University of Alberta

Effect of turbidity on ultraviolet (UV) inactivation of *Cryptosporidium parvum* and *Giardia muris* in an unfiltered drinking water

by

Kingsford Amoah 

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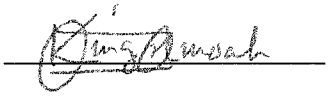

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research for acceptance, a thesis entitled **Effect of turbidity on ultraviolet (UV) inactivation of *Cryptosporidium parvum* and *Giardia muris* in an unfiltered drinking water** submitted by **Kingsford Amoah** in partial fulfillment of the requirements for the degree of Master of Science in Environmental Engineering.

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ABSTRACT

Current research has shown that ultraviolet (UV) light may be effective against the waterborne pathogens, *Cryptosporidium parvum* and *Giardia* spp., but issues surrounding the effects of turbidity are largely undefined. The impact of turbidity on ultraviolet inactivation of *Cryptosporidium parvum* oocysts and *Giardia muris* cysts was studied in samples of unfiltered lake water. Using a modified collimated beam exposure method, parasite suspensions with turbidities ranging from 0.25 to 20.0 NTU were exposed to controlled UV doses of 5 and 40 mJ/cm^2 from a medium pressure lamp at bench-scale. Inactivation of parasites was measured using established mouse infectivity models. Mean inactivation of *C. parvum* and *G. muris* decreased by 0.8 log-unit and 0.4 log-unit, respectively, when turbidity was increased from 0.25 to 20.0 NTU at the UV doses investigated. This turbidity effect was statistically significant at the 95% confidence level for *C. parvum* oocysts but was marginally insignificant for *G. muris* cysts.

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DEDICATION

The author dedicates this thesis to the members of his family, for their encouragement in his pursuit of academic excellence.

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LIST OF ABBREVIATIONS

%T	percent transmittance
ANOVA	analysis of variance
AWWA	American Water Works Association
CI	Confidence Interval
DAPI	4', 6-diaminio-2-phenylindole stain
DNA	deoxyribonucleic acid
ID_{50}	parasite dose required for a 50% rate of infection
IESWTR	Interim Enhanced Surface Water Treatment Rule
ln	natural logarithm
LOF	lack of fit
Log	logarithm
LP	low-pressure ultraviolet lamp
LT1ESWTR	Long Term 1 Enhanced Surface Water Treatment Rule
M	molar concentration
MP	medium-pressure ultraviolet lamp
MS	mean square
na	not applicable
nd	not done
NTU	Nephelometric Turbidity Units
pH	negative logarithm of effective hydrogen ion concentration
PI	propidium iodide
RNA	ribonucleic acid
SE	Standard Error
SS	sum of squares
STDEV	Standard Deviation
SWTR	Surface Water Treatment Rule
UK	United Kingdom
USEPA	United States Environmental Protection Agency
UV	Ultraviolet
WQA	Water Quality Association

LIST OF SYMBOLS

α	acceptable probability level for hypothesis testing
β_1	parameter of the predicted linear model
β_2	parameter of the predicted linear model
°C	degrees Celsius
µm	micrometres
β_o	parameter of the predicted linear model
A	absorbance, %
d	number of live (oo) cysts in inoculum estimated from infectivity model
d_o	total number of (oo)cysts in inoculum based on hemacytometer counts
E	depth averaged irradiance, mW/cm^2
E_g	germicidal average irradiance, mW/cm^2
E_o	incident irradiance, mW/cm^2
F	value of the Fisher-statistic in analysis of variance
g	Gravitational acceleration (= 9.82 m/s^2)
H	total average ultraviolet dose, mJ/cm^2
H_g	germicidal ultraviolet dose, mJ/cm^2
kg	kilograms
L	length, m or litres
L	likelihood function
LP	latent period
m	metres
mg/L	milligram per litre
mL	milliliters
N	concentration of live microorganisms after UV treatment, microbes/mL
N_o	concentration of live microorganisms before UV treatment, microbes/mL
no.	number of measurements
P	proportion of neonatal CD-1 mice that are positive for infection
p-value	probability of type I error in hypothesis testing
s	seconds

1 INTRODUCTION

1.1 EMERGING CHALLENGES IN DRINKING WATER TREATMENT

Chlorine has been the disinfectant of choice for drinking water since its introduction in a hospital in Vienna, Austria (Meulemans, 1987). Although, it has been effective in killing disease-causing bacteria at a relatively low cost, microorganisms like *Giardia lamblia* have been found to be resistant to chlorine disinfection (Rice and Hoff, 1981). *Cryptosporidium* spp., being highly resistant to chlorine has also emerged as one of the most important new contaminants needing control in drinking water in the UK and USA (Smith and Rose, 1998).

Waterborne spread of Giardiasis has been attributed to the environmental resistance and prolonged viability of *Giardia* spp. cysts in water at low temperature, the endemic nature of the *Giardia* spp. infections in humans and animals and cross-species transmission, together with the low infectious dose needed to establish colonization within the new host (Erlandsen, 1994). According to Clark (1999), water treatment deficiencies have been identified in some outbreaks of Cryptosporidiosis but at least one has occurred in association with a modern treatment facility where treatment was well documented. Clark (1999) gave four major factors that are believed to contribute to these outbreaks: (i) the prevalence of *Cryptosporidium* spp. in source water is high; (ii) *Cryptosporidium* spp. oocysts are refractory to chlorine treatment of drinking water; (iii) coarse filtration methods normally performed on surface drinking waters do not efficiently remove *Cryptosporidium* spp. oocysts, due

to their small diameter; and (iv) the infectious dose of *Cryptosporidium* spp. for humans is extremely low.

1.2 ULTRAVIOLET (UV) LIGHT AS AN ALTERNATIVE DISINFECTANT

The removal and inactivation of (oo)cysts of *Cryptosporidium* and *Giardia* spp. from raw water is complicated by their small size and resistance to commonly used oxidants such as chlorine. Unlike most disinfectants, UV radiation inactivates microorganisms by a physical reaction. UV radiation inactivates organisms by absorption of the light which causes a photochemical reaction that alters molecular components essential to cell function (Jagger, 1967; Meulemans, 1987). Although UV light was known to effectively inactivate *Cryptosporidium* spp. and *Giardia* spp. (oo)cysts, it was thought that high dosages were required (Rice and Hoff, 1981; Carlson *et al.*, 1985). Recent studies have however shown that encysted *C. parvum* and *G. lamblia* are quite sensitive to UV radiation and that the methods of viability used in earlier studies may have underestimated the degree of inactivation compared with animal infectivity.

1.3 THE CITY OF KELOWNA, BRITISH COLUMBIA

The City of Kelowna is in the interior of British Columbia, Canada and has a land area of 211.22 km^2 (Statistics Canada, 2002). Its population in 2001 was 96,288 (an increase of 7.7 % from 1996 to 2001). It has a very high quality surface water (from Lake Okanagan) supply and employs chlorination as the only form of

treatment. It has three main pump houses, Poplar point, El Dorado and Cedar creek, all on Lake Okanagan (Weaden, 2002). The City of Kelowna was the reference water source for this study.

1.4 THESIS OVERVIEW

The effect of turbidity due to naturally occurring suspended solids on UV inactivation of *Cryptosporidium parvum* and *Giardia muris* was investigated by first reviewing available literature on *C. parvum*, *G. lamblia/muris* and UV light disinfection. The effect of oocyst concentration on UV inactivation of *C. parvum* oocysts was studied in preliminary collimated beam experiments, using a medium-pressure UV lamp. The effect of parasite concentration was studied to determine if parasite concentration could help explain observed tailing in UV dose-response curves of *C. parvum* and also to assess the use of a low concentration protocol in turbidity experiments. Oocysts purified from Holstein calves were suspended in phosphate buffered de-ionized water and exposed to controlled UV doses of 5 and 40 mJ/cm^2 using a collimated beam apparatus. Parasite inactivation was measured using a neonatal CD-1 mouse model.

For suspended solids experiments, aliquots of concentrated solids were added to samples of unfiltered water from Lake Okanagan, Kelowna, BC, to simulate turbidity levels of 0.25, 5.0, 10.0 and 20.0 NTU. Bench-scale experiments were conducted, with a collimated beam apparatus, using unfiltered drinking water from Lake Okanagan, Kelowna, BC with *C. parvum* and *G. muris* (oo)cysts added to the water. UV exposures were conducted at controlled UV doses of 5 and 40 mJ/cm^2.

Parasite inactivations were determined using infectivity in animal models: neonatal CD-1 mouse for *C. parvum* and C3H/HeN mouse model for *G. muris*. The outcomes of the experimental trials were analyzed using two-way analysis of variance and regression.

2 LITERATURE REVIEW

2.1 DEFINITIONS AND UNITS

2.1.1 Encysted Protozoa

Before looking at *Cryptosporidium parvum* and *Giardia lamblia/muris* and ultraviolet light disinfection, it will help to understand some terms used in water treatment studies (WQA, 2002). *Disinfection* is the treatment of water to inactivate, destroy, and/or remove pathogenic (disease-producing) bacteria, viruses, cysts, and other microorganisms (but not completely eliminating all microorganisms) for the purpose of making the water microbiologically safe for human consumption.

