the ozone inactivation of *C. parvum* in modified natural water at 22°C and pH 6 and pH 8.5;
- the I.g.H model developed for free chlorine did not give an adequate prediction of the free chlorine inactivation of *C. parvum* in modified natural water under the conditions tested;
- greater overall inactivation of *C. parvum* was observed in modified natural water at pH 6.0 compared to pH 8.5, and;
- the level of inactivation increased with free chlorine Ct at the same rate after high and low level ozone pretreatment in modified natural water, at pH 6.0 and 8.5.

More specifically in terms of synergistic effects the following conclusions were drawn:

- synergy was not observed for chlorine dioxide/free chlorine treatment in lab water at 1°C;
- potential synergistic effects were observed for chlorine dioxide/free chlorine treatment after optimal chlorine dioxide pretreatment and a free chlorine $C_{avg}t$ of 1500 and 2500 mg·min/L at 22 and 10°C, respectively in lab water;
- potential synergistic effects were observed for chlorine dioxide/monochloramine treatment after optimal chlorine dioxide pretreatment and a monochloramine $C_{avg}t$ of 600, 3000 and 4000 mg·min/L at 22, 10 and 1°C, respectively in lab water;
- synergy was not observed using ozone/free chlorine treatment in modified NSR water at pH 6.0 or pH 8.5 under the conditions tested.

Therefore, using chlorine dioxide/free chlorine or chlorine dioxide/monochloramine sequentially at higher temperatures may lower the dose of the chemicals required to obtain a certain inactivation of *C. parvum*.

## RECOMMENDATIONS

The effects of sequential chemical treatments on the inactivation of *C. parvum* oocysts found in this study require further investigation. This is for academic purposes, to better understand the inactivation of *C. parvum* by sequential chemical treatment and for practical purposes, to determine how sequential microorganism reduction can be best achieved in water treatment plants. For these purposes the following should be done:

- higher free chlorine and monochloramine Ct's should be applied to determine optimum levels for synergy;
- lower primary treatment levels should be applied to determine if a threshold concentration exits for ozone and what the threshold dose is for chlorine dioxide;
- more trials should be conducted to develop an accurate model for chlorine dioxide/free chlorine and chlorine dioxide/monochloramine sequential disinfection at different temperatures in lab water;
- more natural water trials should be conducted at high free chlorine Ct's (greater than 1500 mg·min/L) to determine if there is a synergistic effect at higher $C_{avg}t$'s;
- natural water experiments should be conducted at low temperatures;
- natural water experiments should be conducted using different disinfection combinations; and,
- natural water experiments should be conducted using a different water source with different water quality characteristics.

# CHAPTER 6

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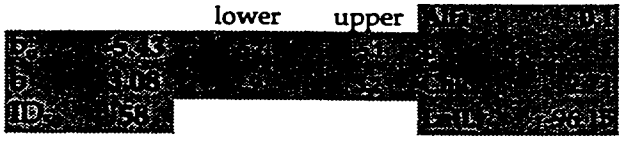
# APPENDIX A

# RAW DATA FOR DOSE RESPONSE TRIALS

## Table A.1a: Dose response modeling
## Batch of oocysts-34

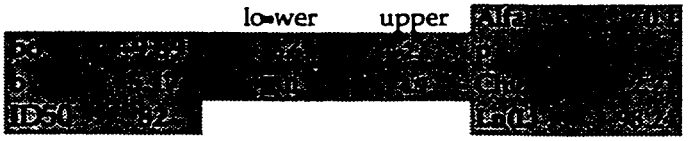



$$\pi' = Ln(\frac{P}{1-P}) = bo + bX$$

$$Ln(L) = \sum_{i=1}^{\alpha} Y_i(bo + bX) - \sum_{i=1}^{\alpha} Ln(1 + \exp(bo + bX))$$

| *Trial* | Dose | Cohort size | Infected | Log(it) | First Part | Second Part | If \|pi-p\| GT 0.25, data is not used | | |
|---|---|---|---|---|---|---|---|---|---|
| # | Xo | No | N | X | L1 | L2 | Pi | Pi-estimated | Diff |
| *747* | 50 | 10 | 6 | 1.70 | -1.16 | 6.01 | 0.60 | 0.45 | 0.15 |
| *747* | 100 | 10 | 9 | 2.00 | 6.61 | 11.26 | 0.90 | 0.68 | 0.22 |
| *747* | 200 | 10 | 7 | 2.30 | 11.63 | 18.35 | 0.70 | 0.84 | 0.14 |
| *747* | 400 | 10 | 10 | 2.60 | 25.89 | 26.61 | 1.00 | 0.93 | 0.07 |
| *762* | 25 | 10 | 2 | 1.40 | -2.24 | 2.82 | 0.20 | 0.25 | 0.05 |
| *762* | 50 | 10 | 4 | 1.70 | -0.77 | 6.01 | 0.40 | 0.45 | 0.05 |
| *762* | 100 | 10 | 4 | 2.00 | 0.00 | 0.00 | 0.40 | 0.68 | 0.28 |
| *762* | 200 | 10 | 9 | 2.30 | 14.95 | 18.35 | 0.90 | 0.84 | 0.06 |
| *772* | 25 | 10 | 2 | 1.40 | -2.24 | 2.82 | 0.20 | 0.25 | 0.05 |
| *772* | 50 | 10 | 7 | 1.70 | -1.35 | 6.01 | 0.70 | 0.45 | 0.25 |
| *772* | 100 | 10 | 8 | 2.00 | 5.87 | 11.26 | 0.80 | 0.68 | 0.12 |
| *772* | 200 | 10 | 10 | 2.30 | 16.61 | 18.35 | 1.00 | 0.84 | 0.16 |
| *773* | 25 | 10 | 7 | 1.40 | 0.00 | 0.00 | 0.70 | 0.25 | 0.45 |
| *773* | 50 | 10 | 4 | 1.70 | -0.77 | 6.01 | 0.40 | 0.45 | 0.05 |
| *773* | 100 | 10 | 7 | 2.00 | 5.14 | 11.26 | 0.70 | 0.68 | 0.02 |
| *773* | 200 | 10 | 6 | 2.30 | 9.97 | 18.35 | 0.60 | 0.84 | 0.24 |
| *798* | 25 | 5 | 3 | 1.40 | 0.00 | 0.00 | 0.60 | 0.25 | 0.35 |
| *798* | 50 | 5 | 4 | 1.70 | 0.00 | 0.00 | 0.80 | 0.45 | 0.35 |
| *798* | 100 | 5 | 5 | 2.00 | 0.00 | 0.00 | 1.00 | 0.68 | 0.32 |
| *798* | 200 | 5 | 5 | 2.30 | 8.31 | 9.18 | 1.00 | 0.84 | 0.16 |
| *807* | 25 | 10 | 1 | 1.40 | -1.12 | 2.82 | 0.10 | 0.25 | 0.15 |
| *807* | 50 | 10 | 1 | 1.70 | 0.00 | 0.00 | 0.10 | 0.45 | 0.35 |
| *807* | 100 | 10 | 5 | 2.00 | 3.67 | 11.26 | 0.50 | 0.68 | 0.18 |
| *807* | 200 | 10 | 6 | 2.30 | 9.97 | 18.35 | 0.60 | 0.84 | 0.24 |
| | | | | | | | | | |
| | 220 | | Sum | | -108.944 | -205.097315 | | | |

## Table A.1b: Dose response modeling
## Batch of oocysts-35




$$\pi' = Ln(\frac{P}{1-P}) = bo + bX$$

$$Ln(L) = \sum_{i=1}^{\alpha} Y_i(bo + bX) - \sum_{i=1}^{\alpha} Ln(1+\exp(bo+bX))$$

| *Trial* | Dose | Cohort size | Infected | Log(it) | First Part | Second Part | If \|pi-p\| GT 0.25, data is not used | | |
|---|---|---|---|---|---|---|---|---|---|
| # | Xo | No | N | X | L1 | L2 | Pi | Pi-estimate | Diff |
| *817* | 25 | 10 | 5 | 1.40 | 0.00 | 0.00 | 0.50 | 0.06 | 0.44 |
| *817* | 50 | 10 | 7 | 1.70 | 0.00 | 0.00 | 0.70 | 0.25 | 0.45 |
| *817* | 100 | 10 | 4 | 2.00 | 1.78 | 9.40 | 0.40 | 0.61 | 0.21 |
| *817* | 200 | 10 | 10 | 2.30 | 20.00 | 21.27 | 1.00 | 0.88 | 0.12 |
| *827* | 25 | 5 | 0 | 1.40 | 0.00 | 0.34 | 0.00 | 0.06 | 0.06 |
| *827* | 50 | 5 | 2 | 1.70 | -2.22 | 1.42 | 0.40 | 0.25 | 0.15 |
| *827* | 100 | 5 | 5 | 2.00 | 0.00 | 0.00 | 1.00 | 0.61 | 0.39 |
| *827* | 200 | 4 | 2 | 2.30 | 0.00 | 0.00 | 0.50 | 0.88 | 0.38 |
| *838* | 25 | 10 | 4 | 1.40 | 0.00 | 0.00 | 0.40 | 0.06 | 0.34 |
| *838* | 50 | 10 | 5 | 1.70 | -5.56 | 2.84 | 0.50 | 0.25 | 0.25 |
| *838* | 100 | 10 | 8 | 2.00 | 3.55 | 9.40 | 0.80 | 0.61 | 0.19 |
| *838* | 200 | 10 | 9 | 2.30 | 18.00 | 21.27 | 0.90 | 0.88 | 0.02 |
| *852* | 25 | 10 | 1 | 1.40 | -2.67 | 0.67 | 0.10 | 0.06 | 0.04 |
| *852* | 50 | 10 | 2 | 1.70 | -2.22 | 2.84 | 0.20 | 0.25 | 0.05 |
| *852* | 100 | 10 | 6 | 2.00 | 2.67 | 9.40 | 0.60 | 0.61 | 0.01 |
| *852* | 200 | 10 | 5 | 2.30 | 0.00 | 0.00 | 0.50 | 0.88 | 0.38 |
| *866* | 25 | 10 | 4 | 1.40 | 0.00 | 0.00 | 0.40 | 0.06 | 0.34 |
| *866* | 50 | 10 | 3 | 1.70 | -3.34 | 2.84 | 0.30 | 0.25 | 0.05 |
| *866* | 100 | 10 | 6 | 2.00 | 2.67 | 9.40 | 0.60 | 0.61 | 0.01 |
| *866* | 200 | 10 | 8 | 2.30 | 16.00 | 21.27 | 0.80 | 0.88 | 0.08 |
| *881* | 25 | 10 | 1 | 1.40 | -2.67 | 0.67 | 0.10 | 0.06 | 0.04 |
| *881* | 50 | 10 | 2 | 1.70 | -2.22 | 2.84 | 0.20 | 0.25 | 0.05 |
| *881* | 100 | 10 | 6 | 2.00 | 2.67 | 9.40 | 0.60 | 0.61 | 0.01 |
| *881* | 200 | 10 | 8 | 2.30 | 16.00 | 21.27 | 0.80 | 0.88 | 0.08 |
| *901* | 25 | 5 | 0 | 1.40 | 0.00 | 0.34 | 0.00 | 0.06 | 0.06 |
| *901* | 50 | 5 | 1 | 1.70 | -1.11 | 1.42 | 0.20 | 0.25 | 0.05 |
| *901* | 100 | 5 | 3 | 2.00 | 1.33 | 4.70 | 0.60 | 0.61 | 0.01 |
| *901* | 200 | 5 | 1 | 2.30 | 0.00 | 0.00 | 0.20 | 0.88 | 0.68 |
| *911* | 25 | 10 | 0 | 1.40 | 0.00 | 0.67 | 0.00 | 0.06 | 0.06 |
| *911* | 50 | 10 | 1 | 1.70 | -1.11 | 2.84 | 0.10 | 0.25 | 0.15 |
| *911* | 100 | 10 | 1 | 2.00 | 0.00 | 0.00 | 0.10 | 0.61 | 0.51 |
| *911* | 200 | 10 | 9 | 2.30 | 18.00 | 21.27 | 0.90 | 0.88 | 0.02 |
| | | | | | | | | | |
| | | 279 | Sum | | | | | | |

**Table A.2a: Calculation of log kill by the dose response**
**Batch No. 34**

| | Inoculum | | Cohort | | Infected | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Trial # | A | B | A | B | A | B | Pa | Pb | Log kill | Cencored |
| *757.1* | 100 | 1,000 | 5 | 5 | 1 | 4 | 0.2 | 0.8 | 0.74 | No |
| *757.2* | 2,000 | 20,000 | 5 | 5 | 4 | 5 | 0.8 | 1.0 | 1.09 | No |
| *757.3* | 10,000 | 100,000 | 5 | 5 | 0 | 4 | 0.0 | 0.8 | 2.79 | No |
| *758.1* | 50 | 500 | 5 | 5 | 1 | 4 | 0.2 | 0.8 | 0.44 | No |
| *758.2* | 100 | 1000 | 5 | 5 | 1 | 4 | 0.2 | 0.8 | 0.74 | No |
| *758.3* | 500 | 5000 | 5 | 5 | 4 | 5 | 0.8 | 1.0 | 0.49 | No |
| *758.4* | 5000 | 50000 | 5 | 5 | 5 | 5 | 1.0 | 1.0 | 1.49 | LT |
| *759.1* | 1000 | 10000 | 5 | 5 | 4 | 5 | 0.8 | 1.0 | 0.79 | No |
| *759.2* | 2,000 | 20,000 | 5 | 5 | 5 | 5 | 1.0 | 1.0 | 1.09 | LT |
| *759.3* | 10,000 | 100,000 | 5 | 5 | 5 | 5 | 1.0 | 1.0 | 1.79 | LT |
| *760.1* | 50 | 500 | 5 | 5 | 4 | 4 | 0.8 | 0.8 | -0.01 | No |
| *760.2* | 1000 | 10000 | 5 | 5 | 3 | 5 | 0.6 | 1.0 | 1.11 | No |
| *760.3* | 2,000 | 20,000 | 5 | 5 | 2 | 5 | 0.4 | 1.0 | 1.67 | No |
| *760.4* | 2,000 | 20,000 | 5 | 5 | 2 | 5 | 0.4 | 1.0 | 1.67 | No |
| *761.1* | 50 | 500 | 5 | 5 | 2 | 5 | 0.4 | 1.0 | 0.07 | No |
| *761.2* | 1,000 | 10,000 | 5 | 5 | 3 | 5 | 0.6 | 1.0 | 1.11 | No |
| *761.3* | 1,000 | 10,000 | 5 | 5 | 2 | 5 | 0.4 | 1.0 | 1.37 | No |
| *761.4* | 10,000 | 100,000 | 5 | 5 | 4 | 5 | 0.8 | 1.0 | 1.79 | No |
| *763.1* | 50 | 500 | 5 | 5 | 4 | 5 | 0.8 | 1.0 | -0.51 | No |
| *763.2* | 1,000 | 10,000 | 5 | 5 | 5 | 5 | 1.0 | 1.0 | 0.79 | LT |
| *763.3* | 5,000 | 50,000 | 5 | 5 | 5 | 5 | 1.0 | 1.0 | 1.49 | LT |
| *763.4* | 10,000 | 100,000 | 5 | 5 | 0 | 2 | 0.0 | 0.4 | 3.37 | No |
| *764.1* | 500 | 5,000 | 5 | 5 | 1 | 4 | 0.2 | 0.8 | 1.44 | No |
| *764.2* | 1,000 | 10,000 | 5 | 5 | 3 | 5 | 0.6 | 1.0 | 1.11 | No |
| *764.3* | 10,000 | 100,000 | 5 | 5 | 0 | 3 | 0.0 | 0.6 | 3.11 | No |
| *765.1* | 500 | 5,000 | 5 | 5 | 0 | 5 | 0.0 | 1.0 | 1.44 | No* |
| *765.2* | 500 | 5,000 | 5 | 5 | 1 | 3 | 0.2 | 0.6 | 1.60 | No |
| *765.3* | 5,000 | 50,000 | 5 | 5 | 4 | 5 | 0.8 | 1.0 | 1.49 | No |
| *766.1* | 500 | 5,000 | 5 | 5 | 1 | 4 | 0.2 | 0.8 | 1.44 | No |
| *766.2* | 1,000 | 10,000 | 5 | 5 | 4 | 5 | 0.8 | 1.0 | 0.79 | No |
| *766.3* | 2,000 | 20,000 | 5 | 5 | 4 | 5 | 0.8 | 1.0 | 1.09 | No |
| *767.1* | 50 | 500 | 5 | 5 | 4 | 5 | 0.8 | 1.0 | -0.51 | No |
| *767.2* | 500 | 5,000 | 5 | 5 | 1 | 5 | 0.2 | 1.0 | 1.39 | No |
| *767.3* | 1,000 | 10,000 | 5 | 4 | 3 | 4 | 0.6 | 1.0 | 1.11 | No |
| *767.4* | 2,000 | 20,000 | 5 | 5 | 5 | 5 | 1.0 | 1.0 | 1.09 | LT |
| *768.1* | 500 | 5,000 | 5 | 5 | 1 | 2 | 0.2 | 0.4 | 1.73 | No |
| *768.2* | 1,000 | 10,000 | 5 | 5 | 1 | 4 | 0.2 | 0.8 | 1.74 | No |
| *768.3* | 10,000 | 100,000 | 5 | 5 | 5 | 5 | 1.0 | 1.0 | 1.79 | LT |
| *769.1* | 50 | 500 | 5 | 5 | 4 | 5 | 0.8 | 1.0 | -0.51 | No |
| *769.2* | 500 | 5,000 | 5 | 5 | 0 | 4 | 0.0 | 0.8 | 1.49 | No |
| *769.3* | 500 | 5,000 | 5 | 5 | 1 | 5 | 0.2 | 1.0 | 1.39 | No |
| *769.4* | 5,000 | 50,000 | 4 | 5 | 3 | 5 | 0.8 | 1.0 | 1.58 | No |
| *770.1* | 100 | 1,000 | 5 | 5 | 3 | 5 | 0.6 | 1.0 | 0.11 | No |
| *770.2* | 500 | 5,000 | 5 | 5 | 4 | 4 | 0.8 | 0.8 | 0.99 | No |
| *770.3* | 5,000 | 50,000 | 5 | 5 | 5 | 5 | 1.0 | 1.0 | 1.49 | LT |
| *771.1* | 100 | 1,000 | 4 | 4 | 0 | 4 | 0.0 | 1.0 | 0.74 | No* |