Protozoa are microscopic, single-celled microorganisms which live in water and are relatively large in comparison to bacteria and other microbes. Protozoa are higher in the food chain than the bacteria that they eat. Many protozoa are parasitic to humans.

Cysts and oocysts are fertilized eggs forms of parasitic protozoa, of *G. lamblia/muris* and *C. parvum*, respectively, that are encapsulated in tough shells. Cysts and oocysts are the environmentally resistant transmissible form of the protozoan parasites and excreted in the feces of an infected host and may remain viable in water supplies.

In vitro (in glass) is a laboratory experiment performed in a test tube or other vessel using tissues, cells or subcellular extracts from an organism.

In vivo (within a living organism) is a laboratory experiment performed in which the substance under study is inserted into a living organism.

Dose-response is the quantitative relationship between the amount of exposure to a substance and the extent of toxic injury produced.

Latent period is the time from the first exposure to a chemical or microorganism until the appearance of an effect.

2.1.2 Ultraviolet Light

Some definitions of UV radiation terms (Verhoeven, 1996): *Irradiance (E)* is defined as the total radiant power of wavelengths incident on an infinitesimal element of surface area, d*S* containing the point under consideration, divided by d*S*. The SI unit of irradiance is W m^{-2} but $mWcm^{-2}$ (= 10 W m^{-2}), which is in common usage in North America, will be used in this work.

UV Dose (H) is defined as the total radiant energy of all wavelengths passing from all directions through an infinitesimally small sphere of cross-sectional area, d*A*, divided by d*A*. The UV dose is the irradiance times the irradiation time in seconds for a collimated beam of UV light. The SI unit of UV dose is Jm^{-2} but the equivalent $mJcm^{-2}$ will be used in this work.

Absorbance (A) is the logarithm to the base 10 of the ratio of the spectral radiant power of incident, essentially monochromatic, radiation (P_λ^o) to the radiant power of transmitted radiation (P_λ):

$$A = \log (P_\lambda^o / P_\lambda) = - \log T \qquad \text{Equation 2-1}$$

In practice, absorbance is the logarithm to the base 10 of the ratio of the spectral radiant power of light transmitted through the reference sample to that of light transmitted through the solution, both observed in identical cells. T is the (internal) transmittance.

The *collimated beam apparatus* is illustrated in Figure 2-1. It was described by Bolton (1999). The UV lamp is housed in the upper cabinet and is separated from the long collimator by a pneumatically driven shutter. The inner walls of the collimator are often painted a flat black, so that any UV light that hits the wall is absorbed. The result is that at the end of the collimator, a reasonably uniform collimated beam of UV can be used to irradiate suspensions of microorganisms.

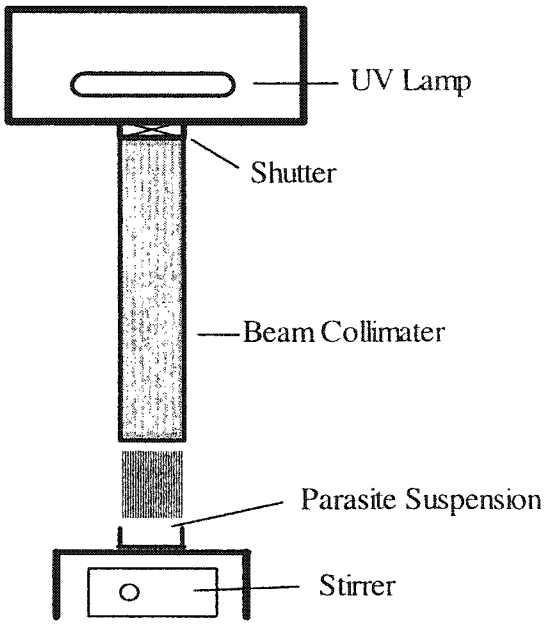

Figure 2-1 UV exposures with a Collimated beam apparatus

2.2 *CRYPTOSPORIDIUM PARVUM* AND *GIARDIA LAMBLIA*

2.2.1 History and Importance

C. parvum and *G. lamblia* are intracellular protozoan parasites that have emerged as important causes of diarrhea among humans and animals. *C. parvum* causes cryptosporidiosis and *G. lamblia,* giardiasis. They are considered to be enteric pathogens with a worldwide distribution.

Meyer *et al.* (1990) have reviewed the history and life cycle of *Giardia* spp. Leewenhoek (1632-1723) was believed to be the first to see and provide a written description of *Giardia* spp. Vilem Lambl (1824-1895), a Czech physician, was credited with the discovery of the flagellate of *Giardia* spp., trophozoites, in 1859. In 1879, the cysts of this organism were first noted by Grassi and he later associated with the flagellate form of the organism. Although reported as a possible waterborne pathogen as early as 1946 in Tokyo, *G. lamblia* was first documented as the causative agent of the intestinal disease, giardiasis, in the United States in 1966 (Moore *et al.*, 1969). *Giardia* spp. is the most commonly reported intestinal parasite in North America and in the world (Farthing, 1989; Adam, 1991).

Tyzzer (1907) first described *Cryptosporidium* spp., which he named *Cryptosporidium muris*, when he isolated it from the gastric glands of mice, in 1907. Tyzzer described much of the morphology and life cycle when he found a second isolate in the intestines of the same species of mice and he named it *Cryptosporidium parvum* (Tyzzer, 1912). In 1971, renewed interest was developed in *C. parvum*, when it was associated with bovine diarrhea (Panciera *et al.*, 1971). The first cases of human cryptosporidiosis were reported in 1976 (Meisel *et al.*, 1976; Nime *et al.*,

1976) and of a waterborne outbreak in 1984 (D'Antonio *et al.*, 1985). Medical, public health and water treatment personnel have become increasingly aware of this parasite and its potential for causing infectious disease especially after the massive Milwaukee, Wis., USA outbreak in 1993 (Sterling and Marshall, 1999). It is now believed to be able to produce a short-term diarrheal illness in immunocompetent persons and life-threatening cholera-like illness in immune deficient patients, especially those with AIDS (Current and Garcia, 1991).

2.2.2 Life Cycles

Both *Giardia* spp. and *Cryptosporidium* spp. are obligate parasites and monoxenus. The *Giardia* spp. cyst is round to oval in dimensions ranging from 8 to 14 μm long by 7 to 10 μm wide, with two or four nuclei and organelles visible (Meyer and Jarroll, 1980). The cysts are transmitted to a new host through contaminated water or ingestion of food contaminated with feces (Feely *et al.*, 1990). Once inside the host, the cyst is stimulated to excyst and to release undifferentiated trophozoites in the duodenum. The trophozoite is an actively feeding, growing, and reproducing stage (Schaefer, 1999). Stimuli in the host's intestinal tract induce *Giardia* spp. to produce a resistant, dormant and environmentally resistant form called the cyst. Cysts can remain viable for several months if they remain wet and cool but cannot withstand desiccation (Feely *et al.*, 1990).

A complete life cycle of *Cryptosporidium* spp. was described by Smith and Rose (1998). *Cryptosporidium* spp. is a coccidian parasite with a life cycle that involves both asexual and sexual reproductive cycles, which it completes within an

individual host (monoxenus). It is in suborder Eimeriorina and family Cryptosporidiidae. The suborder Eimeriorina also contains the medically important coccidian parasites *Isospora belli* and *Toxoplasma* gondii. The transmissible stage is the mature, thick-walled oocyst, 4 to 6 μm in diameter (Fayer *et al.*, 2000). Humans and animals are infected by ingesting these oocysts, which travel through the gut lumen to the small intestines, where they rupture, releasing sporozoites. The sporozoites are motile, they adhere to and invade the absorptive epithelial cells, which line the gastrointestinal tract (Clark, 1999). Sporozoites differentiate into spherical trophozoites. The trophozoite stage divides by asexually forming meront stages containing merozoites. These merozoites can infect neighboring cells or produce stages that will initiate the sexual cycle where zygotes are formed after fertilization. The zygotes then develop into environmentally resistant, thick-walled oocysts. Oocysts are released in feces and can transmit infection from one host to another (Sterling and Marshall, 1999).

2.2.3 Description of the Diseases

G. lamblia is known to produce gastrointestinal distress, including diarrhea, weight loss, flatulence, cramps, belching, distention, anorexia, vomiting, fatigue, mucus in the stool, and bloody and foul-smelling stool. The parasite can produce a continuum of pathologies ranging from no symptoms to illness requiring hospitalization. The time from infection until parasites are seen is called the *prepatent period* and depends on the number of parasites in the dose, as well as the health and susceptibility of the host. The prepatent time was found to be 6 to 16 days (Rendtorff,

1978; Stachan and Kunst, 1983; Nash *et al.*, 1987) and the infective dose could be as low as 1 to 10 cysts (Rendtorff, 1978; Stachan and Kunst, 1983). Rendtorff (1978) determined the ID_{50} (number of cysts ingested resulting in 50% of the test subjects becoming infected) to be 19 cysts, using human-source cysts in humans. Giardiasis infection is diagnosed by finding cysts or trophozoites in feces or by finding trophozoites in duodenojejunal aspirate or biopsy (Wolfe, 1990). *G. lamblia* infections are susceptible to treatment with quinacrine (Atabrine), metronidazole (Flagyl), furizolidone (Furoxone) and paramomycin. The drug of choice depends on the condition of the patient and the experience of the physician (Schaefer, 1999).

The most common symptom of cryptosporidiosis in immunocompetent and immunocompromised persons is profuse and watery diarrhea. It is also associated with weight loss. Other less common symptoms include abdominal pain, nausea, vomiting and low-grade fever. Other nonspecific symptoms are myalgia, weakness, malaise, headache and anorexia (Current and Garcia, 1991). The prepatent time ranged from 2 to 25 days but most symptoms occurred within 3 to 11 days (DuPont *et al.*, 1995). Both the duration of symptoms and outcome vary according to the immune status of the host (Juranek, 1995). In patients with compromised immune systems, the illness may be life-threatening. Most other hosts with intact immune systems are able to clear the infection. Individuals may be infected with as few as 30 oocysts (DuPont, *et al.*, 1995). *Cryptosporidium* spp. infections of a mucosal epithelium result in the release of numerous oocysts. Cryptosporidiosis is diagnosed by finding the environmentally resistant oocyst in specimens of stool, sputum or bile by a variety of diagnostic procedures. There is a lack of effective treatment of cryptosporidiosis in

both immunocompromised and immunocompetent persons (Current and Garcia, 1991).

2.2.4 Mode of Transmission

Studies of experimental infections have demonstrated that *C. parvum* and *G. lamblia* are transmitted by environmentally resistant (oo)cysts (Rice and Hoff, 1981; Current and Garcia, 1991). Giardiasis and cryptosporidiosis may be waterborne, foodborne, transmitted sexually or by the fecal-oral route. Water is the largest potential common source of transmission. Most waterborne outbreaks of giardiasis have been associated with zoonotic transmission; and particularly with beavers (Kirner *et al.*, 1978; Lopez *et al.*, 1980; Lippy, 1981; Isaac-Renton *et al.*, 1993). Human sewage contamination has also been found to be the cause of some waterborne outbreaks of giardiasis (Wallis *et al.*, 1998). Oocysts are more prevalent in calves than in adult animals and infected calves can excrete up to 10^7 oocysts/g feces (Smith and Rose, 1990). Close personal contact also appears to be an important source of transmission. This most likely occurs with person-to-person contact between family and household members, sexual partners, health-care personnel, and day-care employees and attendees. Animal-to-person (zoonosis) route involving pets, farm animals, and laboratory animals has also been observed (Schaefer, 1999; Sterling and Marshall, 1999).

2.2.5 Waterborne Disease Outbreaks

Confirmed outbreaks of waterborne giardiasis in Canada have occurred in British Columbia, Alberta, Ontario, Quebec, New Brunswick and Newfoundland (Health Canada, 1999). There have also been some outbreaks in which giardiasis was suspected but not proven in other communities. Many of these communities were relying on surface water with only chlorination for water treatment but others had filtration plants that were not functioning properly. Wallis *et al.* (1996) carried out a study to estimate the prevalence and potential for human infectivity of *Giardia* spp. cysts in Canadian drinking water supplies and found *Giardia* spp. cysts in 73% of raw sewage samples, 21% of untreated drinking water samples, and 18.2% of treated water samples. Outbreaks have been reported from 24 states in the United States. From 1965 to 1992, 115 outbreaks were reported that resulted in 26,530 known cases of giardiasis in the United States (Moore *et al.*, 1993).

Fayer *et al.* (2000) made a comprehensive review of epidemiology of Cryptosporidium. The first reported waterborne outbreak of cryptosporidiosis, confirmed by stools and serologic tests was in the summer of 1984 in Braun Station, 32 km from San Antonio, Texas. The largest documented waterborne disease outbreak in US history occurred in greater Milwaukee, WI area in 1993. Approximately 403,000 out of approximately 1,610,000 persons were infected (MacKenzie *et al.*, 1994). The prevalence rate of human cryptosporidiosis was found to range from 0.6 to 20% based on a stool survey (Caprioli *et al.*, 1989; Zu *et al.*, 1992; Nimri and Batchoun, 1994). A survey of 1345 Canadian patients revealed a prevalence rate of 1.25% in 1985 (Janoff and Reller, 1987). Infection rates for

patients with AIDS were reported to be at 4% in the United States and 2.5% in Canada (Janoff and Reller, 1987; Soave and Johnson, 1988). Outbreaks associated with community drinking water systems were reported in Ontario (Kitchener-Waterloo and Collingwood) (Welker *et al.*, 1994) and British Columbia (Cranbrook, Kelowna and Chilliwack) (Ong *et al.*, 1997). Nineteen outbreaks were also reported in the United Kingdom and seven in the United States (Craun *et al.*, 1998).

2.2.6 National and International Guidelines

Health Canada has no numerical guidelines proposed for the protozoa *G. lamblia* and *C. parvum* in drinking water (Health Canada, 2001). Currently, it does not recommend monitoring of drinking water because the methods available for routine detection of cysts and oocysts and for the determination of their viability and infectivity in drinking water are considered inadequate. Health Canada recommends that until better monitoring data and information on the viability and infectivity of cysts and oocysts present in drinking water are available, measures to reduce the risk of illness as much as possible should be implemented. If viable cysts or oocysts are present or suspected in treated water and if the protozoan parasites have been responsible for past waterborne outbreaks in a community, a water treatment regime and watershed protection plan (where feasible), or other measures known to reduce the risk of illness, should be implemented (Health Canada, 2001).

Concerning *Giardia* spp., World Health Organization (WHO) guidelines state that drinking water should not contain any pathogenic intestinal protozoa and that drinking water sources not subject to fecal contamination should be used where

possible (Jakubowski, 1990). The United States Environmental Protection Agency (USEPA) finalized the Surface Water Treatment Rule (SWTR) primarily to control waterborne *Giardia* spp. and viruses in public drinking water using either surface water or groundwater under the influence of surface water (LeChevallier *et al.*, 1991). The SWTR requires that systems using surface water or groundwater under the direct influence of surface water to achieve at least 99.9% (3-log) removal/inactivation of *Giardia lamblia* cysts. The Interim Enhanced Surface Water Treatment Rule (IESWTR) and Long Term 1 Enhanced Surface Water Treatment Rule (LT1SWTR) established a treatment technique requiring 99% (2-log) removal of *Cryptosporidium* oocysts from all surface waters, which filter (USEPA, 2002). These Rules also require that unfiltered surface water systems include *Cryptosporidium* spp. as a pathogen of concern in their watershed control programs.

2.3 TURBIDITY

2.3.1 Definition and Source

Turbidity is an expression of the optical property that causes light to be scattered and absorbed rather than transmitted with no change in direction or flux level through the sample (Greenberg *et al.*, 1998). It is therefore a measure of the cloudiness of water. Turbidity in water is caused by suspended solids and colloidal matter such as clay, silt, finely divided organic and inorganic matter, microorganisms, plankton and other macroscopic organisms. Sources of turbidity have been described by (Sawyer *et al.*, 1994). In glacier-fed rivers and lakes, most of the turbidity is due to colloidal rock particles produced by grinding action of glacier. Rivers, which descend

from mountain areas onto plains, receive contributions of turbidity from farming and other operations that disturb the soil. Great amounts of topsoil are washed into receiving streams under flood conditions. Much of this material is inorganic in nature and includes clay and silt, but considerable amounts of organic matter may be included.

2.3.2 Environmental Significance

Turbidity is an important consideration in public water supplies. Once considered as mostly an aesthetic characteristic of drinking water, significant evidence exists that controlling turbidity is a competent safeguard in reducing pathogens in drinking water (LeChevallier and Norton, 1992). Disinfection of public water supplies is usually accomplished by means of chlorine, ozone, chlorine dioxide (Sawyer *et al.*, 1994) or ultraviolet light. According to Hoff (1978), for disinfection to be effective, there must be contact between the agent and the organisms that the disinfectant is to kill. Particles making up part of turbidity may develop particle-organism complexes in which a microorganism is adsorbed onto a larger particle. One or more smaller particles may also be adsorbed onto a microorganism or a particle may completely encase a microorganism. In each of the cases above, varying degrees of protection of microorganisms from the disinfectant may be expected.

2.3.3 Turbidity as a Water Quality Parameter

Turbidity is measured by determining the degree of light scattering by particulates present in a water sample. Turbidity determinations cannot be related either to the quantity (mg/L) or characteristics (size, shape, chemical nature) of the particles in the sample (Hoff, 1978). Despite this limitation, turbidity has been found to be a useful parameter for measuring the quality of public water supplies (Hoff, 1978).

The current standard method for measurement of turbidity depends upon instruments that employ the principles of nephelometry (Sawyer *et al.*, 1994). In the instrument, a light source illuminates the sample and one or more photoelectric detectors are used with a readout device to indicate the intensity of scattered light at right angles to the path of the incident light. Turbidity measurements by the standard nephelometry procedure are reported in nephelometric turbidity units (NTU). Under the IESWTR, a public water system employing filtration must ensure that turbidity never exceeds 1 NTU and must not exceed 0.3 NTU in 95% of daily samples in any month. For unfiltered systems, turbidity should not exceed 5 NTU at any time (USEPA, 2001).