**Table A.2a: Calculation of log kill by the dose response**
**Batch No. 34**

| | Inoculum | | Cohort | | Infected | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Trial # | A | B | A | B | A | B | Pa | Pb | Log kill | Cencored |
| *771.2* | 200 | 2,000 | 5 | 3 | 3 | 3 | 0.6 | 1.0 | 0.41 | No |
| *771.3* | 2,000 | 20,000 | 5 | 4 | 2 | 4 | 0.4 | 1.0 | 1.67 | No |
| *774.1* | 40 | 400 | 5 | 5 | 1 | 4 | 0.2 | 0.8 | 0.34 | No |
| *774.2* | 40 | 400 | 5 | 5 | 0 | 0 | 0.0 | 0.0 | 1.29 | GT |
| *775.1* | 500 | 5,000 | 5 | 5 | 1 | 4 | 0.2 | 0.8 | 1.44 | No |
| *775.2* | 10,000 | 100,000 | 5 | 5 | 0 | 0 | 0.0 | 0.0 | 3.69 | GT |
| *775.3* | 100,000 | 1,000,000 | 5 | 5 | 2 | 2 | 0.4 | 0.4 | 3.87 | No |
| *776.1* | 40 | 400 | 4 | 4 | 1 | 4 | 0.3 | 1.0 | 0.20 | No |
| *776.2* | 40 | 400 | 5 | 4 | 3 | 3 | 0.6 | 0.8 | 0.10 | No |
| *777.1* | 500 | 5,000 | 4 | 5 | 0 | 4 | 0.0 | 0.8 | 1.49 | No |
| *777.2* | 1,000 | 10,000 | 5 | 5 | 3 | 5 | 0.6 | 1.0 | 1.11 | No |
| *777.3* | 2,000 | 20,000 | 5 | 5 | 4 | 5 | 0.8 | 1.0 | 1.09 | No |
| *778.1* | 40 | 400 | 4 | 3 | 2 | 3 | 0.5 | 1.0 | -0.16 | No |
| *778.2* | 40 | 400 | 5 | 4 | 5 | 2 | 1.0 | 0.5 | 99.00 | Error |
| *779.1* | 500 | 5,000 | 5 | 4 | 3 | 3 | 0.6 | 0.8 | 1.19 | No |
| *779.2* | 1,000 | 10,000 | 4 | 5 | 3 | 4 | 0.8 | 0.8 | 1.33 | No |
| *779.3* | 5,000 | 500,000 | 5 | 5 | 1 | 5 | 0.2 | 1.0 | 2.39 | No |
| *780.1* | 40 | 400 | 5 | 5 | 1 | 4 | 0.2 | 0.8 | 0.34 | No |
| *780.2* | 40 | 400 | 5 | 5 | 0 | 0 | 0.0 | 0.0 | 1.29 | GT |
| *781.1* | 500 | 5,000 | 5 | 5 | 4 | 5 | 0.8 | 1.0 | 0.49 | No |
| *781.2* | 5,000 | 50,000 | 5 | 5 | 4 | 4 | 0.8 | 0.8 | 1.99 | No |
| *781.3* | 50,000 | 500,000 | 4 | 5 | 0 | 1 | 0.0 | 0.2 | 4.39 | No |
| *790.1* | 1,000 | 10,000 | 5 | 5 | 3 | 4 | 0.6 | 0.8 | 1.45 | No |
| *790.2* | 1,000 | 10,000 | 5 | 5 | 0 | 3 | 0.0 | 0.6 | 2.11 | No |
| *790.3* | 10,000 | 100,000 | 5 | 5 | 2 | 4 | 0.4 | 0.8 | 2.58 | No |
| *791.1* | 50 | 500 | 5 | 5 | 3 | 5 | 0.6 | 1.0 | -0.19 | No |
| *791.2* | 500 | 5,000 | 5 | 5 | 1 | 5 | 0.2 | 1.0 | 1.39 | No |
| *791.3* | 1,000 | 10,000 | 5 | 5 | 0 | 5 | 0.0 | 1.0 | 1.74 | No* |
| *791.4* | 100,000 | | 4 | | 4 | | 1.0 | | 2.88 | LT |
| *792.1* | 500 | 5,000 | 5 | 5 | 3 | 4 | 0.6 | 0.8 | 1.15 | No |
| *792.2* | 1,000 | 10,000 | 5 | 5 | 0 | 4 | 0.0 | 0.8 | 1.79 | No |
| *792.3* | 2,000 | 20,000 | 7 | 8 | 1 | 8 | 0.1 | 1.0 | 2.12 | No |
| *793.1* | 50 | 500 | 5 | 5 | 1 | 5 | 0.2 | 1.0 | 0.39 | No |
| *793.2* | 500 | 5,000 | 5 | 5 | 4 | 5 | 0.8 | 1.0 | 0.49 | No |
| *793.3* | 2,000 | 20,000 | 7 | 8 | 5 | 7 | 0.7 | 0.9 | 1.57 | No |
| *794.1* | 500 | 5,000 | 5 | 5 | 1 | 5 | 0.2 | 1.0 | 1.39 | No |
| *794.2* | 1,000 | 10,000 | 5 | 5 | 1 | 5 | 0.2 | 1.0 | 1.69 | No |
| *794.3* | 5,000 | 50,000 | 5 | 5 | 1 | 4 | 0.2 | 0.8 | 2.44 | No |
| *795.1* | 50 | 500 | 5 | 5 | 0 | 4 | 0.0 | 0.8 | 0.49 | No |
| *795.2* | 1,000 | 10,000 | 5 | 5 | 3 | 5 | 0.6 | 1.0 | 1.11 | No |
| *795.3* | 5,000 | 50,000 | 5 | 5 | 2 | 5 | 0.4 | 1.0 | 2.07 | No |
| *796.1* | 40 | 400 | 5 | 5 | 4 | 5 | 0.8 | 1.0 | -0.61 | No |
| *796.2* | 400 | 4,000 | 5 | 5 | 3 | 4 | 0.6 | 0.8 | 1.05 | No |
| *797.1* | 40 | 400 | 5 | 5 | 4 | 5 | 0.8 | 1.0 | -0.61 | No |
| *797.2* | 40 | 400 | 5 | 5 | 2 | 4 | 0.4 | 0.8 | 0.18 | No |
| *800.1* | 500 | 5,000 | 5 | 5 | 3 | 4 | 0.6 | 0.8 | 1.15 | No |
| *800.2* | 2,000 | 20,000 | 5 | 5 | 1 | 3 | 0.2 | 0.6 | 2.20 | No |

**Table A.2a: Calculation of log kill by the dose response**
**Batch No. 34**

| | Inoculum | | Cohort | | Infected | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Trial # | A | B | A | B | A | B | Pa | Pb | Log kill | Cencored |
| *800.3* | 10,000 | 100,000 | 5 | 5 | 3 | 5 | 0.6 | 1.0 | 2.11 | No |
| *801.1* | 50 | 500 | 5 | 5 | 1 | 5 | 0.2 | 1.0 | 0.39 | No |
| *801.2* | 200 | 2,000 | 5 | 5 | 0 | 3 | 0.0 | 0.6 | 1.41 | No |
| *801.3* | 500 | 5,000 | 5 | 5 | 1 | 5 | 0.2 | 1.0 | 1.39 | No |
| *801.4* | 5,000 | 50,000 | 5 | 4 | 4 | 4 | 0.8 | 1.0 | 1.49 | No |
| *802.1* | 500 | 5,000 | 5 | 5 | 1 | 4 | 0.2 | 0.8 | 1.44 | No |
| *802.2* | 1,000 | 10,000 | 5 | 5 | 1 | 3 | 0.2 | 0.6 | 1.90 | No |
| *802.3* | 2,000 | 20,000 | 8 | 7 | 3 | 6 | 0.4 | 0.9 | 1.83 | No |
| *803.1* | 50 | 500 | 5 | 5 | 1 | 5 | 0.2 | 1.0 | 0.39 | No |
| *803.2* | 500 | 5,000 | 5 | 5 | 1 | 5 | 0.2 | 1.0 | 1.39 | No |
| *803.3* | 2,000 | 20,000 | 5 | 5 | 1 | 4 | 0.2 | 0.8 | 2.04 | No |
| *804.1* | 100 | 1,000 | 5 | 5 | 2 | 5 | 0.4 | 1.0 | 0.37 | No |
| *804.2* | 500 | 5,000 | 5 | 5 | 4 | 5 | 0.8 | 1.0 | 0.49 | No |
| *804.3* | 1,000 | 10,000 | 5 | 5 | 5 | 5 | 1.0 | 1.0 | 0.79 | LT |
| *805.1* | 50 | 500 | 5 | 5 | 3 | 5 | 0.6 | 1.0 | -0.19 | No |
| *805.2* | 1,000 | 10,000 | 5 | 5 | 3 | 5 | 0.6 | 1.0 | 1.11 | No |
| *805.3* | 1,000 | 10,000 | 5 | 5 | 2 | 4 | 0.4 | 0.8 | 1.58 | No |
| *805.4* | 5,000 | 50,000 | 5 | 5 | 5 | 5 | 1.0 | 1.0 | 1.49 | LT |

**Table A.2b: Calculation of log kill by the dose response**

**Batch No. 35**

| | Inoculum | | Cohort | | Infected | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Trial # | A | B | A | B | A | B | Pa | Pb | Log kill | Cencored |
| *818.1* | 100 | 1,000 | 5 | 5 | 4 | 5 | 0.8 | 1 | -0.18 | No |
| *818.2* | 500 | 5,000 | 5 | 5 | 5 | 5 | 1 | 1 | 0.52 | LT |
| *818.3* | 2,000 | 20,000 | 5 | 5 | 5 | 5 | 1 | 1 | 1.12 | LT |
| *819.1* | 40 | 400 | 5 | 5 | 3 | 5 | 0.6 | 1 | -0.39 | No |
| *819.2* | 40 | 400 | 5 | 5 | 4 | 4 | 0.8 | 0.8 | -0.08 | No |
| *820.1* | 500 | 5000 | 5 | 5 | 4 | 5 | 0.8 | 1 | 0.52 | No |
| *820.2* | 500 | 5000 | 5 | 5 | 3 | 5 | 0.6 | 1 | 0.71 | No |
| *820.3* | 2000 | 20000 | 5 | 5 | 5 | 5 | 1 | 1 | 1.12 | LT |
| *821.1* | 100 | 1,000 | 5 | 5 | 4 | 5 | 0.8 | 1 | -0.18 | No |
| *821.2* | 1,000 | 10,000 | 5 | 5 | 5 | 5 | 1 | 1 | 0.82 | LT |
| *822.1* | 500 | 5000 | 5 | 5 | 5 | 4 | 1 | 0.8 | 99.00 | Error |
| *822.2* | 2,000 | 20,000 | 5 | 5 | 5 | 5 | 1 | 1 | 1.12 | LT |
| *822.3* | 20,000 | 200,000 | 5 | 5 | 5 | 5 | 1 | 1 | 2.12 | LT |
| *823.1* | 40 | 400 | 5 | 5 | 0 | 4 | 0 | 0.8 | 0.42 | No |
| *824.1* | 40 | 400 | 5 | 5 | 3 | 5 | 0.6 | 1 | -0.39 | No |
| *825.1* | 100 | 1,000 | 5 | 5 | 2 | 4 | 0.4 | 0.8 | 0.49 | No |
| *825.2* | 2,000 | 20,000 | 5 | 5 | 5 | 5 | 1 | 1 | 1.12 | LT |
| *825.3* | 10,000 | 100,000 | 5 | 5 | 5 | 5 | 1 | 1 | 1.82 | LT |
| *826.1* | 40 | 400 | 5 | 5 | 3 | 5 | 0.6 | 1 | -0.39 | No |
| *826.2* | 40 | 400 | 5 | 5 | 4 | 4 | 0.8 | 0.8 | -0.08 | No |
| *841.1* | 1,000 | 10,000 | 5 | 5 | 0 | 2 | 0 | 0.4 | 2.16 | No |
| *841.2* | 10,000 | 100,000 | 5 | 5 | 0 | 4 | 0 | 0.8 | 2.82 | No |
| *842.1* | 100 | 1,000 | 5 | 5 | 4 | 5 | 0.8 | 1 | -0.18 | No |
| *843.1* | 100 | 1,000 | 5 | 5 | 5 | 5 | 1 | 1 | -0.18 | LT |
| *843.2* | 100 | 1,000 | 5 | 5 | 3 | 5 | 0.6 | 1 | 0.01 | No |
| *844.1* | 1,000 | 10,000 | 5 | 5 | 2 | 5 | 0.4 | 1 | 1.16 | No |
| *844.2* | 10,000 | 100,000 | 5 | 5 | 4 | 5 | 0.8 | 1 | 1.82 | No |
| *845.1* | 100 | 1,000 | 5 | 5 | 1 | 5 | 0.2 | 1 | 0.35 | No |
| *846.1* | 100 | 1,000 | 5 | 5 | 2 | 5 | 0.4 | 1 | 0.16 | No |
| *846.2* | 100 | 1,000 | 5 | 5 | 3 | 5 | 0.6 | 1 | 0.01 | No |
| *847.1* | 1,000 | 10,000 | 5 | 5 | 3 | 5 | 0.6 | 1 | 1.01 | No |
| *847.2* | 1,000 | 10,000 | 5 | 5 | 1 | 4 | 0.2 | 0.8 | 1.59 | No |
| *848.1* | 100 | 1,000 | 5 | 5 | 3 | 5 | 0.6 | 1 | 0.01 | No |
| *849.1* | 100 | 1,000 | 5 | 5 | 2 | 5 | 0.4 | 1 | 0.16 | No |
| *849.2* | 100 | 1,000 | 5 | 5 | 4 | 4 | 0.8 | 0.8 | 0.32 | No |
| *850.1* | 1,000 | 10,000 | 5 | 5 | 0 | 2 | 0 | 0.4 | 2.16 | No |
| *850.2* | 1,000 | 10,000 | 5 | 5 | 0 | 4 | 0 | 0.8 | 1.82 | No |
| *851.1* | 100 | 1,000 | 5 | 5 | 2 | 5 | 0.4 | 1 | 0.16 | No |
| *855.1* | 100 | 1,000 | 5 | 5 | 2 | 5 | 0.4 | 1 | 0.16 | No |
| *855.2* | 1,000 | 10,000 | 4 | 5 | 3 | 5 | 0.75 | 1 | 0.87 | No |
| *856* | 100 | 1,000 | 5 | 5 | 3 | 5 | 0.6 | 1 | 0.01 | No |
| *857.1* | 60 | 600 | 5 | 5 | 4 | 5 | 0.8 | 1 | -0.40 | No |
| *857.2* | 60 | 600 | 5 | 5 | 2 | 4 | 0.4 | 0.8 | 0.27 | No |
| *858.1* | 100 | 1,000 | 5 | 5 | 0 | 4 | 0 | 0.8 | 0.82 | No |
| *858.2* | 1,000 | 10,000 | 5 | 5 | 5 | 5 | 1 | 1 | 0.82 | LT |
| *859* | 100 | 1,000 | 4 | 5 | 1 | 5 | 0.25 | 1 | 0.30 | No |
| *860.1* | 60 | 600 | 5 | 5 | 2 | 4 | 0.4 | 0.8 | 0.27 | No |
| *860.2* | 60 | 600 | 5 | 5 | 5 | 5 | 1 | 1 | -0.40 | LT |
| *869.1* | 1,000 | 10,000 | 5 | 5 | 1 | 3 | 0.2 | 0.6 | 1.68 | No |
| *869.2* | 10,000 | 100,000 | 5 | 5 | 2 | 5 | 0.4 | 1 | 2.16 | No |
| *870* | 1,000 | 10,000 | 5 | 5 | 2 | 5 | 0.4 | 1 | 1.16 | No |
| *871.1* | 50 | 500 | 4 | 4 | 1 | 4 | 0.25 | 1 | 0.00 | No |
| *871.2* | 50 | 500 | 5 | 5 | 4 | 5 | 0.8 | 1 | -0.48 | No |
| *872.1* | 1,000 | 10,000 | 5 | 5 | 1 | 2 | 0.2 | 0.4 | 1.76 | No |
| *872.2* | 10,000 | 100,000 | 4 | 5 | 1 | 5 | 0.25 | 1 | 2.30 | No |