2.4 ULTRAVIOLET (UV) DISINFECTION

2.4.1 History

Downes and Blount first reported the bactericidal effect of radiant energy from sunlight in 1877, and the technical use of UV was advanced by Hewitt's discovery of mercury vapour lamp in 1901 (Meulemans, 1987). In 1910, Cernvedeau

and Henri experimented with UV disinfection of water in Marseille (Masschelein, 1992). The optimum UV wavelength was narrowed to a range of 250 to 266 nm. Sensitivity was found to be both species and strain dependent.

The use of UV light to disinfect water in the United States dates back to 1916 (USEPA, 1996). The interest in UV for drinking water treatment declined in the 1900s for a variety of reasons, including high operating costs, poor equipment reliability, maintenance problems and the advent of chlorine, which was found to be more efficient, cost effective and reliable (Wolfe, 1990). Due to improvements in equipment reliability and a desire to reduce the by-products of chemical disinfection, there was an increased use of UV technology especially in Europe in the 1980s (Wolfe, 1990).

2.4.2 Source of UV Radiation

UV radiation energy is the range of electromagnetic waves with wavelengths between 100 and 400 nanometers (nm) (between the X-ray and visible light spectrums) (USEPA, 1999a). To produce UV energy, special UV lamps that contain mercury vapour are charged by striking an arc. The energy generated by the excitation of the mercury vapour contained in the lamp results in the emission of UV light (Crites and Tchobanoglous, 1998). In terms of germicidal effects, the optimum UV range is between 245 and 285 nm. UV disinfection uses either: low-pressure lamps that emit maximum energy output at a wavelength of 253.7 nm; medium-pressure lamps that emit energy at wavelengths from 180 to 1370 nm; or lamps that emit at several wavelengths in a high intensity "pulsed" manner (USEPA, 1999a).

2.4.3 Mechanisms of UV Disinfection

The germicidal properties of UV radiation have been used in a variety of applications. With the proper dosage, UV radiation has proven to be an effective bactericide and virucide for wastewater and drinking water. Electromagnetic radiation in the wavelengths ranging from 240 to 280 nm effectively inactivates microorganisms by irreparably damaging their nucleic acid. The most potent wavelength for damaging deoxyribonucleic acid (DNA) is approximately 254 nm (Wolfe, 1990).

The germicidal effects of UV light involve photochemical damage to ribonucleic acid (RNA) and DNA within microorganisms. Microorganism nucleic acids are the most important absorbers of light energy in the wavelength range of 240 to 280 nm (Jagger, 1967). DNA and RNA carry genetic information necessary for reproduction; therefore damage to either of these substances can effectively sterilize the organism. Damage often results from dimerization of pyrimidine molecules (USEPA, 1999a). If the DNA and RNA in a cell are damaged (e.g. through formation of double bonds due to UV radiation) the organism will be unable to reproduce (Crites and Tchobanoglous, 1998).

2.5 UV INACTIVATION OF ENCYSTED PROTOZOANS

2.5.1 *C. parvum*

Ransome *et al.* (1993) studied the effect of UV irradiation on *Cryptosporidium parvum* oocysts. They exposed oocysts suspensions in petri dishes to a low-pressure mercury lamp source. *In vitro* excystation was used to measure the viability of the oocysts after treatment and realized 1-log unit and 2-log units of inactivation at doses of 80 mJ/cm^2 and 120 mJ/cm^2, respectively. There was very little information on the method of UV dose measurement.

Lorenzo-Lorenzo *et al.* (1993) looked at UV irradiation of C. *parvum* using a bench-top system and animal infectivity to measure oocyst inactivation. Oocysts were suspended in demineralized water in petri dishes and exposed to UV light of irradiance 15,000 mW/s. They reported significant reductions in infectivity of neonatal CD-1 mice for exposure times of 150 minutes. The unit of the irradiance reported is unconventional. Furthermore, since the exact log-inactivation and the germicidal UV dose calculations were not reported, the interpretation of these results has been difficult.

Campbell *et al.* (1995) used a novel design of an ultraviolet disinfection system to treat *C. parvum* oocysts suspended in clean water. According to the authors the disinfection unit was designed to provide a maximum total exposure to ultraviolet radiation of 8748 mJ/cm^2. The viability of the oocysts after treatment was assessed using the vital dye assay, which uses 4'6 diamidino-2-phenyl indole (DAPI) and propidium iodide (PI) and *in vitro* excystation. They reported over two logs of

oocysts inactivation. The viability assays used have been shown in recent years to overestimate the survival of oocysts in water after UV treatment.

A study by Clancy *et al.* (1998) examined innovative electrotechnologies to assess their potential for inactivating *C. parvum* oocysts in water. Animal infectivity was used to determine viability of oocysts following treatments and advanced ultraviolet (UV) was found to be effective, achieving >4-log inactivation of *Cryptosporidium* spp. oocysts. Dysen *et al.*, (1998) also evaluated the innovative electrotechnologies from engineering and economic standpoint. The cost comparison indicated that advanced UV technology might be cost-effective for drinking water treatment and competitive with other treatments.

Bukhari *et al.* (1999) investigated the inactivation of *C. parvum* in finished drinking water at bench scale using a collimated beam apparatus with medium pressure lamps and at demonstration scale using a UV reactor. The viability of oocysts was assessed *in vitro* (using 4',6-diamidino-2-phenylindole with propidium iodide and maximized *in vitro* excystation) and *in vivo* (using neonatal mouse infectivity assays). They achieved > 4-log inactivation in the *in vivo* bench scale studies at a UV dose of 41 mJ/cm^2. They found that the *in vitro* surrogate assays showed little or no inactivation at 41 mJ/cm^2 or higher doses. They concluded that *in vitro* assays grossly overestimated UV doses required to inactivate oocysts.

The ability of medium-pressure and low-pressure UV light to inactivate *C. parvum* oocysts was examined by Clancy *et al.* (2000). *C. parvum* oocysts were suspended in deionized water and exposed to UV from medium-pressure and low-pressure UV lamps at bench-scale in collimated beam apparatus. They also examined

the effect of turbidity (from recycled backwash water supernatant) on medium pressure UV inactivation of oocysts. Reduction in oocysts infectivity was determined using neonatal CD-1 mouse model. They found medium-pressure UV and low-pressure UV to be equally effective against *Cryptosporidium parvum* oocysts, achieving, respectively, 3.4-log and 3.0-log inactivation of oocysts at a low UV dose of 3 mJ/cm^2. They also found medium-pressure UV inactivation of oocysts unaffected by turbidity. They achieved oocyst inactivation of >4.5 log-units in an 11-NTU sample.

Craik *et al.* (2001) studied the effect of UV radiation from low- and medium-pressure mercury arc lamps on *C. parvum*, using a collimated beam apparatus. The experiments were conducted using oocysts suspended in both filtered surface water and phosphate buffered laboratory water. Inactivation of oocysts was measured as reduction in infectivity using the neonatal CD-1 mouse model and was found to be a non-linear function of UV dose over the range of germicidal doses tested (0.8 to 119 mJ/cm^2). Oocysts inactivation increased rapidly with UV dose at doses less than 25 mJ/cm^2 with 2 and 3 log-units inactivation at approximately 10 and 25 mJ/cm^2, respectively. The cause of significant leveling-off and tailing in the UV inactivation curve at higher doses was not determined. Maximum measured oocyst inactivation ranged from 3.4 to greater than 4.9 log-units and was dependent on different batches of parasites. They found that water type and temperature, the concentration of the oocysts in the suspension and the UV irradiance did not have significant impacts on oocyst inactivation.

Shin *et al.* (2001) used low-pressure UV to investigate the effectiveness of UV against *C. parvum* oocysts and their potential DNA repair in collimated beam experiments. Parasite suspensions in phosphate buffered saline were exposed to low doses of UV irradiation. Comparable levels of inactivation of oocysts were observed using mouse and cell culture infectivity assays to determine reduction in infectivity of oocysts. They achieved 1.7 log-units and 1.5 log-units of inactivation of oocysts using cell culture and animal infectivity assays, respectively at a UV dose of 2 mJ/cm^2. They did not observe any evidence of either light or dark repair of UV-damaged DNA in *C. parvum* oocysts.

2.5.2 *G. lamblia/muris*

There are limited literature on the use of Ultraviolet (UV) radiation for inactivation of encysted parasites especially *G. lamblia*. Rice and Hoff (1981) studied inactivation of *G. lamblia* cysts by UV irradiation and found them to be resistant to high doses of germicidal UV radiation. Cysts viability was determined by a modified in vitro excystation procedure. With a maximum UV dose of 63 mJ/cm^2, less than 1 log-unit reduction in cyst survival was produced.

The inactivation of *G. muris* and *G. lamblia* in a laboratory batch UV apparatus and two commercially available UV disinfection units were studied by Carlson *et al.* (1985). They were able to achieve greater than 2 log-units of inactivation in the commercial units but at very long exposure times. The degree of inactivation and UV dose applied were measured by *in vitro* excystation and

actinometry respectively. Typical effective UV doses for 2 log-units of inactivation ranged from 1,000 to 30,000 mJ/cm^2 and exposure times were in excess of 60 s.