**Table A.2b: Calculation of log kill by the dose response**

**Batch No. 35**

| | Inoculum | | Cohort | | Infected | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Trial # | A | B | A | B | A | B | Pa | Pb | Log kill | Cencored |
| *873* | 1,000 | 10,000 | 5 | 5 | 4 | 5 | 0.8 | 1 | 0.82 | No |
| *874.1* | 50 | 500 | 5 | 5 | 2 | 5 | 0.4 | 1 | -0.14 | No |
| *874.2* | 50 | 500 | 5 | 5 | 1 | 5 | 0.2 | 1 | 0.05 | No |
| *875.1* | 1,000 | 10,000 | 5 | 5 | 0 | 3 | 0 | 0.6 | 2.01 | No |
| *875.2* | 10,000 | 100,000 | 5 | 5 | 0 | 0 | 0 | 0 | 3.35 | GT |
| *876* | 1,000 | 10,000 | 5 | 5 | 4 | 5 | 0.8 | 1 | 0.82 | No |
| *877.1* | 50 | 500 | 5 | 5 | 2 | 4 | 0.4 | 0.8 | 0.19 | No |
| *877.2* | 50 | 500 | 5 | 5 | 5 | 4 | 1 | 0.8 | 99.00 | Error |
| *878.1* | 1,000 | 10,000 | 5 | 5 | 0 | 1 | 0 | 0.2 | 2.35 | No |
| *878.2* | 10,000 | 100,000 | 5 | 5 | 0 | 1 | 0 | 0.2 | 3.35 | No |
| *879* | 1,000 | 10,000 | 5 | 5 | 4 | 5 | 0.8 | 1 | 0.82 | No |
| *880.1* | 50 | 500 | 5 | 5 | 5 | 1 | 1 | 0.2 | 99.00 | Error |
| *880.2* | 50 | 500 | 5 | 5 | 2 | 5 | 0.4 | 1 | -0.14 | No |
| *912.1* | 100 | 1,000 | 5 | 5 | 0 | 4 | 0 | 0.8 | 0.82 | No |
| *912.2* | 10,000 | 100,000 | 5 | 5 | 1 | 5 | 0.2 | 1 | 2.35 | No |
| *913* | 100 | 1,000 | 5 | 5 | 0 | 1 | 0 | 0.2 | 1.35 | No |
| *914* | 50 | 500 | 5 | 5 | 2 | 4 | 0.4 | 0.8 | 0.19 | No |
| *915.1* | 1,000 | 10,000 | 5 | 5 | 0 | 1 | 0 | 0.2 | 2.35 | No |
| *915.2* | 10,000 | 100,000 | 5 | 5 | 0 | 4 | 0 | 0.8 | 2.82 | No |
| *916* | 100 | 1,000 | 5 | 5 | 1 | 4 | 0.2 | 0.8 | 0.59 | No |
| *917* | 50 | 500 | 5 | 5 | 0 | 3 | 0 | 0.6 | 0.71 | No |
| *918.1* | 100 | 1,000 | 5 | 5 | 0 | 4 | 0 | 0.8 | 0.82 | No |
| *918.2* | 1,000 | 10,000 | 5 | 5 | 2 | 5 | 0.4 | 1 | 1.16 | No |
| *919* | 100 | 1,000 | 5 | 5 | 0 | 4 | 0 | 0.8 | 0.82 | No |
| *920* | 50 | 500 | 5 | 5 | 0 | 4 | 0 | 0.8 | 0.52 | No |
| *921.1* | 3,000 | 30,000 | 5 | 5 | 1 | 3 | 0.2 | 0.6 | 2.16 | No |
| *921.2* | 10,000 | 100,000 | 5 | 5 | 0 | 4 | 0 | 0.8 | 2.82 | No |
| *922* | 100 | 1,000 | 5 | 5 | 2 | 4 | 0.4 | 0.8 | 0.49 | No |

## APPENDIX B

## RAW DATA FOR MICROORAGANISM REDUCTION TRIALS

**Table B.1: Experimental plan for chlorine dioxide followed by free chlorine**

| | | | Chlorine Dioxide Pretreatment | | | | | Free Chlorine Treatment | | | | | Total | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Trial No. | Temp | pH | Co' | Cf' | t' | k' | Logkill A | Co_B | Cf_B | t_B | k'_B | Logkill B | Add log kill | Obs log kill | censored |
| i | °C | | mg/L | mg/L | min | $min^{-1}$ | log-units | mg/L | mg/L | min | $min^{-1}$ | log-units | log-units | log-units | |
| *759.1* | *1* | *6* | 2.38 | 1.91 | 240 | 0.0009 | 1.7 | | | | | | 1.7 | 0.79 | No |
| *759.2* | *1* | *6* | 2.38 | 1.91 | 240 | 0.0009 | 1.7 | 2.90 | 2.80 | 243 | 0.000 | 0.1 | 1.8 | 1.09 | LT |
| *759.3* | *1* | *6* | 2.38 | 1.91 | 240 | 0.0009 | 1.7 | 2.90 | 2.50 | 900 | 0.000 | 0.1 | 1.8 | 1.79 | LT |
| *760.1* | *1* | *6* | Control | | | | | | | | | | | -0.01 | No |
| *760.2* | *1* | *6* | 2.5 | 2.46 | 240 | 0.0001 | 1.7 | | | | | | 1.7 | 1.11 | No |
| *760.3* | *1* | *6* | 2.5 | 2.46 | 240 | 0.0001 | 1.7 | 0.80 | 0.20 | 457 | 0.003 | 0.1 | 1.7 | 1.67 | No |
| *760.4* | *1* | *6* | 2.5 | 2.46 | 240 | 0.0001 | 1.7 | 0.80 | 0.20 | 936 | 0.001 | 0.1 | 1.8 | 1.67 | No |
| *766.1* | *1* | *6* | 2.65 | 1.99 | 240 | 0.0012 | 1.7 | | | | | | 1.7 | 1.44 | No |
| *766.2* | *1* | *6* | 2.65 | 1.99 | 240 | 0.0012 | 1.7 | 3.00 | 2.92 | 240 | 0.000 | 0.1 | 1.8 | 0.79 | No |
| *766.3* | *1* | *6* | 2.65 | 1.99 | 240 | 0.0012 | 1.7 | 3.00 | 2.54 | 960 | 0.000 | 0.1 | 1.8 | 1.09 | No |
| *767.1* | *1* | *6* | Control | | | | | | | | | | | -0.51 | No |
| *767.2* | *1* | *6* | 2.65 | 2.17 | 240 | 0.0008 | 1.7 | | | | | | 1.7 | 1.39 | No |
| *767.3* | *1* | *6* | 2.65 | 2.17 | 240 | 0.0008 | 1.7 | 1.39 | 0.70 | 450 | 0.002 | 0.1 | 1.7 | 1.11 | No |
| *767.4* | *1* | *6* | 2.65 | 2.17 | 240 | 0.0008 | 1.7 | 1.39 | 0.68 | 960 | 0.001 | 0.1 | 1.8 | 1.28 | LT |
| *777.1* | *1* | *6* | 2.54 | 2.09 | 240 | 0.0008 | 1.7 | | | | | | 1.7 | 1.49 | No |
| *777.2* | *1* | *6* | 2.54 | 2.09 | 240 | 0.0008 | 1.7 | 4.19 | 4.01 | 486 | 0.000 | 0.1 | 1.8 | 1.11 | No |
| *777.3* | *1* | *6* | 2.54 | 2.09 | 240 | 0.0008 | 1.7 | 4.19 | 3.91 | 960 | 0.000 | 0.1 | 1.8 | 1.09 | No |
| *827.1* | *1* | *6* | 1.91 | 1.67 | 120 | 0.0011 | 0.7 | | | | | | 1.7 | 0.49 | No |
| *827.2* | *1* | *6* | 1.91 | 1.67 | 120 | 0.0011 | 0.7 | 4.50 | 4.81 | 480 | 0.000 | 0.1 | 1.8 | 0.79 | LT |
| *827.3* | *1* | *6* | 1.91 | 1.67 | 120 | 0.0011 | 0.7 | 4.50 | 4.58 | 960 | 0.000 | 0.1 | 1.8 | 1.49 | No |
| *761.1* | *10* | *6* | Control | | | | | | | | | | | 0.07 | No |
| *761.2* | *10* | *6* | 1.71 | 1.63 | 180 | 0.0003 | 1.7 | | | | | | 1.7 | 1.11 | No |
| *761.3* | *10* | *6* | 1.71 | 1.63 | 180 | 0.0003 | 1.7 | 2.78 | 2.58 | 225 | 0.000 | 0.1 | 1.8 | 1.37 | No |
| *761.4* | *10* | *6* | 1.71 | 1.63 | 180 | 0.0003 | 1.7 | 2.78 | 2.40 | 945 | 0.000 | 0.3 | 1.9 | 1.79 | No |
| *768.1* | *10* | *6* | 1.75 | 1.39 | 180 | 0.0013 | 1.7 | | | | | | 1.7 | 1.73 | No |
| *768.2* | *10* | *6* | 1.75 | 1.39 | 180 | 0.0013 | 1.7 | 3.49 | 3.37 | 225 | 0.000 | 0.1 | 1.8 | 1.74 | No |
| *768.3* | *10* | *6* | 1.75 | 1.39 | 180 | 0.0013 | 1.7 | 3.49 | 3.22 | 970 | 0.000 | 0.3 | 1.9 | 1.79 | LT |
| *769.1* | *10* | *6* | Control | | | | | | | | | | | -0.51 | No |
| *769.2* | *10* | *6* | 1.75 | 1.58 | 180 | 0.0006 | 1.7 | | | | | | 1.7 | 1.49 | No |
| *769.3* | *10* | *6* | 1.75 | 1.58 | 180 | 0.0006 | 1.7 | 1.23 | 1.11 | 210 | 0.000 | 0.1 | 1.7 | 1.39 | No |
| *769.4* | *10* | *6* | 1.75 | 1.58 | 180 | 0.0006 | 1.7 | 1.23 | 0.76 | 960 | 0.001 | 0.2 | 1.8 | 1.58 | No |
| *779.1* | *10* | *6* | 1.96 | 1.42 | 180 | 0.0018 | 1.7 | | | | | | 1.7 | 1.19 | No |
| *779.2* | *10* | *6* | 1.96 | 1.42 | 180 | 0.0018 | 1.7 | 4.31 | 4.05 | 514 | 0.000 | 0.1 | 1.8 | 1.33 | No |
| *779.3* | *10* | *6* | 1.96 | 1.42 | 180 | 0.0018 | 1.7 | 4.31 | 4.01 | 960 | 0.000 | 0.2 | 2.0 | 2.39 | No |
| *770.1* | *10* | *6* | 1.3 | 1.16 | 90 | 0.0013 | 0.7 | | | | | | 0.7 | 0.11 | No |
| *770.2* | *10* | *6* | 1.3 | 1.16 | 90 | 0.0013 | 0.7 | 4.33 | 4.15 | 230 | 0.000 | 0.1 | 0.9 | 0.99 | No |
| *770.3* | *10* | *6* | 1.3 | 1.16 | 90 | 0.0013 | 0.7 | 4.33 | 4.03 | 960 | 0.000 | 0.3 | 1.0 | 1.49 | LT |
| *771.1* | *10* | *6* | 1.3 | 1.18 | 90 | 0.0011 | 0.7 | | | | | | 0.7 | 0.74 | No* |
| *771.2* | *10* | *6* | 1.3 | 1.18 | 90 | 0.0011 | 0.7 | 1.39 | 0.90 | 426 | 0.001 | 0.1 | 0.9 | 0.41 | No |
| *771.3* | *10* | *6* | 1.3 | 1.18 | 90 | 0.0011 | 0.7 | 1.39 | 0.80 | 960 | 0.001 | 0.2 | 0.9 | 1.67 | No |
| *763.1* | *22* | *6* | control | | | | | | | | | | | -0.51 | No |
| *763.2* | *22* | *6* | 1.36 | 1.15 | 90 | 0.0019 | 1.7 | | | | | | 1.7 | 0.79 | LT |
| *763.3* | *22* | *6* | 1.36 | 1.15 | 90 | 0.0019 | 1.7 | 3.06 | 2.78 | 220 | 0.000 | 0.4 | 2.1 | 1.49 | LT |
| *763.4* | *22* | *6* | 1.36 | 1.15 | 90 | 0.0019 | 1.7 | 3.06 | 2.36 | 950 | 0.000 | 0.8 | 2.5 | 3.37 | No |
| *764.1* | *22* | *6* | 1.42 | 1.16 | 90 | 0.0022 | 1.7 | | | | | | 1.7 | 1.44 | No |
| *764.2* | *22* | *6* | 1.42 | 1.16 | 90 | 0.0022 | 1.7 | 2.66 | 2.44 | 270 | 0.000 | 0.4 | 2.1 | 1.11 | No |
| *764.3* | *22* | *6* | 1.42 | 1.16 | 90 | 0.0022 | 1.7 | 2.66 | 2.20 | 940 | 0.000 | 0.8 | 2.5 | 3.11 | No |
| *765.1* | *22* | *6* | 1.42 | 1.39 | 90 | 0.0002 | 1.7 | | | | | | 1.7 | 1.44 | No* |
| *765.2* | *22* | *6* | 1.42 | 1.39 | 90 | 0.0002 | 1.7 | 0.95 | 0.64 | 255 | 0.002 | 0.2 | 2.0 | 1.60 | No |
| *765.3* | *22* | *6* | 1.42 | 1.39 | 90 | 0.0002 | 1.7 | 0.95 | 0.40 | 920 | 0.001 | 0.4 | 2.2 | 1.67 | No |
| *775.1* | *22* | *6* | 1.41 | 1.17 | 90 | 0.0021 | 1.7 | | | | | | 1.7 | 1.44 | No |
| *775.2* | *22* | *6* | 1.41 | 1.17 | 90 | 0.0021 | 1.7 | 4.09 | 3.71 | 495 | 0.000 | 0.2 | 2.5 | 3.69 | GT |
| *775.3* | *22* | *6* | 1.41 | 1.17 | 90 | 0.0021 | 1.7 | 4.09 | 3.53 | 960 | 0.000 | 0.4 | 3.5 | 3.87 | No |
| *757.1* | *22* | *6* | 0.95 | 0.84 | 45 | 0.0027 | 0.7 | | | | | | 0.7 | 0.74 | No |
| *757.2* | *22* | *6* | 0.95 | 0.84 | 45 | 0.0027 | 0.7 | 2.80 | 2.50 | 240 | 0.000 | 0.4 | 1.1 | 1.09 | No |
| *757.3* | *22* | *6* | 0.95 | 0.84 | 45 | 0.0027 | 0.7 | 2.80 | 2.10 | 940 | 0.000 | 0.7 | 1.4 | 2.79 | No |
| *758.1* | *22* | *6* | control | | | | | | | | | | | 0.44 | No |
| *758.2* | *22* | *6* | 0.95 | 0.84 | 45 | 0.0027 | 0.7 | | | | | | 0.7 | 0.74 | No |
| *758.3* | *22* | *6* | 0.95 | 0.84 | 45 | 0.0027 | 0.7 | 1.10 | 1.00 | 240 | 0.000 | 0.2 | 0.9 | 0.49 | No |
| *758.4* | *22* | *6* | 0.95 | 0.84 | 45 | 0.0027 | 0.7 | 1.10 | 0.70 | 950 | 0.000 | 0.3 | 1.1 | 1.49 | LT |
| *781.1* | *22* | *6* | 0.95 | 0.80 | 45 | 0.0038 | 0.7 | | | | | | 0.7 | 0.49 | No |
| *781.2* | *22* | *6* | 0.95 | 0.80 | 45 | 0.0038 | 0.7 | 4.76 | 4.07 | 460 | 0.000 | 0.4 | 2.0 | 1.99 | No |
| *781.3* | *22* | *6* | 0.95 | 0.80 | 45 | 0.0038 | 0.7 | 4.76 | 3.97 | 985 | 0.000 | 0.7 | 3.0 | 4.39 | No |
| *822.1* | *22* | *6* | 0.95 | 0.80 | 45 | | | 4.76 | 3.97 | 985 | | 0.0 | | 1.12 | LT |
| *822.2* | *22* | *6* | 0.95 | 0.80 | 45 | | | 4.76 | 3.97 | 985 | 0.000 | 0.0 | | 2.12 | LT |
| *825.1* | *22* | *6* | 0.86 | 0.72 | 45 | 0.0003 | 0.7 | | | | | | 0.7 | 0.49 | No |
| *825.2* | *22* | *6* | 0.86 | 0.72 | 45 | 0.0003 | 0.7 | 2.40 | 1.74 | 240 | 0.000 | 0.1 | 0.8 | 1.12 | LT |
| *825.3* | *22* | *6* | 0.86 | 0.72 | 45 | 0.0003 | 0.7 | 2.40 | 1.63 | 960 | 0.000 | 0.1 | 0.8 | 1.82 | LT |