Karanis *et al.* (1992) examined the UV sensitivity of different protozoan parasites, *Trichomonas vaginalis, Giardia lamblia, Acanthamoeba and Naegleria* spp. and realized 2 log-units inactivation of *Giardia lamblia* after 1800 mJ/cm^2. A special apparatus for batch experiments was used and the irradiation density of the lamp was measured using a potassium ferrioxalate actinometer method described by Zemke and Schoenen (1989). The reduction in cysts infectivity after UV exposure was measured using excystation. Cysts from *G. lamblia* for irradiation were obtained from patients and gerbils after infecting the gerbils (*Meriones unguiculatus*) with trophozoites cultivated *in vitro*.

Craik *et al.* (2000) studied the effects of medium pressure UV radiation on *G. muris* using a collimated beam apparatus with filtered surface water. They achieved 2 to 3 log-units of reduction in infectivity measured by a C3H/HeN mouse model with UV doses ranging from 5 to 83 mJ/cm^2. The parasite suspensions were placed 280 mm beneath the open end of the collimating tube. The test water volume was 20 mL and the liquid height in petri dish was 8 mm. The UV irradiance was measured by using a radiometer equipped with a SED240 UV detector. They realized that *in vitro* excystation and nucleic acid staining with Live/Dead BacLight greatly underestimated the inactivation of *Giardia* spp. when compared with animal infectivity.

The sensitivity of human-derived *G. lamblia* cysts to UV light has been reported by Campbell *et al.* (2002). They observed significant inactivation of the

Giardia spp. cysts, 2 and 3 log-units at approximately UV doses of 10 and 20 mJ/cm^2, respectively, using a gerbil infectivity assay. No reduction in viability of cysts was observed with using propidium iodide followed by 4',6-diamidino-2-phenylindole (PI/DAPI). Cysts, isolated and purified from gerbils were suspended in 20 mL samples and exposed to UV at 254 nm. The researchers measured incident irradiance with a calibrated International Light Inc. Model IL 1400A radiometer and the potassium iodide method of wet chemistry. They concluded that vital dye assays significantly underestimated UV inactivation of *Giardia* spp. cysts.

Mofidi *et al.* (2002) measured the effect of germicidal ultraviolet (UV) light on cysts of *G. lamblia* and *G. muris* in bench-scale collimated beam experiments. Reduction in infectivity of *G. lamblia* cysts in Mongolian gerbils and *G. muris* cysts in CD-1 mice was quantified by using most probable number techniques. Cysts were suspended in filtered natural water and irradiated with light from low-pressure UV lamp. They realized > 2-log inactivation of both *G. lamblia* and *G. muris* cysts at UV dose of 3 mJ/cm^2. The method used to determine the reduction in cyst infectivity reduction based on the most probably number is not well understood.

2.6 EFFECT OF SUSPENDED SOLIDS ON UV INACTIVATION OF WATERBORNE PATHOGENS

Qualls *et al.* (1983) studied the effects of absorbed and scattered UV light and their role in irradiance measurement using a collimated beam apparatus. They also evaluated the effects of exclusion of various particle sizes on the dose-survival curves of coliforms in wastewater and determined the effects of mixed-media filtration in a

pilot scale UV disinfection system. They concluded that suspended particles in wastewater effluents could play two roles in UV disinfection: they can absorb and scatter the UV light, and they can harbour bacteria that are partially protected from UV light. They found that UV disinfection was significantly better in a sand-anthracite filtered secondary effluent than unfiltered secondary effluent. They used a bioassay method to measure average intensity.

Crites and Tchobanoglous (1998), published establishment of a total coliform dose-response curves for UV disinfection of wastewater effluents, containing different total suspended solids (TSS) concentrations. The data were collected from experiments using collimated beam device. The UV dose required to achieve a five to six-log reduction in the number of dispersed non-particle associated coliform organisms (total coliform/100 mL) was typically in the range from 10 to 40 mJ/cm^2. They made the following findings and observations: (1) The effectiveness of UV disinfection will vary with each treatment plant depending on the number of organisms that are either clumped or particle associated and the distribution of particles in the effluent, and (2) To overcome the effects of particle shielding either the particle size distribution must be modified, by making appropriate modifications in the design and operation of the upstream treatment processes to produce an effluent with particles that can be disinfected more effectively, or the particles must be removed by some means (e.g. granular medium or membrane filtration).

According to USEPA (1999a) when microorganisms are exposed to UV radiation, a constant fraction of the living population is inactivated with each progressive increment in time. The survival of microorganisms can be calculated as a

function of dose and exposure time based on first order kinetics (White, 1992). Particles can affect disinfection efficiency of UV by harbouring bacteria and other pathogens, partially protecting them from UV radiation, and scattering UV light (USEPA, 1999b). Typically, the low turbidity of groundwater results in minimal impact on disinfection efficiency. However, the higher turbidities of surface water can impact disinfection efficiency. Pathogen removal has been found to coincide with turbidity/particle removal in studies of pathogens and turbidity occurrences in source waters (Fox, 1995).

Loge *et al.* (1999) studied the effect of wastewater particles on UV disinfection. They developed a technique for measuring UV absorbance and internal scattering characteristics of wastewater solids. Wastewater solids were irradiated in medium-pressure UV lamp exposures at bench-scale. They concluded that UV light could only penetrate wastewater particles because of high particle porosity and not by transmission through the solid material.

Emerick *et al.* (2000) investigated UV inactivation of particle-associated coliform bacteria. They derived an equation describing the measured inactivation of particle-associated coliform bacteria in wastewater secondary effluent exposed to UV light disinfection. They found inactivation rate coefficient of mono-dispersed bacteria and the total number of particles that contain coliform to be important in determining UV dose-inactivation. They also found that a minimum particle size (10 μm) seemed to govern the ability of a particle to shield coliform bacteria from UV and that particles smaller than this size do not contain regions shielded from UV light.

The effect of water quality on long-term performance of nine pilot-scale UV disinfection systems treating finished water at five drinking water treatment plants was studied by Mackey *et al.* (2001). The plants used various types of UV lamps: low-pressure (LP) mercury arc lamps, low-pressure high-output (LPHO) lamps and medium-pressure lamps (MP). The UV transmittance of the water was found to vary significantly from one plant to the other. Temperature and sleeve fouling were found not to impact UV dose delivery, which was assessed by MS2 bioassay. The UV dose-response of MS2 was measured using a collimated beam apparatus and a water sample taken from the reactor influent. They failed to find a correlation between UV dose and water transmittance.

Christensen *et al.* (2001) conducted research to find out the impacts of suspended solids on both UV dose transmission and spectrophotometric absorbance measurements for unfiltered drinking water with turbidity up to 10 NTU. They used chemical actinometry and a special quartz reactor dish to measure the UV dose in collimated beam experiments. They concluded that particles would impact the delivery of UV dose. They added that for turbidity up to 10 NTU, the impact of scattering on average UV dose appeared negligible and that the main effect was simple absorbance.

Malley *et al.* (2001) have looked at the effect of turbidity and algae on the dose requirements for ultraviolet systems treating unfiltered drinking water supplies. Tests were conducted using medium pressure collimated beam apparatus, with laboratory prepared water and real water spiked with MS2 at turbidity levels up to 12 NTU and algal content up to 42 μg/L as chlorophyll. They found UV performance

unaffected by clay turbidity up to 12 NTU and algal content up to 42 μg/L as chlorophyll. UV was found to be practical and effective for all the reservoir samples tested.

2.7 SUMMARY

Current studies have amply shown that ultraviolet (UV) light may be very effective against the waterborne pathogen, *C. parvum*. There are limited data on the effectiveness of UV light inactivation of the human pathogen, *G. lamblia* and its surrogate, *G. muris*. The following observations were made from the review, which may account for the difficulties encountered in evaluating and comparing results:

1. Studies utilized various viability assays. Among them were *in vitro* methods, such as excystation and its variations, cell tissue culture, DAPI/PI and other vital dye stains. Most of the recent studies used animal infectivity, which has been found to better estimate UV inactivation rather than *in vitro* methods used in older studies. It is essential that researchers agree on the most suitable test of viability.
2. In animal infectivity studies, there is lack of agreement on how infection is defined, determined and measured and also how data are analyzed and interpreted. Some of the studies did not accurately describe dose-response for the parasite used, which is important in the interpretation of results.
3. Data were generated under various experimental conditions, including bench- and pilot scale studies, different types of UV lamps and various water quality matrices.

4. Some of the studies did not provide sufficient detail about experimental conditions, results and methodology, especially the method of dose measurement. This is needed to allow accurate assessment of UV dose-response data. A method of determining and reporting UV dose should be agreed on.
5. UV inactivation was beyond detection (i.e. no infections in the animals) in some of the studies. They were reported as 'greater than' a given log inactivation value. These values are open to many interpretations but reporting the maximum detection limit is preferred.
6. There was only one study on the impact of particles on UV inactivation of *C. parvum* and *Giardia* spp. in water treatment. There were few studies on impact of particles on UV disinfection of drinking water. Most of these failed to report the characteristics (size, shape and chemical nature) of the particles used in their experiments. Reporting only turbidity of test samples may be misleading since it cannot be related to the quantity or characteristics of the particles.

3 PROBLEM STATEMENT AND RESEARCH OBJECTIVES

3.1 PROBLEM STATEMENT

Waterborne pathogens, especially protozoans have been known to cause life-threatening diseases in the immunosuppressed populations of the world and illness in the general population (AWWA, 1999). Reports in print and electronic media of waterborne disease outbreaks, in which *C. parvum* and *G. lamblia* have been implicated, have expanded public awareness of water quality issues. This awareness places greater demands on utilities to provide additional barriers against these pathogens.