**Table B.2: Experimental plan for chlorine dioxide followed by monochloramine**

| | | | Chlorine Dioxide Pretreatment | | | | | Monochloramine Treatment | | | | | Total | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Trial No. i | Temp °C | pH | Co' mg/L | Cf' mg/L | t' min | k' $min^{-1}$ | Logkill A log-units | Co_B mg/L | Cf_B mg/L | t_B min | k'_B $min^{-1}$ | Logkill B log-units | Add log kill log-units | Obs log kill log-units | censored |
| 792.1 | 1 | 8 | 2.6 | 2.00 | 240 | 0.0011 | 1.7 | | | | | | 1.7 | 1.15 | No |
| 792.2 | 1 | 8 | 2.6 | 2.00 | 240 | 0.0011 | 1.7 | 4.78 | 4.72 | 495 | 0.000 | 0.1 | 1.8 | 1.79 | No |
| 792.3 | 1 | 8 | 2.6 | 2.00 | 240 | 0.0011 | 1.7 | 4.78 | 4.62 | 965 | 0.000 | 0.1 | 1.8 | 2.12 | No |
| 793.1 | 1 | 8 | Control | | | | | | | | | | | 0.39 | No |
| 793.2 | 1 | 8 | 2.6 | 2.14 | 240 | 0.0008 | 1.7 | | | | | | 1.7 | 0.49 | No |
| 793.3 | 1 | 8 | 2.6 | 2.14 | 240 | 0.0008 | 1.7 | 3.20 | 3.10 | 960 | 0.000 | 0.1 | 1.8 | 1.57 | No |
| 802.1 | 1 | 8 | 2.64 | 2.09 | 240 | 0.0010 | 1.7 | | | | | | 1.7 | 1.44 | No |
| 802.2 | 1 | 8 | 2.64 | 2.09 | 240 | 0.0010 | 1.7 | 4.70 | 4.64 | 470 | 0.000 | 0.1 | 1.8 | 1.90 | No |
| 802.3 | 1 | 8 | 2.64 | 2.09 | 240 | 0.0010 | 1.7 | 4.70 | 4.41 | 985 | 0.000 | 0.1 | 1.8 | 1.83 | No |
| 803.1 | 1 | 8 | Control | | | | | | | | | | | 0.39 | No |
| 803.2 | 1 | 8 | 2.64 | 2.51 | 240 | 0.0002 | 1.7 | | | | | | 1.7 | 1.39 | No |
| 803.3 | 1 | 8 | 2.64 | 2.51 | 240 | 0.0002 | 1.7 | 3.22 | 3.02 | 960 | 0.000 | 0.1 | 1.8 | 2.04 | No |
| 810.1 | 1 | 8 | 1.91 | 1.67 | 120 | 0.0011 | 0.7 | | | | | | 1.7 | 0.49 | No |
| 810.2 | 1 | 8 | 1.91 | 1.67 | 120 | 0.0011 | 0.7 | 4.86 | 4.81 | 465 | 0.000 | 0.1 | 1.8 | 0.79 | LT |
| 810.3 | 1 | 8 | 1.91 | 1.67 | 120 | 0.0011 | 0.7 | 4.86 | 4.58 | 990 | 0.000 | 0.1 | 1.8 | 1.49 | No |
| 811.1 | 1 | 8 | Control | | | | | | | | | | | 0.39 | No |
| 811.2 | 1 | 8 | 1.91 | 1.76 | 120 | 0.0007 | 0.7 | | | | | | 1.7 | 0.49 | LT |
| 811.3 | 1 | 8 | 1.91 | 1.76 | 120 | 0.0007 | 0.7 | 3.29 | 2.96 | 980 | 0.000 | 0.1 | 1.8 | 1.09 | LT |
| 820.1 | 1 | 8 | 1.91 | 1.67 | 120 | 0.0011 | 0.7 | | | | | | 1.7 | 0.52 | No |
| 820.2 | 1 | 8 | 1.91 | 1.67 | 120 | 0.0011 | 0.7 | 4.50 | 4.81 | 480 | 0.000 | 0.1 | 1.8 | 0.71 | LT |
| 820.3 | 1 | 8 | 1.91 | 1.67 | 120 | 0.0011 | 0.7 | 4.50 | 4.58 | 960 | 0.000 | 0.1 | 1.8 | 1.12 | No |
| 821.1 | 1 | 8 | Control | | | | | | | | | | | -0.18 | No |
| 821.2 | 1 | 8 | 1.91 | 1.76 | 120 | 0.0007 | 0.7 | | | | | | 1.7 | 0.00 | LT |
| 821.3 | 1 | 8 | 1.91 | 1.76 | 120 | 0.0007 | 0.7 | 2.98 | 2.96 | 960 | 0.000 | 0.1 | 1.8 | 0.82 | LT |
| 794.1 | 10 | 8 | 1.79 | 1.15 | 180 | 0.0025 | 1.7 | | | | | | 1.7 | 1.39 | No |
| 794.2 | 10 | 8 | 1.79 | 1.15 | 180 | 0.0025 | 1.7 | 4.64 | 4.60 | 480 | 0.000 | 0.1 | 1.8 | 1.69 | No |
| 794.3 | 10 | 8 | 1.79 | 1.15 | 180 | 0.0025 | 1.7 | 4.64 | 4.41 | 965 | 0.000 | 0.3 | 1.9 | 2.44 | No |
| 795.1 | 10 | 8 | Control | | | | | | | | | | | 0.49 | No |
| 795.2 | 10 | 8 | 1.79 | 1.50 | 180 | 0.0010 | 1.7 | | | | | | 1.7 | 1.11 | No |
| 795.3 | 10 | 8 | 1.79 | 1.50 | 180 | 0.0010 | 1.7 | 3.18 | 3.06 | 960 | 0.000 | 0.2 | 1.8 | 2.07 | No |
| 804.1 | 10 | 8 | 1.52 | 0.80 | 180 | 0.0036 | 1.7 | | | | | | | 0.37 | No |
| 804.2 | 10 | 8 | 1.52 | 0.80 | 180 | 0.0036 | 1.7 | 4.51 | 4.48 | 480 | 0.000 | 0.1 | | 0.49 | No |
| 804.3 | 10 | 8 | 1.52 | 0.80 | 180 | 0.0036 | 1.7 | 4.51 | 4.43 | 970 | 0.000 | 0.3 | | 0.79 | LT |
| 805.1 | 10 | 8 | Control | | | | | | | | | | | -0.19 | No |
| 805.2 | 10 | 8 | 1.68 | 1.14 | 180 | 0.0022 | | | | | | | | 1.11 | No |
| 805.3 | 10 | 8 | 1.68 | 1.14 | 180 | 0.0022 | 1.7 | 3.06 | 3.04 | 480 | 0.000 | | 1.7 | 1.58 | No |
| 805.4 | 10 | 8 | 1.68 | 1.14 | 180 | 0.0022 | 1.7 | 3.06 | 2.94 | 960 | 0.000 | 0.2 | 1.8 | 1.49 | LT |
| 812.1 | 10 | 8 | 1.38 | 1.23 | 90 | 0.0013 | 0.7 | | | | | | | 0.58 | No |
| 812.2 | 10 | 8 | 1.38 | 1.23 | 90 | 0.0013 | 0.7 | 4.79 | 4.76 | 460 | 0.000 | 0.1 | | 1.07 | No |
| 812.3 | 10 | 8 | 1.38 | 1.23 | 90 | 0.0013 | 0.7 | 4.79 | 4.66 | 975 | 0.000 | 0.3 | | 1.09 | LT |
| 813.1 | 10 | 8 | Control | | | | | | | | | | | -0.01 | No |
| 813.2 | 10 | 8 | 1.5 | 1.44 | 90 | 0.0005 | 0.7 | | | | | | | 0.79 | No |
| 813.3 | 10 | 8 | 1.5 | 1.44 | 90 | 0.0005 | 0.7 | 1.53 | 1.51 | 450 | 0.000 | 0.1 | 1.7 | 0.75 | No |
| 813.4 | 10 | 8 | 1.5 | 1.44 | 90 | 0.0005 | 0.7 | 1.53 | 1.17 | 960 | 0.000 | 0.2 | 1.8 | 1.09 | LT |
| 790.1 | 22 | 8 | 1.33 | 0.95 | 90 | 0.0037 | 1.7 | | | | | | 1.7 | 1.45 | No |
| 790.2 | 22 | 8 | 1.33 | 0.95 | 90 | 0.0037 | 1.7 | 4.78 | 4.58 | 435 | 0.000 | 0.4 | 2.1 | 2.11 | No |
| 790.3 | 22 | 8 | 1.33 | 0.95 | 90 | 0.0037 | 1.7 | 4.78 | 4.39 | 930 | 0.000 | 0.8 | 2.5 | 2.58 | No |
| 791.1 | 22 | 8 | Control | | | | | | | | | | | -0.19 | No |
| 791.2 | 22 | 8 | 1.33 | 1.22 | 90 | 0.0010 | 1.7 | | | | | | 1.7 | 1.39 | No |
| 791.3 | 22 | 8 | 1.33 | 1.22 | 90 | 0.0010 | 1.7 | 3.18 | 3.04 | 430 | 0.000 | 0.4 | 2.1 | 1.74 | No* |
| 791.4 | 22 | 8 | 1.33 | 1.22 | 90 | 0.0010 | 1.7 | 3.18 | 2.86 | 920 | 0.000 | 0.8 | 2.5 | 2.88 | LT |
| 800.1 | 22 | 8 | 1.45 | 1.19 | 90 | 0.0022 | 1.7 | | | | | | 1.7 | 1.15 | No |
| 800.2 | 22 | 8 | 1.45 | 1.19 | 90 | 0.0022 | 1.7 | 3.19 | 3.02 | 460 | 0.000 | 0.4 | 2.1 | 2.20 | No |
| 800.3 | 22 | 8 | 1.45 | 1.19 | 90 | 0.0022 | 1.7 | 3.19 | 2.90 | 975 | 0.000 | 0.8 | 2.5 | 2.11 | No |
| 801.1 | 22 | 8 | Control | | | | | | | | | | | 0.39 | No |
| 801.2 | 22 | 8 | 1.45 | 1.21 | 90 | 0.0020 | 1.7 | | | | | | 1.7 | 1.41 | No |
| 801.3 | 22 | 8 | 1.45 | 1.21 | 90 | 0.0020 | 1.7 | 1.71 | 1.63 | 450 | 0.000 | 0.4 | 2.1 | 1.39 | No |
| 801.4 | 22 | 8 | 1.45 | 1.21 | 90 | 0.0020 | 1.7 | 1.71 | 1.51 | 960 | 0.000 | 0.8 | 2.5 | 1.49 | No |
| 808.1 | 22 | 8 | 0.98 | 0.84 | 45 | 0.0034 | 0.7 | | | | | | 1.7 | 1.44 | No |
| 808.2 | 22 | 8 | 0.98 | 0.84 | 45 | 0.0034 | 0.7 | 4.82 | 4.78 | 450 | 0.000 | 0.4 | 2.1 | 1.99 | No |
| 808.3 | 22 | 8 | 0.98 | 0.84 | 45 | 0.0034 | 0.7 | 4.82 | 4.41 | 965 | 0.000 | 0.8 | 2.5 | 2.49 | LT |
| 809.1 | 22 | 8 | Control | | | | | | | | | | | -0.19 | No |
| 809.2 | 22 | 8 | 0.98 | 0.88 | 45 | 0.0024 | 0.7 | | | | | | 1.7 | -0.21 | No |
| 809.3 | 22 | 8 | 0.98 | 0.88 | 45 | 0.0024 | 0.7 | 1.45 | 1.33 | 455 | 0.000 | 0.4 | 2.1 | 1.07 | No |
| 809.4 | 22 | 8 | 0.98 | 0.88 | 45 | 0.0024 | 0.7 | 1.45 | 0.92 | 975 | 0.000 | 0.8 | 2.5 | 1.09 | No |
| 818.1 | 22 | 8 | 0.98 | 0.84 | 45 | 0.0034 | 0.7 | | | | | | 1.7 | -0.18 | No |
| 818.2 | 22 | 8 | 0.98 | 0.84 | 45 | 0.0034 | 0.7 | 4.50 | 4.78 | 480 | 0.000 | 0.4 | 2.1 | 0.52 | LT |
| 818.3 | 22 | 8 | 0.98 | 0.84 | 45 | 0.0034 | 0.7 | 4.50 | 4.41 | 960 | 0.000 | 0.8 | 2.5 | 1.12 | LT |

## Table B3: Experimental plan for free chlorine and monochloramine

| Trial No. i | Temp °C | pH | Disinfectant | Co_B mg/L | Cf_B mg/L | t_B min | k'_B $min^{-1}$ | Predicted kill log-units | Obs log kill log-units | censored |
|---|---|---|---|---|---|---|---|---|---|---|
| *774.1* | *22* | *6* | | Control | | | | **0.0** | 0.34 | No |
| *774.2* | *22* | *6* | **Free chlorine** | 4.09 | 3.61 | 970 | 0.000129 | **0.4** | 1.29 | GT |
| *776.1* | *1* | *6* | | Control | | | | **0.0** | 0.20 | No |
| *776.2* | *1* | *6* | **Free chlorine** | 5.44 | 4.92 | 960 | 0.000105 | **0.1** | 0.10 | No |
| *778.1* | *10* | *6* | | Control | | | | **0.0** | -0.16 | No |
| *778.2* | *10* | *6* | **Free chlorine** | 4.45 | 4.29 | 960 | 0.000038 | **0.3** | 99.00 | Error |
| *780.1* | *22* | *6* | | Control | | | | **0.0** | 0.34 | No |
| *780.2* | *22* | *6* | **Free chlorine** | 2.74 | 2.32 | 995 | 0.000167 | **0.0** | 1.29 | GT |
| *823.1* | *10* | *6* | **Free chlorine** | 3.85 | 3.51 | 990 | 0.000093 | **0.0** | 0.42 | No |
| *824.1* | *10* | *6* | **Free chlorine** | 1.09 | 0.80 | 990 | 0.000312 | **0.0** | -0.39 | No |
| *826.1* | *1* | *6* | | Control | | | | | -0.39 | No |
| *826.2* | *1* | *6* | **Free chlorine** | 2.74 | 2.07 | 960 | 0.000292 | **0.0** | -0.08 | No |
| *796.1* | *22* | *8* | | Control | | | | **0.0** | -0.61 | No |
| *796.2* | *22* | *8* | **Monochloramin** | 4.62 | 4.23 | 960 | 0.000092 | **0.0** | 1.05 | No |
| *797.1* | *10* | *8* | | Control | | | | **0.0** | -0.61 | No |
| *797.2* | *10* | *8* | **Monochloramin** | 4.66 | 4.43 | 960 | 0.000053 | **0.0** | 0.18 | No |
| *806.1* | *10* | *8* | **Monochloramin** | 4.45 | 4.43 | 480 | 0.000009 | **0.0** | 0.71 | No |
| *806.2* | *10* | *8* | **Monochloramin** | 4.45 | 4.41 | 960 | 0.000009 | **0.0** | 0.71 | No |
| *814.1* | *22* | *8* | **Monochloramin** | 1.49 | 1.47 | 460 | 0.000029 | **0.0** | 0.34 | No |
| *814.2* | *22* | *8* | **Monochloramin** | 1.49 | 1.27 | 975 | 0.000164 | **0.0** | 0.29 | No |
| *815.1* | *1* | *8* | **Monochloramin** | 4.50 | | 480 | 0.000120 | **0.0** | 0.29 | No |
| *815.2* | *1* | *8* | **Monochloramin** | 4.50 | 4.23 | 960 | 0.000064 | **0.0** | 0.20 | No |
| *816.1* | *1* | *8* | | Control | | | | **0.0** | -0.61 | No |
| *816.2* | *1* | *8* | **Monochloramin** | 3.00 | | 480 | | | 0.01 | No |
| *816.3* | *1* | *8* | **Monochloramin** | 3.00 | 4.43 | 960 | -0.000406 | **0.0** | -0.61 | No |
| *819.1* | *22* | *8* | | Control | | | | | -0.39 | No |
| *819.2* | *22* | *8* | **Monochloramin** | 1.45 | 1.43 | 980 | 0.000014 | **2.5** | -0.08 | No |

**Table B.4 a: Experimental plan for ozone followed by free chlorine in natural water (treatment conditions)**

| Trial no. | Database no. | Water quality | pH | Temp., °C |
|---|---|---|---|---|
| 1 | 841 | Settled, pre-ozonated | 8.5 | 22 |
| 2 | 844 | Settled, pre-ozonated | 8.5 | 22 |
| 3 | 847 | Settled, pre-ozonated | 8.5 | 22 |
| 4 | 850 | Settled, pre-ozonated | 8.5 | 22 |
| 5 | 855 | Settled | 8.5 | 22 |
| 6 | 858 | Settled | 8.5 | 22 |
| 7 | 869 | Settled | 8.5 | 22 |
| 8 | 872 | Settled | 8.5 | 22 |
| 9 | 875 | Settled | 6.05 | 22 |
| 10 | 878 | Settled | 5.95 | 22 |
| 11 | 912 | Settled | 6.05 | 22 |
| 12 | 915 | Settled | 5.97 | 22 |
| 13 | 918 | Settled | 5.98 | 22 |
| 14 | 921 | Settled | 6.02 | 22 |

**Table B.4 b: Experimental plan for ozone followed by free chlorine in natural water (ozone pretreatment)**

| Trial no. | Applied dose mg/L | $C_f$ mg/L | $C_{avg}t$ mg·min/L | k' $min.^{-1}$ | IgH pred. Kill log-units | '+' Control (5 min), log-units | Net obs. kill ($O_3$), log-units |
|---|---|---|---|---|---|---|---|
| 1 | 2.06 | 0.45 | 4.42 | 0.34 | 1.9 | -0.18 | 2.34 |
| 2 | 2.10 | 0.12 | 3.20 | 0.62 | 1.3 | 0.16 | 1.00 |
| 3 | 1.96 | 0.21 | 3.42 | 0.49 | 1.5 | 0.16 | 0.85 |
| 4 | 1.94 | 0.37 | 4.02 | 0.37 | 1.7 | 0.00 | 2.16 |
| 5 | 1.20 | 0.24 | 1.05 | 0.57 | 0.7 | -0.40 | 0.56 |
| 6 | 1.22 | 0.46 | 1.24 | 0.34 | 0.8 | 0.27 | 0.55 |
| 7 | 2.45 | 0.13 | 5.86 | 0.56 | 1.9 | 0.00 | 1.68 |
| 8 | 2.46 | 0.47 | 6.89 | 0.31 | 2.5 | -0.14 | 1.90 |
| 9 | 1.48 | 0.73 | 5.02 | 0.18 | 2.0 | 0.19 | 1.82 |
| 10 | 1.50 | 0.80 | 5.57 | 0.12 | 2.3 | -0.14 | 2.49 |
| 11 | 0.96 | 0.59 | 1.38 | 0.19 | 0.9 | 0.00 | 0.82 |
| 12 | 1.47 | 0.72 | 4.95 | 0.10 | 2.1 | 0.00 | 2.35 |
| 13 | 0.98 | 0.60 | 1.34 | 0.12 | 0.8 | 0.00 | 0.82 |
| 14 | 1.48 | 0.96 | 5.97 | 0.08 | 2.4 | 0.00 | 2.16 |

**Table B.4 c: Experimental plan for ozone followed by free chlorine in natural water (free chlorine secondary treatment)**

| Trial no. | Applied dose mg/L | $C_o$ mg/L | $C_f$ mg/L | t min. | Ct mgmin/L | k' $min.^{-1}$ | IgH pred. Kill log-units | Obs. $Cl_2$ kill log-units |
|---|---|---|---|---|---|---|---|---|
| 1 | 4.1 | 3.4 | 1.0 | 960 | 1771 | 0.00110 | 0.1 | -0.18 |
| 2 | 3.6 | 1.9 | 0.1 | 960 | 455 | 0.00290 | 0.1 | 0.35 |
| 3 | 1.5 | 0.4 | 0.0 | 1000 | *100 | ND | 0.0 | 0.01 |
| 4 | 1.5 | 0.6 | 0.0 | 960 | *150 | ND | 0.0 | 0.16 |
| 5 | 5.2 | 3.6 | 1.6 | 940 | 2287 | 0.00090 | 0.1 | 0.01 |
| 6 | 3.2 | 2.7 | 1.6 | 970 | 2024 | 0.00090 | 0.1 | 0.30 |
| 7 | 4.1 | 3.2 | 0.6 | 960 | 1370 | 0.00170 | 0.1 | 1.16 |
| 8 | 4.1 | 3.2 | 2.1 | 240 | 625 | 0.00040 | 0.1 | 0.82 |
| 9 | 4.1 | 2.3 | 0.5 | 995 | 1109 | 0.00150 | 0.5 | 0.82 |
| 10 | 4.1 | 2.4 | 0.7 | 995 | 1303 | 0.00120 | 0.5 | 0.82 |
| 11 | 4.2 | 3.0 | 1.0 | 1095 | 1890 | 0.00100 | 0.7 | 1.35 |
| 12 | 3.6 | 2.4 | 1.3 | 230 | 404 | 0.00250 | 0.3 | 0.59 |
| 13 | 3.6 | 2.3 | 1.4 | 235 | 413 | 0.00210 | 0.3 | 0.82 |
| 14 | 3.6 | 2.4 | 1.3 | 240 | 430 | 0.00250 | 0.3 | 0.49 |

**Table B.4 d: Experimental plan for ozone followed by free chlorine in natural water (total)**

| Trial no. | '+' Control (1,000 min), log-units | total kill log-units | Ozone log-units | Chorine log-units | Additive log-units | Total observed log-units | Synergy log-units |
|---|---|---|---|---|---|---|---|
| 1 | 0.01 | 2.81 | 2.16 | -0.18 | 1.98 | 2.82 | 0.84 |
| 2 | 0.01 | 1.81 | 1.16 | 0.35 | 1.51 | 1.82 | 0.31 |
| 3 | 0.32 | 1.27 | 1.01 | 0.01 | 1.02 | 1.59 | 0.57 |
| 4 | 0.15 | 1.67 | 2.16 | 0.16 | 2.32 | 1.82 | -0.50 |
| 5 | 0.27 | 0.60 | 0.16 | 0.01 | 0.17 | 0.87 | 0.70 |
| 6 | <-0.40 | ND | 0.82 | 0.30 | 1.12 | <0.82 | <-0.75 |
| 7 | -0.48 | 2.64 | 1.68 | 1.16 | 2.84 | 2.16 | -0.68 |
| 8 | 0.05 | 2.25 | 1.76 | 0.82 | 2.58 | 2.30 | -0.28 |
| 9 | 0.15 | >3.2 | 2.01 | 0.82 | 2.83 | >3.35 | >0.68 |
| 10 | -0.14 | 3.49 | 2.35 | 0.82 | 3.17 | 3.35 | 0.18 |
| 11 | 0.19 | 2.16 | 0.82 | 1.35 | 2.17 | 2.35 | 0.18 |
| 12 | 0.71 | 2.11 | 2.35 | 0.59 | 2.94 | 2.82 | -0.12 |
| 13 | 0.52 | 0.64 | 0.82 | 0.82 | 1.64 | 1.16 | -0.48 |
| 14 | 0.15 | 2.67 | 2.16 | 0.49 | 2.65 | 2.82 | 0.17 |

# APPENDIX C

# MAXIMUM LIKELIHOOD CALCULATIONS

**Table C.1: Maximum likelihood estimate for the parameters of Chick-Watson model for Sequential Inactivation of *Cryptosporidium***
**(High Level Chlorine Dioxide Pretreatment followed by Free Chlorine)**

$$Ln(L) = -vLn\sigma - \frac{1}{2}\sum_{i=1}^{v}\left(\frac{y_i - \mu_i}{\sigma}\right)^2 + \sum_{i=1}^{v_i} Ln\Phi\left(\frac{y_i^{\leq} - \mu_i}{\sigma}\right)$$

***organism*** Crypto ***pH*** 6
***disinfectant*** ClO2 ***Temp.*** 1 to 22

| | | |
|---|---|---|
| Number of data | v | 21 |
| Cencored data | vi | 0 |
| Parameters | p | 2 |

| | | | |
|---|---|---|---|
| m | 1.00 | | |
| n | 1.00 | Upper | Lower |
| Af | 1.159 | [illegible] | [illegible] |
| k | 0.00077 | | |
| SG | 0.40 | | |

| | | |
|---|---|---|
| alfa | 0.1 | |
| P | 1 | |
| Chi | 2.71 | |
| Maximum | Ln(L) | 8.69 |

| Trial No. i | Cencored data mark=1 Mark | temp. Temp | pH | Initial Conc (mg/L) $C_o$ | Final Conc (mg/L) $C_f$ | Time (min.) T | log removal (total) $y_i$ | ClO2 kill Yo | 1st order Decay rate Kt | Model predicted gross kill Lg(N/No) | Expected log removal SM |
|---|---|---|---|---|---|---|---|---|---|---|---|
| *760.2* | | 1 | 6 | 0.8 | 0.2 | 457 | 1.7 | 1.1 | 0.0000 | 1.1 | 0.305 |
| *760.3* | | 1 | 6 | 0.8 | 0.2 | 936 | 1.7 | 1.1 | 0.0000 | 1.1 | 0.296 |
| *767.2* | | 1 | 6 | 1.4 | 0.7 | 450 | 1.1 | 1.4 | 0.0000 | 1.4 | 0.088 |
| *759.2* | 1 | 1 | 6 | 2.9 | 2.8 | 243 | 1.1 | 0.8 | 0.0000 | 0.8 | 0.000 |
| *766.2* | | 1 | 6 | 3.0 | 2.9 | 240 | 0.8 | 1.4 | 0.0000 | 1.5 | 0.455 |
| *767.3* | 1 | 1 | 6 | 1.4 | 0.7 | 960 | 1.1 | 1.4 | 0.0000 | 1.4 | 0.000 |
| *777.2* | | 1 | 6 | 4.2 | 4.0 | 486 | 1.1 | 1.5 | 0.0000 | 1.6 | 0.202 |
| *759.3* | 1 | 1 | 6 | 2.9 | 2.5 | 900 | 1.8 | 0.8 | 0.0000 | 0.9 | 0.000 |
| *766.3* | | 1 | 6 | 3.0 | 2.5 | 960 | 1.1 | 1.4 | 0.0000 | 1.5 | 0.196 |
| *777.3* | | 1 | 6 | 4.2 | 3.9 | 960 | 1.1 | 1.5 | 0.0000 | 1.6 | 0.287 |
| 768.2 | | 10 | 6 | 1.2 | 1.1 | 225 | 1.4 | 1.5 | 0.0001 | 1.5 | 0.018 |
| 761.2 | | 10 | 6 | 2.8 | 2.6 | 225 | 1.4 | 1.1 | 0.0001 | 1.2 | 0.033 |
| 769.2 | | 10 | 6 | 3.5 | 3.4 | 210 | 1.7 | 1.7 | 0.0001 | 1.8 | 0.007 |
| 768.3 | 1 | 10 | 6 | 1.2 | 0.8 | 970 | 1.6 | 1.5 | 0.0001 | 1.6 | 0.000 |
| 779.2 | | 10 | 6 | 4.3 | 4.1 | 514 | 1.3 | 1.2 | 0.0001 | 1.5 | 0.030 |
| 761.3 | | 10 | 6 | 2.8 | 2.4 | 920 | 1.8 | 1.1 | 0.0001 | 1.4 | 0.135 |
| 769.3 | | 10 | 6 | 3.5 | 3.2 | 960 | 1.8 | 1.7 | 0.0001 | 2.2 | 0.132 |
| 779.3 | | 10 | 6 | 4.3 | 4.0 | 960 | 2.4 | 1.2 | 0.0001 | 1.7 | 0.456 |
| *765.2* | | 22 | 6 | 1.0 | 0.6 | 255 | 1.6 | 1.4 | 0.0008 | 1.6 | 0.000 |
| *765.3* | | 22 | 6 | 1.0 | 0.4 | 920 | 1.5 | 1.4 | 0.0008 | 1.9 | 0.182 |
| *763.2* | | 22 | 6 | 3.1 | 2.8 | 220 | 1.5 | 0.8 | 0.0008 | 1.3 | 0.043 |
| *764.2* | | 22 | 6 | 2.7 | 2.4 | 270 | 2.1 | 1.4 | 0.0008 | 2.0 | 0.018 |
| *775.2* | 1 | 22 | 6 | 4.1 | 3.7 | 495 | 3.7 | 1.4 | 0.0008 | 2.9 | 0.000 |
| *764.3* | | 22 | 6 | 2.7 | 2.2 | 950 | 3.1 | 1.4 | 0.0008 | 3.2 | 0.011 |
| *763.3* | | 22 | 6 | 3.1 | 2.4 | 960 | 3.4 | 0.8 | 0.0008 | 2.8 | 0.339 |
| *775.3* | | 22 | 6 | 4.1 | 3.5 | 960 | 3.9 | 1.4 | 0.0008 | 4.2 | 0.144 |

Sigma [illegible]

**Table C.1: Maximum likelihood estimate for the parameters of Chick-Watson model for Sequential Inactivation of *Cryptosporidium***
**(Low Level Chlorine Dioxide Pretreatment followed by Free Chlorine)**

$$Ln(L) = -\nu Ln\sigma - \frac{1}{2}\sum_{i=1}^{\nu}\left(\frac{y_i - \mu_i}{\sigma}\right)^2 + \sum_{i=1}^{\nu_i} Ln\Phi\left(\frac{y_i^{\leq} - \mu_i}{\sigma}\right)$$

*organism* Crypto *pH* 6
*disinfectant* ClO2 *Temp.* 1 to 22

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | | | m | 1.00 | | | alfa 0.1 |
| | | | n | 1.00 | Upper | Lower | P 1 |
| Number of data | v | 8 | Af | 1.015 | [illegible] | [illegible] | Chi 2.71 |
| Cencored data | vi | 0 | k | 0.00087 | | | |
| Parameters | p | 2 | SG | 0.33 | | Maximum | Ln(L) 4.80 |

| Trial No. i | Cencored data mark=1 Mark | Temp | pH | Initial Conc (mg/L) $C_o$ | Final Conc (mg/L) $C_r$ | Time (min.) T | log removal (total) $y_i$ | ClO2 kill Yo | 1st order Decay rate Kt | Model predicted gross kill Lg(N/No) | Expected log removal SM |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 771.2 | | 10 | 6 | 1.4 | 0.9 | 426 | 0.4 | 0.7 | 0.0007 | 1.1 | 0.4708 |
| 770.2 | | 10 | 6 | 4.3 | 4.2 | 230 | 1.0 | 0.1 | 0.0007 | 0.8 | 0.0282 |
| 771.3 | | 10 | 6 | 1.4 | 0.8 | 960 | 1.7 | 0.7 | 0.0007 | 1.5 | 0.0264 |
| 770.3 | 1 | 10 | 6 | 4.3 | 4.0 | 960 | 1.5 | 0.1 | 0.0007 | 3.0 | 0.0000 |
| 758.2 | | 22 | 6 | 1.1 | 1.0 | 240 | 0.5 | 0.7 | 0.0009 | 1.0 | 0.2202 |
| 825.2 | 1 | 22 | 6 | 2.4 | 1.7 | 240 | 1.1 | 0.6 | 0.0009 | 1.0 | 0.0000 |
| 757.2 | | 22 | 6 | 2.8 | 2.5 | 240 | 1.1 | 0.7 | 0.0009 | 1.3 | 0.0414 |
| 758.3 | 1 | 22 | 6 | 1.1 | 0.7 | 950 | 1.5 | 0.7 | 0.0009 | 1.5 | 0.0000 |
| 825.3 | 1 | 22 | 6 | 2.4 | 1.6 | 960 | 1.8 | 0.6 | 0.0009 | 2.3 | 0.0000 |
| 781.2 | | 22 | 6 | 4.8 | 4.1 | 460 | 2.0 | 0.5 | 0.0009 | 2.3 | 0.0713 |
| 757.3 | | 22 | 6 | 2.8 | 2.1 | 940 | 2.8 | 0.7 | 0.0009 | 2.7 | 0.0021 |
| 781.3 | | 22 | 6 | 4.8 | 4.0 | 985 | 4.4 | 0.5 | 0.0009 | 4.2 | 0.0253 |

Sigma [illegible]

**Table C.1: Maximum likelihood estimate for the parameters of Chick-Watson model for Sequential Inactivation of *Cryptosporidium***
**(High Level Chlorine Dioxide Pretreatment followed by Monochloramine)**

$$Ln(L) = -vLn\sigma - \frac{1}{2}\sum_{i=1}^{v}\left(\frac{y_i - \mu_i}{\sigma}\right)^2 + \sum_{i=1}^{v_i} Ln\Phi\left(\frac{y_i^S - \mu_i}{\sigma}\right)$$

| | | | |
|---|---|---|---|
| *organism* | Crypto | *pH* | 8 |
| *disinfectant* | ClO2 | *Temp.* | 1 to 22 |

| | | |
|---|---|---|
| Number of data pts | v | 18 |
| Cencored data | vi | 0 |
| Parameters in Hom | p | 2 |

| | | lower | upper |
|---|---|---|---|
| m | 1.00 | | |
| n | 1.00 | | |
| Af | 1.009 | [illegible] | [illegible] |
| k | 0.00026 | [illegible] | [illegible] |
| SG | 0.29 | | |

| | |
|---|---|
| alfa | 0.1 |
| P | 1 |
| Chi | 2.71 |
| Maximum Ln(L) | 13.40 |

| Trial No. i | Cencored data mark=1 Mark | Temp | pH | Initial Conc (mg/L) $C_o$ | Final Conc (mg/L) $C_f$ | Time (min.) T | log removal (total) $y_i$ | ClO2 kill Yo | 1st order Decay rate Kt | Model predicted gross kill Lg(N/No) | Expected log removal SM |
|---|---|---|---|---|---|---|---|---|---|---|---|
| *802.2* | | 1 | 8 | 4.7 | 4.6 | 470 | 1.9 | 1.4 | 0.0002 | 1.9 | 0.0004 |
| *792.2* | | 1 | 8 | 4.8 | 4.7 | 495 | 1.8 | 1.2 | 0.0002 | 1.7 | 0.0157 |
| *803.2* | | 1 | 8 | 3.2 | 3.0 | 960 | 2.0 | 1.4 | 0.0002 | 2.0 | 0.0000 |
| *793.2* | | 1 | 8 | 3.2 | 3.1 | 960 | 1.6 | 0.5 | 0.0002 | 1.2 | 0.1747 |
| *802.3* | 1 | 1 | 8 | 4.7 | 4.4 | 985 | 1.8 | 1.4 | 0.0002 | 2.4 | 0.0000 |
| *792.3* | | 1 | 8 | 4.8 | 4.6 | 965 | 2.2 | 1.2 | 0.0002 | 2.1 | 0.0045 |
| *805.2* | | 10 | 8 | 3.1 | 3.0 | 480 | 1.6 | 1.1 | 0.0002 | 1.5 | 0.0151 |
| *804.2* | | 10 | 8 | 4.5 | 4.5 | 480 | 0.5 | 0.4 | 0.0002 | 0.9 | 0.1531 |
| *794.2* | | 10 | 8 | 4.6 | 4.6 | 480 | 1.7 | 1.4 | 0.0002 | 1.9 | 0.0509 |
| *805.3* | 1 | 10 | 8 | 3.1 | 2.9 | 960 | 1.5 | 1.1 | 0.0002 | 1.8 | 0.0000 |
| *795.2* | | 10 | 8 | 3.2 | 3.1 | 960 | 2.1 | 1.1 | 0.0002 | 1.8 | 0.0626 |
| *804.3* | | 10 | 8 | 4.5 | 4.4 | 960 | 0.8 | 0.4 | 0.0002 | 1.4 | 0.3563 |
| *794.3* | | 10 | 8 | 4.6 | 4.4 | 965 | 2.4 | 1.4 | 0.0002 | 2.4 | 0.0002 |
| *801.2* | | 22 | 8 | 1.7 | 1.6 | 450 | 1.4 | 1.4 | 0.0003 | 1.6 | 0.0475 |
| *800.2* | | 22 | 8 | 3.2 | 3.0 | 460 | 2.2 | 1.2 | 0.0003 | 1.5 | 0.4540 |
| *791.2* | | 22 | 8 | 3.2 | 3.0 | 430 | 1.7 | 1.4 | 0.0003 | 1.7 | 0.0000 |
| *801.3* | | 22 | 8 | 1.7 | 1.5 | 960 | 1.5 | 1.4 | 0.0003 | 1.8 | 0.1070 |
| *790.2* | | 22 | 8 | 4.8 | 4.6 | 435 | 2.1 | 1.5 | 0.0003 | 2.0 | 0.0153 |
| *791.3* | 1 | 22 | 8 | 3.2 | 2.9 | 920 | 2.9 | 1.4 | 0.0003 | 2.1 | 0.0000 |
| *800.3* | | 22 | 8 | 3.2 | 2.9 | 960 | 2.1 | 1.2 | 0.0003 | 1.9 | 0.0361 |
| *790.3* | | 22 | 8 | 4.8 | 4.4 | 930 | 2.6 | 1.5 | 0.0003 | 2.6 | 0.0000 |

Sigma [illegible]