From the review in Chapter 2, UV light has been shown be to very effective against *C. parvum* and *G. lamblia* at low doses when animal infectivity was used as a test of viability as opposed to other methods of viability, including *in vitro* excystation, used in earlier studies. Experiments have been conducted in different laboratories, with different types of UV lamps and water quality. UV dose – inactivation curves of *C. parvum* and *G. muris* have been observed to exhibit 'tailing', wherein large increases in UV dose results in minimal increase in inactivation. The tailing characteristics of UV dose – response curves may mean that very high levels or complete inactivation of these protozoans may be difficult to achieve with UV.

The effect of suspended solids on UV disinfection efficiency in wastewater treatment has been studied extensively but only limited studies have been conducted on drinking waters. Meanwhile, the only point of application of UV disinfection that is supported by current research findings is post-filtration (Malley *et al.*, 2001). Issues

surrounding effects of turbidity and particle associations on unfiltered water UV applications are largely undefined and require research into the nature and impact of unfiltered water turbidity (particles) on UV disinfection efficiency.

The City of Kelowna experienced the largest *Cryptosporidium* outbreak ever reported in British Columbia, in which approximately 14,500 people were reported infected (Fayer *et al.*, 2000). The cause was not determined but unfiltered drinking water from Lake Okanagan was suspected (Fayer *et al.*, 2000). The City of Kelowna is now faced with developing a cost-effective disinfection method to prevent the re-occurrence of the 1996 Cryptosporidiosis outbreak. The City is considering UV disinfection of its unfiltered lake water. The turbidity of the lake water is very low (<0.5 NTU) but can exceed 3.0 NTU (or higher) during storm events. The City is interested in knowing the effect of particulates in the lake on the effectiveness of UV against *C. parvum* and *Giardia* spp.

3.2 RESEARCH OBJECTIVES

The proposed study was to further assess the effect of medium-pressure UV light on encysted *C. parvum* and *G. muris*. The effect of turbidity on the UV dose - inactivation of C. *parvum* and *G. muris* were investigated. The effect of oocyst concentration on UV dose – inactivation of *C. parvum* was also investigated to see if it could help explain previously observed 'tailing'. Answers to these questions were necessary to help develop the use of medium-pressure UV systems for control of *C. parvum* and *G. lamblia* in unfiltered drinking water application at the City of Kelowna.

The specific aims of the project were to:

1. Determine the effects of turbidity on the UV dose – inactivation relationship for C. *parvum* and *G. muris* and;
2. Determine the effect of oocyst concentration on the UV dose-inactivation relationship for *C. parvum* in medium-pressure UV lamp exposure experiments at bench-scale.

G. muris, the rodent parasite, was being used as a surrogate for the human pathogen, *G. lamblia* in this work. *G. muris* cysts have been found to be useful model for *G. lamblia* cysts for use in UV disinfection studies (Mofidi *et al.*, 2002). Chemical disinfection studies, using *G. muris* and *G. lamblia*, have reported similar resistances of the parasites to disinfectants (Leahy *at al.*, 1987, Finch *et al.*, 1993b). There are advantages in using the murine form in experiments. For instance, the cysts are easily produced compared to *G. lamblia cysts* and are also not pathogenic.

4 MATERIALS AND METHODS

4.1 TEST WATERS

The experimental trials in this work were done at ambient room temperature (20 to 22°C) by either using an unfiltered drinking water from Lake Okanagan, Kelowna, BC or a phosphate buffered laboratory water, pH 6. The samples of unfiltered water were shipped in from Kelowna in 4-litre containers in the week of the trials and stored at a temperature of 4°C. The 0.05 M phosphate buffered laboratory water at pH 6 was prepared by dissolving appropriate amounts of reagent grade potassium dihydrogen orthophosphate (KH_2PO_4) and disodium hydrogen orthophosphate (Na_2HPO4)(Analar, BDH Inc., Toronto, ON) in deionized water from an Elga Ultra-pure system (Fisher Scientific, Pittsburgh, PA). The final pH was measured and recorded. A Hach Model 2100P Portable Turbidimeter (Hach Company, Loveland, Colorado, USA) was used to determine the turbidities of the water samples before experimental trials.

4.2 PREPARATION, CONCENTRATION AND CALIBRATION OF SAMPLE OF LAKE SUSPENDED SOLIDS

One thousand litres or more of unfiltered water from Lake Okanagan, at Eldorado pumping station, were sampled through a 1.0 μm porosity polypropylene yarn cartridge filter by City of Kelowna staff. The intake of the Eldorado pumping station is 20 m deep and 100 m from the lake shore. The filters were shipped to the University of Alberta and stored at 4°C. The filters were cut lengthwise down to the

core and hand washed in 0.05 M phosphate buffered laboratory water at pH 6. The particulates in suspension were concentrated by centrifugation for 10 minutes at 600 x g and 4°C. The supernatant was then removed to 25 mL and the pellet resuspended by gently pipetting up and down. To calibrate the final lake suspension, 4 Erlenmeyer flasks were set-up with 1 L of phosphate buffered de-ionized water and gently stirred. The initial turbidities of the water samples were determined and aliquots of 1, 2, 3 and 4 mL of the concentrated sample added to the 4 flasks, respectively, and stirred. The turbidities of the resulting suspensions were recorded at 30-minute intervals over 90 minutes and average turbidities determined. A plot of turbidity versus volume of lake suspended solids (Table D-4 and Figure D-1, Appendix D) was produced and the line of best fit determined. This curve was used to estimate the various volumes of suspended solids needed to produce turbidity levels of 5.0, 10.0 and 20.0 NTU. Particle size distribution was determined by using a Potable Water Sensor (L & H Environmental, Inc., Roseburg, OR, USA). Gravimetric analysis based on Standard Methods (Greenberg et al., 1998), conducted on the sample showed that 67% of the total suspended solids was mainly volatile material (Table D-6, Appendix D). Microscopic examination of the turbid sample revealed the presence of a diverse microbial fauna, in which algae was well represented. There were no microbial analyses done on the sample of lake suspended solids.

4.3 UV EXPOSURE

The parasite suspensions, 20 mL in volume, in 150 mL beakers were exposed to UV radiation from a 1 kW medium-pressure UV lamp (model QC-1000-45000071,

HNG, Germany) using a collimated beam apparatus (model PSI-1-120, Calgon Carbon Corporation, Pittsburgh, PA). The dimensions of the 150 mL beaker are 5.0 cm in diameter and height, 8.0 cm. The liquid depth was 0.85 cm. The UV apparatus produced a nearly parallel beam of UV radiation with the help of a plastic collimating tube. This tube was placed directly below a UV lamp mounted horizontally in an enclosure. During the UV exposure the suspensions were gently and constantly stirred. The plastic collimating tube measures 60 mm in diameter and 930 mm in length. The parasite suspensions were placed 280 mm beneath the open end of the tube during the experimental trials. The duration of exposure to UV radiation was controlled by means of a pneumatic shutter located at the top of the collimating tube, beneath the UV lamp and measured with the help of a stopwatch.

4.4 DETERMINATION OF UV DOSE

Before each UV treatment, the UV irradiance at the surface of the parasite suspension and at the center of the UV beam was measured with a radiometer (International Light, Model IL1400A) equipped with a SED240 UV detector. The UV irradiance was also measured after each UV exposure. The total UV irradiance in the suspension was determined by using previously described method (Bukhari *et al.*, 1999; Craik *et al.*, 2000; Craik *et al.*, 2001). The detailed method is provided in Appendix E. The irradiance at the center of the UV beam was multiplied by factors, which account for radial variation, reflection at the liquid surface, attenuation of the beam within the liquid, and variation in sensor sensitivity and lamp output with

wavelength. The depth averaged total irradiance, E, was determined by using an integrated form of Beer's law:

$$\frac{E}{E_o} = \frac{\left(1-10^{-A}\right)}{\ln(10)A} \qquad \text{Equation 4-1}$$

Where E_o is the incident irradiance at the surface and A is the absorbance within the liquid. The 200 to 300 nm absorbance spectrum of a sample of suspension was measured in a 10 mm quartz cell using a UV-vis spectrophotometer (Hewlett Packard, Model HP 8452A). The total average irradiance, E, and germicidal average irradiance, E_g were determined using Equation 4-1 and taking into account necessary corrections. The UV dose, either total (H) or germicidal (H_g) was then determined from:

UV Dose (H or H_g) = Irradiance (E or E_g) x Exposure time (t)

mJ/cm^2 = mW/cm^2 x s Equation 4-2

4.5 *CRYPTOSPORIDIUM PARVUM* METHODS

4.5.1 Production of oocysts

The Iowa strain isolate of *C. parvum* oocysts was used in this study. They were originally obtained from Dr. Harley Moon (National Animal Disease Control Center). The production and purification of *C. parvum* oocysts from the Holstein calf were done according to established methods (Finch *et al.*, 1994; Finch *et al.*, 1995;

Craik *et al.*, 2001). A 2 to 4 day old calf was infected with *C. parvum* oocysts and maintained on an electrolyte solution diet. Feces from the calf was collected from day 4 through day 10 and first passed through a series of sieves (400 to 75 μm). Cesium chloride gradient centrifugations were then used to purify the sieved feces (Kilani and Sekla, 1987). The stock suspension of purified oocysts was stored in deionized water with antibiotics (100 μg/mL streptomycin, 100 μg/mL gentamycin, 100 U/mL penicillin) and 0.01% Tween 20 at 4°C. One batch of purified oocysts was used in the experiments over a five-month period.