**Table C.1: Maximum likelihood estimate for the parameters of Chick-Watson model for Sequential Inactivation of *Cryptosporidium***
**(Low Level Chlorine Dioxide Pretreatment followed by Monochloramine)**

$$Ln(L) = -vLn\sigma - \frac{1}{2}\sum_{i=1}^{v}\left(\frac{y_i - \mu_i}{\sigma}\right)^2 + \sum_{i=1}^{v_i} Ln\Phi\left(\frac{y_i^{\leq} - \mu_i}{\sigma}\right)$$

***organism*** Crypto ***pH*** 8
***disinfectant*** ClO2 ***Temp.*** 1 to 22

| | | |
|---|---|---|
| Number of data pts | v | 8 |
| Cencored data | vi | 0 |
| Parameters in Hom | p | 2 |

| | | | |
|---|---|---|---|
| m | 1.00 | | |
| n | 1.00 | lower | upper |
| Af | 1.039 | [illegible] | [illegible] |
| k | 0.00047 | [illegible] | [illegible] |
| Std. Dev | 0.40 | | Maximum |

| | |
|---|---|
| alfa | 0.1 |
| P | 1 |
| Chi | 2.71 |
| Ln(L) | 3.39 |

| Trial No. i | Cencored data mark=1 Mark | Temp | pH | Initial Conc (mg/L) $C_o$ | Final Conc (mg/L) $C_f$ | Time (min.) T | log removal (total) $y_i$ | ClO2 kill Yo | 1st order Decay rate Kt | Model predicted gross kill Lg(N/No) | Expected log removal SM |
|---|---|---|---|---|---|---|---|---|---|---|---|
| *820.2* | | 1 | 8 | 4.6 | 4.6 | 480 | 0.8 | 0.5 | 0.0002 | 1.0 | 0.0204 |
| *810.2* | 1 | 1 | 8 | 4.9 | 4.8 | 465 | 0.8 | 0.5 | 0.0002 | 1.0 | 0.0000 |
| *821.2* | | 1 | 8 | 3.0 | 3.0 | 960 | 0.8 | 0 | 0.0002 | 0.6 | 0.0360 |
| *811.2* | 1 | 1 | 8 | 3.3 | 3.0 | 980 | 1.1 | 0.5 | 0.0002 | 1.1 | 0.0000 |
| *820.3* | 1 | 1 | 8 | 4.6 | 4.5 | 960 | 1.1 | 0.5 | 0.0002 | 1.4 | 0.0000 |
| *810.3* | | 1 | 8 | 4.9 | 4.6 | 965 | 1.5 | 0.5 | 0.0002 | 1.4 | 0.0017 |
| *813.2* | | 10 | 8 | 1.5 | 1.5 | 450 | 0.8 | 0.8 | 0.0003 | 1.0 | 0.0588 |
| *813.3* | 1 | 10 | 8 | 1.5 | 1.2 | 960 | 1.1 | 0.8 | 0.0003 | 1.2 | 0.0000 |
| *812.2* | | 10 | 8 | 4.8 | 4.8 | 460 | 1.1 | 0.6 | 0.0003 | 1.2 | 0.0256 |
| *812.3* | 1 | 10 | 8 | 4.8 | 4.7 | 975 | 1.1 | 0.6 | 0.0003 | 1.9 | 0.0000 |
| *818.2* | 1 | 22 | 8 | 1.1 | 1.1 | 240 | 0.5 | 0.0 | 0.0005 | 0.1 | 0.0000 |
| *809.2* | | 22 | 8 | 1.5 | 1.3 | 455 | 1.1 | 0.0 | 0.0005 | 0.3 | 0.6011 |
| *818.3* | 1 | 22 | 8 | 1.1 | 1.0 | 980 | 1.1 | 0.0 | 0.0005 | 0.5 | 0.0000 |
| *822.2* | 1 | 22 | 8 | 4.6 | 4.5 | 200 | 1.1 | 0.7 | 0.0005 | 1.1 | 0.0000 |
| *809.3* | | 22 | 8 | 1.5 | 0.9 | 965 | 1.1 | 0.0 | 0.0005 | 0.5 | 0.3104 |
| *808.2* | | 22 | 8 | 4.8 | 4.8 | 450 | 2.0 | 1.4 | 0.0005 | 2.4 | 0.2084 |
| *822.3* | 1 | 22 | 8 | 4.6 | 4.1 | 975 | 2.1 | 0.7 | 0.0005 | 2.7 | 0.0000 |
| *808.3* | 1 | 22 | 8 | 4.8 | 4.4 | 960 | 2.5 | 1.4 | 0.0005 | 3.5 | 0.0000 |

Sigma [illegible]

**Figure C.1: Chick-Watson model fit for chlorine dioxide/free chlorine treatment (high level pretreatment)**

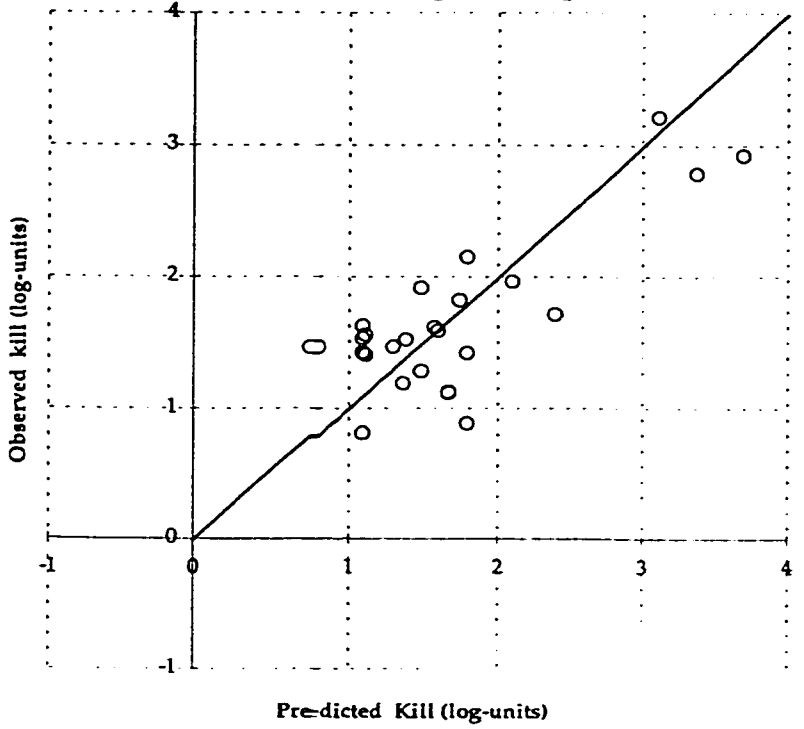



**Figure C.2: Chick-Watson model fit for chlorine dioxide/free chlorine treatment (low level pretreatment)**

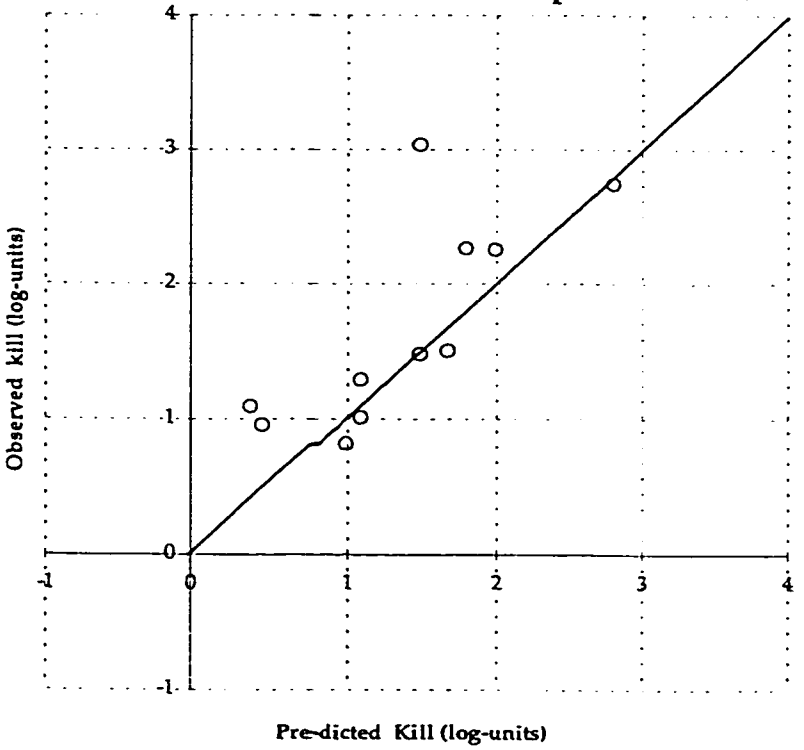


**Figure C.3: Chick-Watson Model fit for chlorine dioxide/monochloramine treatment (high level pretreatment)**

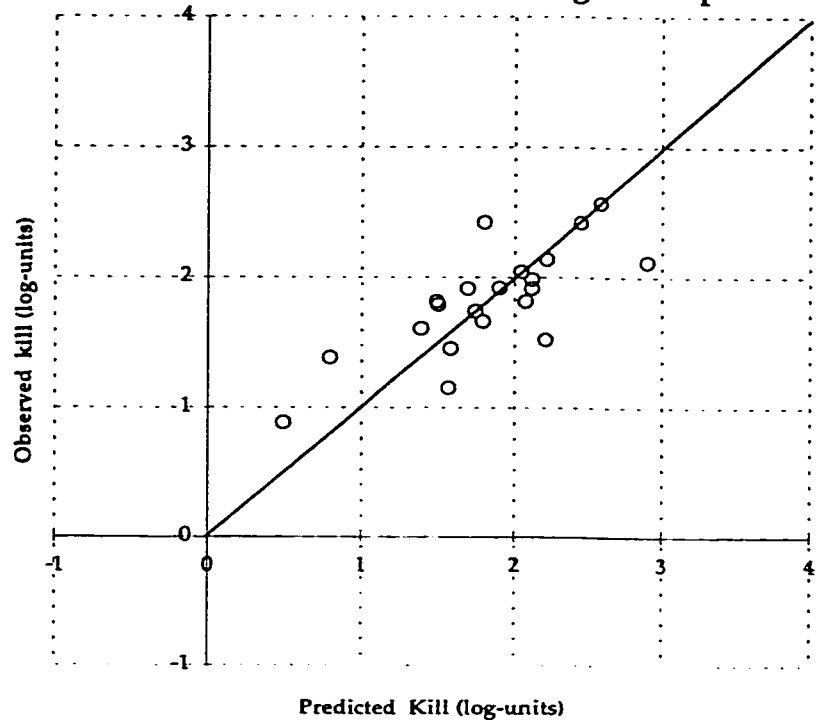
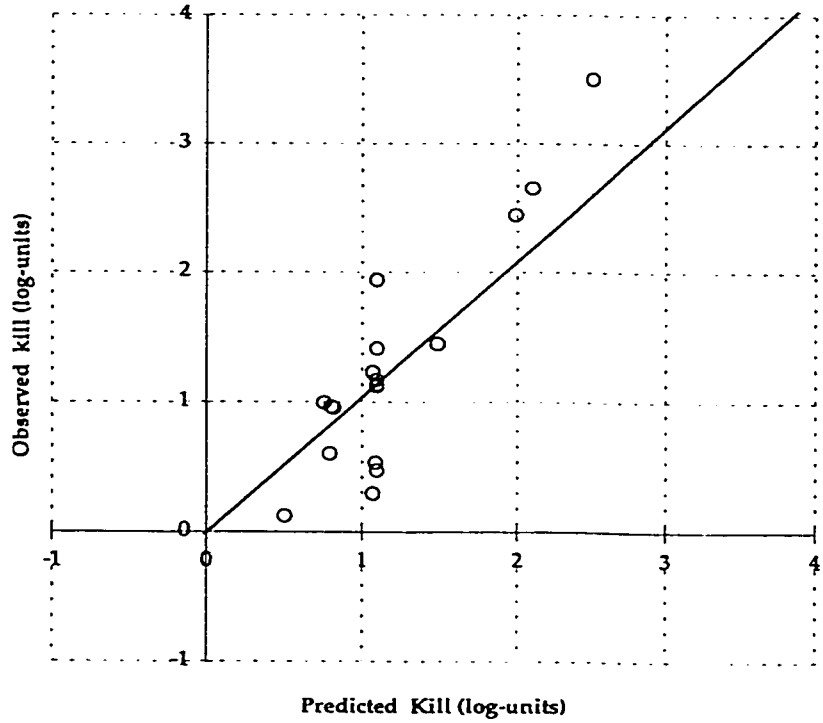



**Figure C.4: Chick-Watson Model fit for chlorine dioxide/monochloramine treatment (low level pretreatment)**



# APPENDIX D

# OZONE RESIDUAL DECAY IN NATURAL WATER

## Trial 841 - Ozone Residual Decay in a Natural Water

Date of Test: Oct. 25, 1999
Water Sample: Settled Water From Rossdale WTP, Collected Oct. 21/99, Filtered and pre-ozonated with approx. 1.9 mg/L O3

Initial Water Sample Volume: 200 mL
Volume of Ozone Stock Added 20.18 mL
Total Solution Volume: 220.18
Lab Temperature: 22.5 C

Ozone Stock Solution Conc, mg/L:

| | | | | |
|---|---|---|---|---|
| Initial: | 23.10 | 22.5700 | 21.9 | |
| Final: | 23.30 | 23.1000 | 20.6 | 23 |
| Average: | 22.51 | | | |

Transferred Ozone Dose, mg/L: 2.06

**Ozone Residual By Direct UV Absorbance @ 260 nm**

Time of O3 Dose: 150 s

Decay Model Parameters: k1 0.34 min-1
Co 1.94 mg/L

| Time (s) | Adjusted Time (min) | Abs @ 260 | Measured O3 Conc., mg/L | Predicted O3 Conc., mg/L | Square Error |
|---|---|---|---|---|---|
| 140 | -0.17 | 0.03972 | | | |
| 166 | 0.30 | 0.15411 | 1.76 | 1.75 | 0.0001 |
| 206 | 0.93 | 0.12973 | 1.41 | 1.41 | 0.0000 |
| 245 | 1.58 | 0.10954 | 1.12 | 1.14 | 0.0003 |
| 280 | 2.17 | 0.09604 | 0.92 | 0.93 | 0.0001 |
| 328 | 2.97 | 0.08247 | 0.73 | 0.71 | 0.0002 |
| 372 | 3.70 | 0.07182 | 0.57 | 0.56 | 0.0002 |
| 389 | 3.98 | 0.06717 | 0.51 | 0.51 | 0.0000 |

Average Baseline 0.0322

SSE= 0.0010

Calculated Ozone Demand:
Based on Estimated Co: 0.13 mg/L
Based on Measured Co: 0.30 mg/L

**Ozone Residual By Indigo Trisulphonate Method**

Time of O3 Dose: 0 s

Decay Model Parameters: k1 0.32 min-1
Co 2.12 mg/L

| Time (s) | Adjusted Time (min) | Abs @ 600 | Sample Volume, mL | Indigo Solution Vol, mL | Measured O3 Conc., mg/L | Predicted O3 Conc., mg/L | Square Error |
|---|---|---|---|---|---|---|---|
| 30 | 0.50 | 0.119 | 9.96 | 5 | 2.05 | 1.80 | 0.0598 |
| 65 | 1.08 | 0.223 | 9.96 | 5 | 1.68 | 1.50 | 0.0330 |
| 150 | 2.50 | 0.427 | 9.96 | 5 | 0.95 | 0.95 | 0.0000 |
| 220 | 3.67 | 0.51 | 9.96 | 5 | 0.65 | 0.65 | 0.0000 |

Blank Absorbance: 0.692

SSE= 0.0330

Calculated Ozone Demand:
Based on Estimated Co: -0.06 mg/L
Based on Measured Co: 0.01 mg/L

## Trial 844 - Ozone Residual Decay in a Natural Water

Date of Test: 10/24/99
Water Sample: Settled Water From Rossdale WTP, Collected Oct. 21/99, filtered and pre-ozonated with approx. 0.95 mg/L O3

Initial Water Sample Volume: 190 mL
Volume of Ozone Stock Added 20 mL
Total Solution Volume: 210
Lab Temperature: 22 C

Ozone Stock Solution Conc, mg/L:

| | | | | |
|---|---|---|---|---|
| Initial: | 22.20 | 22.2000 | 22.5 | |
| Final: | 21.50 | 21.6000 | 21.5 | 23 |
| Average: | 22.07 | | | |

Transferred Ozone Dose, mg/L: 2.10

**Ozone Residual By Direct UV Absorbance @ 260 nm**

Time of O3 Dose: 195 s

Decay Model Parameters: k1 0.62 min-1
Co 1.75 mg/L

| Time (s) | Adjusted Time (min) | Abs @ 260 | Measured O3 Conc., mg/L | Predicted O3 Conc., mg/L | Square Error |
|---|---|---|---|---|---|
| 210 | 0.25 | 0.14413 | 1.48 | 1.50 | 0.0003 |
| 232 | 0.62 | 0.12602 | 1.22 | 1.19 | 0.0007 |
| 259 | 1.07 | 0.10417 | 0.90 | 0.90 | 0.0000 |
| 295 | 1.67 | 0.08415 | 0.61 | 0.62 | 0.0000 |
| 359 | 2.73 | 0.06352 | 0.31 | 0.32 | 0.0000 |
| 414 | 3.65 | 0.05377 | 0.17 | 0.18 | 0.0000 |
| 446 | 4.18 | 0.05081 | 0.13 | 0.13 | 0.0000 |

Average Baseline 0.04184

SSE= 0.0011

Calculated Ozone Demand:
Based on Estimated Co: 0.35 mg/L
Based on Measured Co: 0.62 mg/L

**Ozone Residual By Indigo Trisulphonate Method**

Time of O3 Dose: 0 s

Decay Model Parameters: k1 0.65 min-1
Co 2.11 mg/L

| Time (s) | Adjusted Time (min) | Abs @ 600 | Sample Volume, mL | Indigo Solution Vol, mL | Measured O3 Conc., mg/L | Predicted O3 Conc., mg/L | Square Error |
|---|---|---|---|---|---|---|---|
| 28 | 0.47 | 0.693 | 10 | 10 | 1.59 | 1.56 | 0.0010 |
| 60 | 1.00 | 0.611 | 10 | 10 | 1.03 | 1.10 | 0.0051 |
| 126 | 2.10 | 0.532 | 10 | 5 | 0.54 | 0.54 | 0.0000 |
| 225 | 3.75 | 0.631 | 10 | 5 | 0.18 | 0.18 | 0.0000 |

Blank Absorbance: 1.027
0.682

SSE= 0.0051

Calculated Ozone Demand:
Based on Estimated Co: -0.01 mg/L
Based on Measured Co: 0.51 mg/L

## Trial 847 - Ozone Residual Decay in a Natural Water

Date of Test: Oct. 27, 1999
Water Sample: Settled Water From Rossdale WTP, Collected Oct. 21/99, filtered and pre-ozonated with approx. 0.95 mg/L O3

Initial Water Sample Volume: 200 mL
Volume of Ozone Stock Added 20 mL
Total Solution Volume: 220
Lab Temperature: 22 C

| Ozone Stock Solution Conc, mg/L: | | | | |
|---|---|---|---|---|
| | Initial: | 22.40 | 21.9000 | 21.1 |
| | Final: | 22.00 | 20.7000 | 21.4 |
| | Average: | 21.58 | | |

Transferred Ozone Dose, mg/L: 1.96

**Ozone Residual By Direct UV Absorbance @ 260 nm**

Time of O3 Dose: 180 s

Decay Model Parameters: k1 0.49 min-1
Co 1.68 mg/L

| Time (s) | Adjusted Time (min) | Abs @ 260 | Measured O3 Conc., mg/L | Predicted O3 Conc., mg/L | Square Error |
|---|---|---|---|---|---|
| 193 | 0.22 | 0.14975 | 1.50 | 1.51 | 0.0000 |
| 225 | 0.75 | 0.12729 | 1.18 | 1.16 | 0.0003 |
| 261 | 1.35 | 0.10504 | 0.86 | 0.87 | 0.0001 |
| 300 | 2.00 | 0.08922 | 0.63 | 0.63 | 0.0000 |
| 339 | 2.65 | 0.07716 | 0.45 | 0.46 | 0.0000 |
| 382 | 3.37 | 0.06824 | 0.32 | 0.32 | 0.0000 |
| 428 | 4.13 | 0.06161 | 0.23 | 0.22 | 0.0000 |

Average Baseline 0.04581

SSE= 0.0005

Calculated Ozone Demand:
Based on Estimated Co: 0.29 mg/L
Based on Measured Co: 0.46 mg/L

**Ozone Residual By Indigo Trisulphonate Method**

Time of O3 Dose: 0 s

Decay Model Parameters: k1 0.55 min-1
Co 2.29 mg/L

| Time (s) | Adjusted Time (min) | Abs @ 600 | Sample Volume, mL | Indigo Solution Vol, mL | Measured O3 Conc., mg/L | Predicted O3 Conc., mg/L | Square Error |
|---|---|---|---|---|---|---|---|
| 24 | 0.40 | 0.67 | 10 | 10 | 1.83 | 1.84 | 0.0000 |
| 55 | 0.92 | 0.764 | 10 | 10 | 1.39 | 1.38 | 0.0000 |
| 128 | 2.13 | 0.493 | 10 | 5 | 0.70 | 0.71 | 0.0000 |
| 228 | 3.80 | 0.609 | 10 | 5 | 0.29 | 0.28 | 0.0000 |

Blank Absorbance: 1.055
0.69

SSE= 0.0001

Calculated Ozone Demand:
Based on Estimated Co: -0.33 mg/L
Based on Measured Co: 0.13 mg/L

## Trial 850 - Ozone Residual Decay in a Natural Water

Date of Test: 10/28/99
Water Sample: Settled Water From Rossdale WTP, Collected Oct. 21/99, filtered and pre-ozonated with approx. 0.95 mg/L O3

Initial Water Sample Volume: 200 mL
Volume of Ozone Stock Added 20 mL
Total Solution Volume: 220
Lab Temperature: 22 C

Ozone Stock Solution Conc, mg/L:

| | | | |
|---|---|---|---|
| Initial: | 21.70 | 21.6000 | 21.9 |
| Final: | 20.60 | 20.7000 | 21.6 |
| Average: | 21.35 | | |

Transferred Ozone Dose, mg/L: 1.94

Ozone Residual By Direct UV Absorbance @ 260 nm

Time of O3 Dose: 10 s

Decay Model Parameters: k1 0.37 min-1
Co 1.82 mg/L

| Time (s) | Adjusted Time (min) | Abs @ 260 | Measured O3 Conc., mg/L | Predicted O3 Conc., mg/L | Square Error |
|---|---|---|---|---|---|
| 27 | 0.28 | 0.15337 | 1.64 | 1.64 | 0.0000 |
| 56 | 0.77 | 0.13487 | 1.37 | 1.37 | 0.0000 |
| 85 | 1.25 | 0.11801 | 1.13 | 1.14 | 0.0002 |
| 125 | 1.92 | 0.10176 | 0.89 | 0.89 | 0.0000 |
| 166 | 2.60 | 0.08804 | 0.69 | 0.69 | 0.0000 |
| 208 | 3.30 | 0.07727 | 0.54 | 0.53 | 0.0001 |
| 259 | 4.15 | 0.06631 | 0.38 | 0.39 | 0.0000 |

Average Baseline 0.04005

SSE= 0.0003

Calculated Ozone Demand:
Based on Estimated Co: 0.12 mg/L
Based on Measured Co: 0.30 mg/L

Ozone Residual By Indigo Trisulphonate Method

Time of O3 Dose: 0 s

Decay Model Parameters: k1 0.34 min-1
Co 1.89 mg/L

| Time (s) | Adjusted Time (min) | Abs @ 600 | Sample Volume, mL | Indigo Solution Vol, mL | Measured O3 Conc., mg/L | Predicted O3 Conc., mg/L | Square Error |
|---|---|---|---|---|---|---|---|
| 23 | 0.38 | 0.646 | 10 | 10 | 1.93 | 1.66 | 0.0728 |
| 60 | 1.00 | 0.764 | 10 | 10 | 1.37 | 1.34 | 0.0005 |
| 120 | 2.00 | 0.432 | 10 | 5 | 0.91 | 0.96 | 0.0023 |
| 240 | 4.00 | 0.542 | 10 | 5 | 0.51 | 0.48 | 0.0010 |

Blank Absorbance: 1.051
0.666

SSE= 0.0038

Calculated Ozone Demand:
Based on Estimated Co: 0.05 mg/L
Based on Measured Co: 0.01 mg/L

## Trial 855 - Ozone Residual Decay in a Natural Water

Date of Test: 11/8/99
Water Sample: Settled Water From Rossdale WTP, Collected Oct. 21/99, filtered

Initial Water Sample Volume: 200 mL
Volume of Ozone Stock Added 8.27 mL
Total Solution Volume: 208.27
Lab Temperature: 22 C

| Ozone Stock Solution Conc, mg/L: | | | | |
|---|---|---|---|---|
| | Initial: | 31.70 | 29.3000 | 29.9 |
| | Final: | 30.10 | 30.4000 | 29.4 |
| | Average: | 30.13 | | |

Transferred Ozone Dose, mg/L: 1.20

**Ozone Residual By Direct UV Absorbance @ 260 nm**

Time of O3 Dose: 93 s

Decay Model Parameters: k1 0.57 min-1
Co 0.82 mg/L

| Time (s) | Adjusted Time (min) | Abs @ 260 | Measured O3 Conc., mg/L | Predicted O3 Conc., mg/L | Square Error |
|---|---|---|---|---|---|
| 99 | 0.10 | 0.096462 | 0.77 | 0.77 | 0.0000 |
| 125 | 0.53 | 0.0851 | 0.61 | 0.60 | 0.0000 |
| 156 | 1.05 | 0.07451 | 0.45 | 0.45 | 0.0000 |
| 186 | 1.55 | 0.0665 | 0.34 | 0.34 | 0.0000 |
| 218 | 2.08 | 0.05978 | 0.24 | 0.25 | 0.0000 |

Average Baseline 0.04309 SSE= 0.0001

Calculated Ozone Demand:
Based on Estimated Co: 0.38 mg/L
Based on Measured Co: 0.43 mg/L

**Ozone Residual By Indigo Trisulphonate Method**

Time of O3 Dose: 0 s

Decay Model Parameters: k1 0.62 min-1
Co 1.09 mg/L

| Time (s) | Adjusted Time (min) | Abs @ 600 | Sample Volume, mL | Indigo Solution Vol, mL | Measured O3 Conc., mg/L | Predicted O3 Conc., mg/L | Square Error |
|---|---|---|---|---|---|---|---|
| 15 | 0.25 | 0.858 | 10 | 10 | 0.98 | 0.93 | 0.0024 |
| 53 | 0.88 | 0.932 | 10 | 10 | 0.63 | 0.63 | 0.0000 |
| 90 | 1.50 | 0.591 | 10 | 5 | 0.43 | 0.43 | 0.0000 |

Blank Absorbance: 1.064
0.711 SSE= 0.0000

Calculated Ozone Demand:
Based on Estimated Co: 0.11 mg/L
Based on Measured Co: 0.22 mg/L

## Trial 858 - Ozone Residual Decay in a Natural Water

Date of Test: 11/9/99
Water Sample: Settled Water From Rosedale WTP, Collected Oct. 21/99, filtered

Initial Water Sample Volume: 200 mL
Volume of Ozone Stock Added 6.74 mL
Total Solution Volume: 206.74
Lab Temperature: 22 C

Ozone Stock Solution Conc, mg/L:

| | | | |
|---|---|---|---|
| Initial: | 38.60 | 38.8000 | 38.9 |
| Final: | 38.20 | 37.5000 | 35.9 |
| Average: | 37.32 | | |

Transferred Ozone Dose, mg/L: 1.22

**Ozone Residual By Direct UV Absorbance @ 260 nm**

Time of O3 Dose: 173 s

Decay Model Parameters: k1 0.34 min-1
Co 0.92 mg/L

| Time (s) | Adjusted Time (min) | Abs @ 260 | Measured O3 Conc., mg/L | Predicted O3 Conc., mg/L | Square Error |
|---|---|---|---|---|---|
| 180 | 0.12 | 0.09641 | 0.87 | 0.89 | 0.0002 |
| 193 | 0.33 | 0.09393 | 0.84 | 0.82 | 0.0002 |
| 214 | 0.68 | 0.08681 | 0.73 | 0.73 | 0.0000 |
| 243 | 1.17 | 0.07932 | 0.63 | 0.62 | 0.0000 |
| 267 | 1.57 | 0.07332 | 0.54 | 0.54 | 0.0000 |
| 285 | 1.87 | 0.06987 | 0.49 | 0.49 | 0.0000 |

Average Baseline 0.036

SSE= 0.0004

Calculated Ozone Demand:
Based on Estimated Co: 0.30 mg/L
Based on Measured Co: 0.34 mg/L

**Ozone Residual By Indigo Trisulphonate Method**

Time of O3 Dose: 0 s

Decay Model Parameters: k1 0.37 min-1
Co 1.20 mg/L

| Time (s) | Adjusted Time (min) | Abs @ 600 | Sample Volume, mL | Indigo Solution Vol, mL | Measured O3 Conc., mg/L | Predicted O3 Conc., mg/L | Square Error |
|---|---|---|---|---|---|---|---|
| 19 | 0.32 | 0.811 | 10 | 10 | 1.16 | 1.07 | 0.0071 |
| 46 | 0.77 | 0.863 | 10 | 10 | 0.91 | 0.91 | 0.0000 |
| 90 | 1.50 | 0.501 | 10 | 5 | 0.70 | 0.70 | 0.0000 |
| 115 | 1.92 | 0.529 | 10 | 5 | 0.60 | 0.60 | 0.0000 |

Blank Absorbance: 1.054
0.696

SSE= 0.0000

Calculated Ozone Demand:
Based on Estimated Co: 0.01 mg/L
Based on Measured Co: 0.06 mg/L

## Trial 869 - Ozone Residual Decay in a Natural Water

Date of Test: 11/22/99
Water Sample: Settled Water From Rossdale WTP, Collected Oct. 21/99, filtered

Initial Water Sample Volume: 200 mL
Volume of Ozone Stock Added 12.94 mL
Total Solution Volume: 212.94
Lab Temperature: 22 C

| Ozone Stock Solution Conc, mg/L: | | | | |
|---|---|---|---|---|
| | Initial: | 40.70 | 42.1000 | 40.3 |
| | Final: | 41.00 | 39.8000 | 38.2 |
| | Average: | 40.35 | | |

Transferred Ozone Dose, mg/L: 2.45

**Ozone Residual By Direct UV Absorbance @ 260 nm**

Time of O3 Dose: 127 s

Decay Model Parameters: k1 0.56 min-1
Co 2.16 mg/L

| Time (s) | Adjusted Time (min) | Abs @ 260 | Measured O3 Conc., mg/L | Predicted O3 Conc., mg/L | Square Error |
|---|---|---|---|---|---|
| 149 | 0.37 | 0.14291 | 1.76 | 1.76 | 0.0000 |
| 174 | 0.78 | 0.11848 | 1.41 | 1.40 | 0.0002 |
| 200 | 1.22 | 0.09563 | 1.08 | 1.10 | 0.0003 |
| 223 | 1.60 | 0.08116 | 0.87 | 0.89 | 0.0002 |
| 267 | 2.33 | 0.06145 | 0.59 | 0.59 | 0.0000 |
| 336 | 3.48 | 0.04379 | 0.33 | 0.31 | 0.0004 |
| 434 | 5.12 | 0.0307 | 0.14 | 0.13 | 0.0003 |

Average Baseline 0.02086
SSE= 0.0014

Calculated Ozone Demand:
Based on Estimated Co: 0.29 mg/L
Based on Measured Co: 0.69 mg/L

**Ozone Residual By Indigo Trisulphonate Method**

Time of O3 Dose: 0 s

Decay Model Parameters: k1 0.57 min-1
Co 2.46 mg/L

| Time (s) | Adjusted Time (min) | Abs @ 600 | Sample Volume, mL | Indigo Solution Vol, mL | Measured O3 Conc., mg/L | Predicted O3 Conc., mg/L | Square Error |
|---|---|---|---|---|---|---|---|
| 28 | 0.47 | 0.631 | 10 | 10 | 2.03 | 1.89 | 0.0196 |
| 69 | 1.15 | 0.787 | 10 | 10 | 1.29 | 1.28 | 0.0000 |
| 147 | 2.45 | 0.535 | 10 | 5 | 0.60 | 0.62 | 0.0001 |
| 233 | 3.88 | 0.624 | 10 | 5 | 0.29 | 0.27 | 0.0002 |

Blank Absorbance: 1.057
0.704
SSE= 0.0003

Calculated Ozone Demand:
Based on Estimated Co: -0.01 mg/L
Based on Measured Co: 0.42 mg/L

## Trial 872 - Ozone Residual Decay in a Natural Water

Date of Test: 11/23/99
Water Sample: Settled Water From Rosedale WTP, Collected Oct. 21/99, filtered

Initial Water Sample Volume: 200 mL
Volume of Ozone Stock Added 12.93 mL
Total Solution Volume: 212.93
Lab Temperature: 22 C

| Ozone Stock Solution Conc, mg/L: | | | | |
|---|---|---|---|---|
| | Initial: | 41.70 | 41.4000 | 41.2 |
| | Final: | 40.90 | 37.9000 | 39.9 |
| | Average: | 40.50 | | |

Transferred Ozone Dose, mg/L: 2.46

**Ozone Residual By Direct UV Absorbance @ 260 nm**

Time of O3 Dose: 182 s

Decay Model Parameters: k1 0.31 min-1
Co 2.22 mg/L

| Time (s) | Adjusted Time (min) | Abs @ 260 | Measured O3 Conc., mg/L | Predicted O3 Conc., mg/L | Square Error |
|---|---|---|---|---|---|
| 207 | 0.42 | 0.16066 | 1.71 | 1.95 | 0.0591 |
| 232 | 0.83 | 0.14001 | 1.41 | 1.72 | 0.0951 |
| 265 | 1.38 | 0.11676 | 1.07 | 1.45 | 0.1435 |
| 297 | 1.92 | 0.10006 | 0.83 | 1.24 | 0.1613 |
| 339 | 2.62 | 0.09395 | 0.75 | 1.00 | 0.0638 |
| 397 | 3.58 | 0.08689 | 0.38 | 0.74 | 0.1295 |
| 469 | 5.12 | 0.08097 | 0.27 | 0.47 | 0.0387 |

Average Baseline 0.04237

SSE= 0.6910

Calculated Ozone Demand:
Based on Estimated Co: 0.24 mg/L
Based on Measured Co: 0.75 mg/L

**Ozone Residual By Indigo Trisulphonate Method**

Time of O3 Dose: 0 s

Decay Model Parameters: k1 0.55 min-1
Co 2.63 mg/L

| Time (s) | Adjusted Time (min) | Abs @ 600 | Sample Volume, mL | Indigo Solution Vol, mL | Measured O3 Conc., mg/L | Predicted O3 Conc., mg/L | Square Error |
|---|---|---|---|---|---|---|---|
| 25 | 0.42 | 0.607 | 10 | 10 | 2.17 | 2.09 | 0.0064 |
| 57 | 0.95 | 0.736 | 10 | 10 | 1.55 | 1.56 | 0.0001 |
| 140 | 2.33 | 0.498 | 10 | 5 | 0.76 | 0.72 | 0.0016 |
| 220 | 3.67 | 0.627 | 10 | 5 | 0.30 | 0.35 | 0.0018 |

Blank Absorbance: 1.063
0.712

SSE= 0.0035

Calculated Ozone Demand:
Based on Estimated Co: -0.17 mg/L
Based on Measured Co: 0.29 mg/L