4.5.2 Oocysts Sample Preparation, UV exposure, Collection and Concentration

Oocyst suspensions containing about 30 x 10^6oocysts in 0.50 mL of deionized water were prepared from the stock suspension for experimental trials. This was done by washing oocysts from the stock suspension in Milli-Q® water and concentrated by centrifugation (14,500 x g, 10 minutes). They were counted using hemacytometer. The oocysts suspension was vortexed for 30 s before being added to the test water in all the UV exposure trials.

For trials with high oocyst concentrations, oocyst suspension, approximately 0.5 mL, was added directly to 19.5 mL of phosphate buffered de-ionized water in a 150 mL beaker. The parasite suspension in the beaker was then thoroughly mixed for 2 minutes using a magnetic stirrer set at high and a 10 mm x 3 mm Teflon® coated stir bar. The stirrer speed was reduced to low to allow gentle and continuous mixing during UV exposure. At the end of the UV exposure, the contents of the beaker were transferred to a 50 mL Teflon coated Nalgene® centrifuge tube and the beaker rinsed

with some deionized water into the centrifuge tube. For low oocyst concentration trials, the oocyst suspension was added to 1000 mL of test water in a 2000 mL Erlenmeyer flask and stirred for about 15 minutes to ensure thorough mixing. Twenty millimeters of this suspension was measured out into the 150 mL beaker, stirred gently and continuously and exposed to UV irradiation for the appropriate length of time. The UV irradiated samples were collected into a 250 mL plastic centrifuge tube and the beaker rinsed with about 20 mL of deionized water. The procedure was repeated with 49 other aliquots from the flask for a total of 1 L of suspension. The turbidity of the test water was determined before and after addition of oocyst suspension.

In the suspended solids experiments, trials were conducted at four turbidity levels, natural lake water turbidity (0.25 NTU), 5.0, 10.0 and 20.0 NTU. For trials with natural lake water turbidity, 1000 mL of the test water (unfiltered drinking water from Lake Okanagan) was measured into a 2000 mL Erlenmeyer flask, stirred for 15 minutes on a magnetic stirrer and the turbidity recorded. The oocyst suspension was then added to the test water and stirred for one hour and the final turbidity recorded. Aliquots of 20 mL, 50 in number, were measured out of this suspension, subjected to the stirring procedures described above and exposed to UV irradiation for the required length of time. For trials involving elevated turbidities, the test water was stirred initially for 15 minutes and the turbidity measured. The turbidity was then adjusted to the required level by addition of pre-determined volume of lake suspended solids sample and stirred for 30 minutes and the turbidity recorded at the end of this period. The oocyst suspension was finally added to the test water and stirred for one

hour. The final turbidity was measured and 50 aliquots of 20 mL each were exposed to UV irradiation. The UV irradiated samples were collected in four 250 mL plastic centrifuge tubes.

The UV treated samples were centrifuged at 27,000 x g for 10 minutes to concentrate the oocysts for inoculation of neonatal CD-1 mice. The supernatant was aspirated and the cell pellet re-suspended in deionized water. The oocyst concentration was determined in quadruplicate by microscopic counting using a hemacytometer. For each UV treated sample 3 dilutions of oocyst were prepared in deionized water and a cohort of 5 mice were inoculated. The processing of the samples and inoculation of the mice occurred within 8 hours on the same day as the UV exposure. In all the trials, control samples were subjected to all the processing and handling steps as the treated samples with the exception of exposure to UV irradiation.

4.5.3 Infectivity in Neonatal CD-1 Mice

A neonatal mouse model was used to evaluate infectivity of *C. parvum* (Ernest *et al.*, 1986; Craik *et al.*, 2001). The detailed method is found in Appendix G. Five-day old mice were inoculated with oocysts suspended in 50 µL of deionized water using a ball-point neonate feeding needle (24 gauge syringe, Popper and Sons, Inc.). The infectivity of the oocysts was determined 7 days after infection. The mice were killed and the intestinal homogenates subjected to various processes and finally examined for the presence of parasites using fluorescent activated cell sorting (FACS) with immunoflourescence.

4.5.4 Interpretation of Animal Infectivity Data

The infectivity data were analyzed as described in Craik *et al.*, (2001). The reduction in oocysts infectivity was interpreted as the inactivation ratio (N/N_o), where N_o and N are the number of live oocysts in the suspension in the beaker before and after UV exposure, respectively. The inactivation ratio (log-units) for each UV trial was estimated from:

$$-\log\left(\frac{N}{N_o}\right) = -\log\left(\frac{d}{d_o}\right) \qquad \text{Equation 4-3}$$

Where d is the estimated number of infectious oocysts in the inoculum to each mouse in a trial and d_o is the total number of oocysts in the same inoculum as determined by hemacytometer count. The proportion of mice positive for infection 7 days post-inoculation, P, was determined for each cohort. The estimated infectious dose, d, was then estimated using a logistic dose-response model for *C. parvum* oocyst infectivity in the neonatal CD-1 mice. The form of the model (Neter *et al.*, 1989) is:

$$\ln\left[\frac{P}{(1-P)}\right] = \beta_o + \beta_1 \log d \qquad \text{Equation 4-4}$$

Where P is the proportion of animals in a cohort that become infected after ingesting a specified live inoculum, d, of oocysts. The parameters of the logit model, β_o and β_1, were determined for the batch of oocysts used in the UV trials in oocyst dose-response experiments. Cohorts of 10 neonatal CD-1 mice were inoculated with levels

of inoculum ranging from 25 to 400 oocysts per mouse. The number of mice positive for infection at each level was determined as described previously. Parameters of the logit model were estimated from the results of the dose-response experiments by maximizing the *ln* likelihood function, *L,* for binary data, given by (Brand *et al.*, 1973) using Solver function in MS Excel 2000.

$$\ln L = \sum_{i=1}^{\lambda} Y_i(\beta_o + \beta_1 X_i) - \sum_{i=1}^{\lambda} \ln[1 + \exp(\beta_o + \beta_1 X_i)] \qquad \text{Equation 4-5}$$

Where, X_i is log (base 10) of the inoculum size for each mouse, Y_i is the binary score (0 = not infected, 1 = positive) of each mouse 7 days post infection and the subscripts $i = 1, 2, \ldots, \lambda$ represents each individual mouse used in the dose response experiment.

4.6 *GIARDIA MURIS* METHODS

4.6.1 Production of Cysts

The strain of *G. muris* used in this work was originally isolated from a golden hamster by Roberts-Thomson *et al.* (1976). The parasite has been maintained in the laboratory since 1981 using bi-weekly passages in CD-1 mice. The methods for mouse inoculation, eventual cysts collection and purification from mouse feces followed those of previous studies (Belosevic *et al.*, 1984; Labatiuk *et al.*, 1991; Craik et al., 2000) and finally purification on a Percoll-Percoll gradient (Nieminski *et al.*, 1995). Cysts were produced by inoculating groups of 4 to 5 week old female CD-1 mice with purified suspensions of fresh cysts. Feces of the infected mice were

collected over a 2 hr period on two days. The collection of feces was done from 5 to 7 days post-infection. To isolate cysts, the samples of feces collected each day were emulsified in de-ionized water, layered on 1 M sucrose solution and then centrifuged for 15 minutes at 400 x g and 4°C. At the end, the cyst-rich layer at the water-sucrose interface was collected and then washed once in de-ionized water by centrifugation at 600 x g for 10 minutes. The concentrated cysts were layered on a two-step Percoll-Percoll gradient (specific gravities, 1.05 and 1.09 kg/m^3) and purified further by centrifugation at 750 x g for 35 minutes at 10°C (Nieminski *et al.*, 1995). The procedure for the preparation of working solutions of Percoll of specific gravities, 1.05 and 1.09 kg/m^3 is found in Appendix E. The Percoll-Percoll gradient was added in order to produce cysts cleaner than those produced in previous studies. At the end of the Percoll-Percoll centrifugation, the cyst-rich layer at the Percoll-Percoll gradient interface was carefully collected and washed once in de-ionized water by centrifugation at 600 x g for 10 minutes and hemacytometer used to determine the concentration.