## Trial 875 - Ozone Residual Decay in a Natural Water

Date of Test: 11/24/99 pH= 6.0500
Water Sample: Settled Water From Rossdale WTP, Collected Oct. 21/99, filtered

Initial Water Sample Volume: 200 mL
Volume of Ozone Stock Added 8.21 mL
Total Solution Volume: 208.21
Lab Temperature: 25 C

| Ozone Stock Solution Conc, mg/L: | | | | |
|---|---|---|---|---|
| | Initial: | 38.60 | 38.3000 | 38 |
| | Final: | 36.60 | 37.5000 | 36.3 |
| | Average: | 37.55 | | |

Transferred Ozone Dose, mg/L: 1.48

**Ozone Residual By Direct UV Absorbance @ 260 nm**

Time of O3 Dose: 36 s

Decay Model Parameters: k1 0.18 min-1
Co 1.40 mg/L
Cl 0.73 mg/L

| Time (s) | Adjusted Time (min) | Abs @ 260 | Measured O3 Conc., mg/L | Predicted O3 Conc., mg/L | Square Error |
|---|---|---|---|---|---|
| 51 | 0.25 | 0.131 | 1.30 | 1.34 | 0.0016 |
| 102 | 1.10 | 0.11957 | 1.13 | 1.14 | 0.0001 |
| 143 | 1.78 | 0.1105 | 1.00 | 1.01 | 0.0001 |
| 198 | 2.70 | 0.10414 | 0.91 | 0.85 | 0.0031 |
| 259 | 3.72 | 0.09868 | 0.83 | 0.71 | 0.0148 |
| 301 | 4.42 | 0.09709 | 0.81 | 0.62 | 0.0338 |
| 337 | 5.02 | 0.09328 | 0.75 | 0.56 | 0.0375 |
| 347 | 5.18 | 0.04289 | 0.02 | 0.54 | 0.2679 |

Average Baseline 0.041275
SSE= 0.3590

Calculated Ozone Demand:
Based on Estimated Co: 0.06 mg/L
Based on Measured Co: 0.18 mg/L

**Ozone Residual By Indigo Trisulphonate Method**

Time of O3 Dose: 0 s

Decay Model Parameters: k1 0.13 min-1
Co 1.80 mg/L

| Time (s) | Adjusted Time (min) | Abs @ 600 | Sample Volume, mL | Indigo Solution Vol, mL | Measured O3 Conc., mg/L | Predicted O3 Conc., mg/L | Square Error |
|---|---|---|---|---|---|---|---|
| 18 | 0.30 | 0.757 | 10 | 10 | 1.42 | 1.54 | 0.0146 |
| 58 | 0.97 | 0.751 | 10 | 10 | 1.45 | 1.42 | 0.0011 |
| 179 | 2.98 | 0.424 | 10 | 5 | 0.99 | 1.10 | 0.0108 |
| 267 | 4.45 | 0.427 | 10 | 5 | 0.98 | 0.91 | 0.0053 |

Blank Absorbance: 1.056
0.702
SSE= 0.0172

Calculated Ozone Demand:
Based on Estimated Co: -0.12 mg/L
Based on Measured Co: 0.06 mg/L

## Trial 878 - Ozone Residual Decay In a Natural Water

Date of Test: 11/25/99 pH= 5.9500
Water Sample: Settled Water From Rossdale WTP, Collected Oct. 21/99, filtered

Initial Water Sample Volume: 200 mL
Volume of Ozone Stock Added 7.87 mL
Total Solution Volume: 207.87
Lab Temperature: 24 C

Ozone Stock Solution Conc, mg/L:

| | | | |
|---|---|---|---|
| Initial: | 42.30 | 41.7000 | 40.3 |
| Final: | 41.30 | 40.9000 | 40.1 |
| Average: | 41.10 | | |

Transferred Ozone Dose, mg/L: 1.56

**Ozone Residual By Direct UV Absorbance @ 260 nm**

Time of O3 Dose: 87 s

Decay Model Parameters: k1 0.12 min-1
Co 1.39 mg/L

| Time (s) | Adjusted Time (min) | Abs @ 260 | Measured O3 Conc., mg/L | Predicted O3 Conc., mg/L | Square Error |
|---|---|---|---|---|---|
| 95 | 0.13 | 0.13882 | 1.42 | 1.37 | 0.0025 |
| 101 | 0.23 | 0.13269 | 1.33 | 1.36 | 0.0005 |
| 146 | 1.02 | 0.12762 | 1.26 | 1.23 | 0.0006 |
| 183 | 1.60 | 0.1152 | 1.08 | 1.15 | 0.0051 |
| 240 | 2.55 | 0.11029 | 1.01 | 1.03 | 0.0004 |
| 302 | 3.58 | 0.10475 | 0.93 | 0.91 | 0.0004 |
| 350 | 4.38 | 0.09663 | 0.81 | 0.83 | 0.0002 |
| 388 | 5.02 | 0.0959 | 0.80 | 0.77 | 0.0012 |

Average Baseline 0.04042

SSE= 0.0109

Calculated Ozone Demand:
Based on Estimated Co: 0.16 mg/L
Based on Measured Co: 0.13 mg/L

**Ozone Residual By Indigo Trisulphonate Method**

Time of O3 Dose: 0 s

Decay Model Parameters: k1 0.13 min-1
Co 1.74 mg/L

| Time (s) | Adjusted Time (min) | Abs @ 600 | Sample Volume, mL | Indigo Solution Vol, mL | Measured O3 Conc., mg/L | Predicted O3 Conc., mg/L | Square Error |
|---|---|---|---|---|---|---|---|
| 22 | 0.37 | 0.72 | 10 | 10 | 1.58 | 1.65 | 0.0058 |
| 56 | 0.93 | 0.724 | 10 | 10 | 1.56 | 1.53 | 0.0006 |
| 171 | 2.85 | 0.388 | 10 | 5 | 1.12 | 1.19 | 0.0046 |
| 278 | 4.63 | 0.427 | 10 | 5 | 0.98 | 0.93 | 0.0020 |

Blank Absorbance: 1.051
0.701

SSE= 0.0072

Calculated Ozone Demand:
Based on Estimated Co: -0.16 mg/L
Based on Measured Co: -0.02 mg/L

## Trial 912 - Ozone Residual Decay In a Natural Water

Date of Test: 1/17/00 pH= 6.0500
Water Sample: Settled Water From Rossdale WTP, Collected Oct. 21/99, filtered

Initial Water Sample Volume: 200 mL
Volume of Ozone Stock Added 5.04 mL
Total Solution Volume: 205.04
Lab Temperature: 22 C

Ozone Stock Solution Conc, mg/L:

| | | | |
|---|---|---|---|
| Initial: | 39.50 | 39.6000 | 39.8 |
| Final: | 39.10 | 39.5000 | 37.9 |
| Average: | 39.23 | | |

Transferred Ozone Dose, mg/L: 0.96

### Ozone Residual By Direct UV Absorbance @ 260 nm

Time of O3 Dose: 24 s

Decay Model Parameters: k1 0.19 min-1
Co 0.83 mg/L

| Time (s) | Adjusted Time (min) | Abs @ 260 | Measured O3 Conc., mg/L | Predicted O3 Conc., mg/L | Square Error |
|---|---|---|---|---|---|
| 36 | 0.20 | 0.09744 | 0.80 | 0.80 | 0.0000 |
| 47 | 0.36 | 0.09512 | 0.77 | 0.77 | 0.0000 |
| 64 | 0.67 | 0.09288 | 0.74 | 0.73 | 0.0000 |
| 92 | 1.13 | 0.08812 | 0.67 | 0.67 | 0.0000 |
| 117 | 1.55 | 0.08444 | 0.62 | 0.62 | 0.0001 |
| 141 | 1.95 | 0.08244 | 0.59 | 0.58 | 0.0001 |

Average Baseline 0.041835

SSE= 0.0002

Calculated Ozone Demand:
Based on Estimated Co: 0.13 mg/L
Based on Measured Co: 0.16 mg/L

### Ozone Residual By Indigo Trisulphonate Method

Time of O3 Dose: 0 s

Decay Model Parameters: k1 0.09 min-1
Co 0.78 mg/L

| Time (s) | Adjusted Time (min) | Abs @ 600 | Sample Volume, mL | Indigo Solution Vol, mL | Measured O3 Conc., mg/L | Predicted O3 Conc., mg/L | Square Error |
|---|---|---|---|---|---|---|---|
| 15 | 0.25 | 0.261 | 10 | 10 | 0.92 | 0.76 | 0.0264 |
| 40 | 0.67 | 0.304 | 10 | 10 | 0.72 | 0.73 | 0.0002 |
| 79 | 1.32 | 0.11 | 10 | 5 | 0.73 | 0.70 | 0.0014 |
| 111 | 1.85 | 0.135 | 10 | 5 | 0.64 | 0.66 | 0.0005 |

Blank Absorbance: 0.455
0.315

SSE= 0.0021

Calculated Ozone Demand:
Based on Estimated Co: 0.19 mg/L
Based on Measured Co: 0.04 mg/L

## Trial 915 - Ozone Residual Decay In a Natural Water

Date of Test: 1/18/00 pH= 5.9700
Water Sample: Settled Water From Rosedale WTP, Collected Oct. 21/99, filtered

Initial Water Sample Volume: 200 mL
Volume of Ozone Stock Added 7.66 mL
Total Solution Volume: 207.66
Lab Temperature: 22 C

| Ozone Stock Solution Conc, mg/L: | | | | |
|---|---|---|---|---|
| | Initial: | 41.70 | 38.9000 | 40.2 |
| | Final: | 39.80 | 39.5000 | 39.4 |
| | Average: | 39.92 | | |

Transferred Ozone Dose, mg/L: 1.47

**Ozone Residual By Direct UV Absorbance @ 260 nm**

Time of O3 Dose: 22 s

Decay Model Parameters: k1 0.10 min-1
Co 1.23 mg/L

| Time (s) | Adjusted Time (min) | Abs @ 260 | Measured O3 Conc., mg/L | Predicted O3 Conc., mg/L | Square Error |
|---|---|---|---|---|---|
| 36 | 0.23 | 0.11919 | 1.20 | 1.20 | 0.0000 |
| 58 | 0.60 | 0.11618 | 1.16 | 1.15 | 0.0000 |
| 101 | 1.32 | 0.11053 | 1.08 | 1.07 | 0.0000 |
| 132 | 1.83 | 0.10556 | 1.01 | 1.02 | 0.0001 |
| 172 | 2.50 | 0.10167 | 0.95 | 0.95 | 0.0000 |
| 227 | 3.42 | 0.09401 | 0.84 | 0.86 | 0.0005 |
| 264 | 4.03 | 0.09204 | 0.81 | 0.81 | 0.0000 |
| 296 | 4.60 | 0.09024 | 0.78 | 0.76 | 0.0005 |
| 327 | 5.08 | 0.08601 | 0.72 | 0.72 | 0.0000 |

Average Baseline 0.036

SSE= 0.0012

Calculated Ozone Demand:
Based on Estimated Co: 0.24 mg/L
Based on Measured Co: 0.27 mg/L

**Ozone Residual By Indigo Trisulphonate Method**

Time of O3 Dose: 0 s

Decay Model Parameters: k1 0.10 min-1
Co 1.33 mg/L

| Time (s) | Adjusted Time (min) | Abs @ 600 | Sample Volume, mL | Indigo Solution Vol, mL | Measured O3 Conc., mg/L | Predicted O3 Conc., mg/L | Square Error |
|---|---|---|---|---|---|---|---|
| 20 | 0.33 | 0.132 | 10 | 10 | 1.30 | 1.28 | 0.0005 |
| 105 | 1.75 | 0.165 | 10 | 10 | 1.15 | 1.12 | 0.0010 |
| 180 | 3.00 | 0.006 | 10 | 5 | 0.92 | 0.99 | 0.0043 |
| 270 | 4.50 | 0.016 | 10 | 5 | 0.89 | 0.85 | 0.0012 |

Blank Absorbance: 0.406
0.264

SSE= 0.0065

Calculated Ozone Demand:
Based on Estimated Co: 0.15 mg/L
Based on Measured Co: 0.17 mg/L

## Trial 918 - Ozone Residual Decay in a Natural Water

Date of Test: 1/19/00 pH= 5.9800
Water Sample: Settled Water From Rosedale WTP, Collected Oct. 21/99, filtered

Initial Water Sample Volume: 200 mL
Volume of Ozone Stock Added 4.85 mL
Total Solution Volume: 204.85
Lab Temperature: 22 C

Ozone Stock Solution Conc, mg/L:

| | | | |
|---|---|---|---|
| Initial: | 43.10 | 41.8000 | 40.5 |
| Final: | 41.40 | 40.9000 | 41.3 |
| Average: | 41.50 | | |

Transferred Ozone Dose, mg/L: 0.98

**Ozone Residual By Direct UV Absorbance @ 260 nm**

Time of O3 Dose: 27 s

Decay Model Parameters: k1 0.12 min-1
Co 0.75 mg/L

| Time (s) | Adjusted Time (min) | Abs @ 260 | Measured O3 Conc., mg/L | Predicted O3 Conc., mg/L | Square Error |
|---|---|---|---|---|---|
| 39 | 0.20 | 0.08463 | 0.72 | 0.74 | 0.0004 |
| 54 | 0.45 | 0.08725 | 0.76 | 0.72 | 0.0016 |
| 70 | 0.72 | 0.08217 | 0.68 | 0.69 | 0.0001 |
| 94 | 1.12 | 0.08002 | 0.65 | 0.66 | 0.0001 |
| 131 | 1.73 | 0.07753 | 0.61 | 0.62 | 0.0000 |
| 146 | 1.98 | 0.07686 | 0.60 | 0.60 | 0.0000 |

Average Baseline 0.035

SSE= 0.0023

Calculated Ozone Demand:
Based on Estimated Co: 0.23 mg/L
Based on Measured Co: 0.27 mg/L

**Ozone Residual By Indigo Trisulphonate Method**

Time of O3 Dose: 0 s

Decay Model Parameters: k1 0.34 min-1
Co 0.97 mg/L

| Time (s) | Adjusted Time (min) | Abs @ 600 | Sample Volume, mL | Indigo Solution Vol, mL | Measured O3 Conc., mg/L | Predicted O3 Conc., mg/L | Square Error |
|---|---|---|---|---|---|---|---|
| 19 | 0.32 | 0.247 | 10 | 10 | 0.78 | 0.87 | 0.0081 |
| 45 | 0.75 | 0.243 | 10 | 10 | 0.80 | 0.75 | 0.0024 |
| 70 | 1.17 | 0.241 | 10 | 5 | 0.61 | 0.65 | 0.0019 |
| 93 | 1.55 | 0.236 | 10 | 5 | 0.63 | 0.57 | 0.0030 |

Blank Absorbance: 0.411
0.411

SSE= 0.0073

Calculated Ozone Demand:
Based on Estimated Co: 0.01 mg/L
Based on Measured Co: 0.20 mg/L

## Trial 921 - Ozone Residual Decay in a Natural Water

Date of Test: 1/20/00 pH= 8.0200
Water Sample: Settled Water From Rossdale WTP, Collected Oct. 21/99, filtered

Initial Water Sample Volume: 200 mL
Volume of Ozone Stock Added 7.32 mL
Total Solution Volume: 207.32
Lab Temperature: 22 C

| Ozone Stock Solution Conc, mg/L: | | | | |
|---|---|---|---|---|
| | Initial: | 42.30 | 41.6000 | 42 |
| | Final: | 43.00 | 41.1000 | 42 |
| | Average: | 42.00 | | |

Transferred Ozone Dose, mg/L: 1.46

**Ozone Residual By Direct UV Absorbance @ 260 nm**

Time of O3 Dose: 34 s

Decay Model Parameters: k1 0.08 min-1
Co 1.42 mg/L

| Time (s) | Adjusted Time (min) | Abs @ 260 | Measured O3 Conc., mg/L | Predicted O3 Conc., mg/L | Square Error |
|---|---|---|---|---|---|
| 45 | 0.18 | 0.12617 | 1.39 | 1.40 | 0.0001 |
| 85 | 0.52 | 0.12592 | 1.39 | 1.38 | 0.0005 |
| 133 | 1.65 | 0.11659 | 1.25 | 1.24 | 0.0000 |
| 179 | 2.42 | 0.10887 | 1.14 | 1.17 | 0.0009 |
| 244 | 3.50 | 0.10475 | 1.08 | 1.07 | 0.0001 |
| 293 | 4.32 | 0.09912 | 1.00 | 1.00 | 0.0000 |
| 335 | 5.02 | 0.09641 | 0.96 | 0.95 | 0.0001 |

Average Baseline 0.03
SSE= 0.0018

Calculated Ozone Demand:
Based on Estimated Co: 0.06 mg/L
Based on Measured Co: 0.09 mg/L

**Ozone Residual By Indigo Trisulphonate Method**

Time of O3 Dose: 0 s

Decay Model Parameters: k1 0.18 min-1
Co 1.84 mg/L

| Time (s) | Adjusted Time (min) | Abs @ 600 | Sample Volume, mL | Indigo Solution Vol, mL | Measured O3 Conc., mg/L | Predicted O3 Conc., mg/L | Square Error |
|---|---|---|---|---|---|---|---|
| 19 | 0.32 | 0.075 | 10 | 10 | 1.60 | 1.74 | 0.0182 |
| 92 | 1.53 | 0.107 | 10 | 10 | 1.45 | 1.40 | 0.0026 |
| 168 | 2.80 | 0.13 | 10 | 5 | 1.01 | 1.12 | 0.0127 |
| 266 | 4.43 | 0.159 | 10 | 5 | 0.90 | 0.84 | 0.0043 |

Blank Absorbance: 0.412
0.412
SSE= 0.0195

Calculated Ozone Demand:
Based on Estimated Co: -0.36 mg/L
Based on Measured Co: -0.12 mg/L