4.6.2 Cysts Sample Preparation, UV Exposure, Collection and Concentration

The experiments with *G. muris* cysts were conducted at two levels of turbidity, the turbidity of the natural water from Lake Okanagan and an elevated turbidity of 20.0 NTU. Two final volumes of water, 20 mL and 1000 mL, were used in the trials and 20 mL used in each UV exposure. Twenty millimeters was used in trials involving the natural lake water turbidity (0.25 NTU) and 1000 mL, elevated turbidity of 20.0 NTU. Before the UV exposures, about 1.0 to 1.5 mL volume of *G.*

muris cysts of the stock suspension containing between 2 x 10^6 and 4 x 10^6 cysts were added to the test water to make a final volume of 20 mL and 1000 mL in a 150 mL beaker and 2000 mL Erlenmeyer flask, respectively. In the low turbidity trials 20 mL of test water sample was stirred for about 5 minutes and the turbidity recorded. The parasite suspension was then added and stirred for about 30 minutes and the final turbidity measured. The test water was then exposed to UV in a 150 mL beaker stirring gently and constantly. At the end of the exposure the sample was transferred into a 50 mL plastic centrifuge tube and the beaker rinsed with de-ionized water into centrifuge tube. The high turbidity trials followed the procedures above except that the turbidity was first adjusted to 20.0 NTU and it involved 50 UV exposures. UV irradiated samples were collected into four 250 mL plastic centrifuge tubes. The centrifuge tubes with the irradiated water samples were centrifuged for 10 minutes at 800 x g and 4°C. At the end, the supernatant was removed, the concentrated pellet re-suspended in 3 mL of liquid and the concentration was determined by hemacytometer counts. The cyst suspensions were then used to infect mice for animal infectivity analysis using C3H/HeN mouse model. The concentration of cysts in the treated samples and inoculation of mice were done within 8 hours of UV exposure. For all the trials, control samples were subjected to all the experimental conditions except exposure to UV.

4.6.3 Interpretation of Animal Infectivity Data

There is a period following inoculation of mice, called the latent period, in which no cysts are seen in the feces. At the end of this period there is a rapid increase

in cyst output until it levels off at about 10^6 cysts per gram feces, which is independent of the infective inoculum (Belosevic *et al.*, 1983; Belosevic *et al.*, 1984; Craik *et al.*, 2000). The size of the infective inoculum determines the length of latent period and the position in time of the rapid increase phase (Craik *et al.*, 2000). The relationship between inoculum size and latent period was determined in dose–response experiments. Cohorts of five C3H/HeN mice were infected with 5.0×10^5, 5.0×10^3 and 5.0×10^1 cysts each. Cysts for the dose-response experiment were isolated and used for infection on the same day. The mice were placed in individual false bottom cages for a 2-hour period for feces collection. The weight of wet feces collected from each mouse was determined and analyzed for cysts. The recovery and purification process followed previously described sucrose gradient centrifugation method in Section 4.6.1. The cysts were washed once in de-ionized water and then counted using hemacytometer. The average cysts output per gram feces were determined for each cohort of mice on days following infection until it leveled off at about 10^6 cysts per gram of wet feces.

Using the latent period in days estimated from the dose-response experiment, a linear model of the following form was developed:

$$Log(d) = \beta_1 Log(LP) + \beta_o \qquad \text{Equation 4-6}$$

Where: d is the number of infectious cysts in the mouse inoculum, LP is the latent period, β_o and β_1 are parameters of the model.

At the end of each experimental trial, including the controls, cysts were concentrated by centrifugation from the treated water samples and used to inoculate five mice. The volume of the suspensions of approximately 5.0 x 10^4 cysts ranged from 0.10 to 0.20 mL. The production of cysts post infection was followed as in the dose-response experiments and the various latent periods determined. The inactivation was determined from Equation 4-3, where d_o was the total number of cysts in the inoculum as determined by hemacytometer count and d was the number of infectious cysts in the inoculum estimated by the latent period model (Equation 4-6).

4.7 STATISTICAL ANALYSES

Statistical analyses were performed on the outcomes from the experiments in this work. A number of tools, graphs, tables, sums, averages, were used to organize and summarize data. Other tools used were standard error, standard deviation and confidence intervals. To determine the relationship between pairs of variables, maximum likelihood, simple linear and multiple regression analyses were employed, and decisions made based on the 90 and 95 percent confidence levels. Statistical tests were done using analysis of variance (ANOVA) to be able to generalize beyond individual pairs of mean observations. All the statistical analyses were done using the various functions available in Analysis Tool Pak of Microsoft Excel 2000.

5 UV INACTIVATION OF *CRYPTOSPORIDIUM PARVUM*

5.1 LOGISTIC DOSE-RESPONSE IN NEONATAL CD-1 MICE

The oocysts used in this study were isolated and purified from an infected Holstein calf between June 20 and 26, 2001. Logit dose response models are useful for assaying the infectivity of disinfectant-treated oocysts (Korich *et al.*, 2000). Dose-response experiments were conducted every two weeks over the period of this study to determine the average infectivity characteristics during the life of the batch of oocysts. The oocysts came from a single batch and six dose-response experiments were conducted. Data from the dose-response trials are provided in Table A-1 of Appendix A. The number of mice in a cohort that were infected in all trials but one increased with increasing oocyst dose. In trial 5, a higher number of mice were positive for infection in a cohort inoculated with a lower dose (100 oocysts) than a cohort inoculated with a higher dose (200 oocysts). This might have been due to the variability of the mice's receptivity to infectivity or problems with the doses delivered.

The analyses of the dose-response data was as described by Craik (2001). The results from the individual dose-response were pooled and the logit dose-response model (Equation 4-4) was fit to the pooled data using the maximum likelihood method (Equation 4-5). A plot of results of the pooled data and the predicted logit model are shown in Figure 5-1. The predicted logit model parameters, with corresponding 90% confidence intervals of the model parameters (Equation 4-4) enclosed in brackets are:

$$\beta_o = -6.86\ (-7.2, -6.6) \quad \text{and} \quad \beta_1 = 3.46\ (3.3, 3.6)$$

The estimated oocyst dose required for 50% infection in the mice (ID_{50}) was calculated from the model parameters as follows:

$$ID_{50} = \text{antilog}\ (-\beta_o/\beta_1) = 96$$

5.2 EFFECT OF OOCYST CONCENTRATION ON UV DOSE-RESPONSE OF *C. PARVUM*

5.2.1 Experimental Design

A 2^2 factorial experimental design approach was used to assess how oocyst concentration impacted the UV dose-response of *C. parvum*. It involved looking at two factors, oocyst concentration and UV dose. The factors and their level-settings are found in Table 5-1. The high level setting of 1.5 x 10^6 oocysts/mL of oocyst concentration factor coincides with the concentration used in previous studies (Craik *et al.*, 2001) and the low, a 50-fold dilution of the high level. The UV dose factor was assessed at low and high doses of 5.0 mJ/cm^2 and 40.0 mJ/cm^2, respectively. The 2^2 factorial design resulted in 4 experimental conditions. The design matrix and results are presented in Table 5-2. Three replicates were performed for each combination of oocyst concentration and UV dose for a total of 12 trials. In four of these trials the results were beyond detection limit and the trials were repeated. There were, therefore, a total of 16 trials.

5.2.2 Determination of Inactivation

Summary of UV exposure parameters and calculated log inactivations are shown in Table A-2 of Appendix A. Detailed infectivity results for the trials, including controls, are found in Table A-3 of Appendix A. They are reported as the number of mice positive for infection 7 days post-inoculation in the 3 cohorts used in each trial. The log inactivation of *C. parvum* was measured using neonatal CD-1 mice with flow cytometry detection and interpreted with a logistic dose-response model as described elsewhere (Craik *et al.*, 2001) and in Section 4.5.4 of Chapter 4.

Log inactivation ratios were calculated for those cohorts for which the infectivity result was in the measurable range, i.e. for those cohorts in which the number of mice positive for infection was greater than zero, but less than the total number of mice in a cohort. For trials in which the results from more than one cohort were in the measurable range, the arithmetic average of the log inactivation ratio was reported. If none of the mice were infected in any of the cohorts (i.e. 0/5), the results was reported as greater than (>) the value that would be calculated if one of the mice had been infected in the highest inoculum (i.e. 1/5). If, on the other hand, all the mice were infected in a cohort (i.e. 5/5), the result was reported as less than (<) the value that would be calculated if all but one of the mice in the lowest inoculum cohort were infected (i.e. 4/5). In a trial in which all the mice in the highest inoculum were infected (i.e. 5/5) and no mice infected in the immediate lower inoculum (i.e. 0/5), the result was reported as the arithmetic mean of the inactivation ratios determined in the two previous cases.

The reported log inactivations were not corrected for inactivation in the control samples, which ranged from –0.4 to +0.8 log-unit, with a mean of +0.1 log-unit. This range of values is considered typical of the neonatal CD-1 mouse model and is due to the normal variation of the response in the mice (Craik *et al.*, 2001). The effect of extraneous UV light on parasite inactivation was expected to be small relative to experimental UV exposure. Any such effects that were present to significant extents would have been observed in the controls.

5.2.3 Effect of Oocyst concentration and UV Dose

The mean is a particularly informative measure of the central tendency of a variable if it is reported along with its confidence intervals (Helsel and Hirsch, 1992). The mean log inactivation and associated 95% confidence intervals for each trial were calculated and are shown in Table 5-3. At a UV dose of 5.0 mJ/cm^2, mean inactivation (log-units) increased slightly from 3.8 to 3.9 as the concentration increased from 3 x 10^4 to 1.5 x 10^6 oocysts per mL. Mean inactivation at a UV dose of 40.0 mJ/cm^2, however, declined from 4.3 to 3.7, from the low to high oocyst concentrations. The results are illustrated in Figure 5-2. Each bar in this figure represents the arithmetic mean of the outcomes of three independent replicated exposure trials conducted at the specified UV dose and oocyst concentration combination. The error bars represent the 95% confidence intervals on the mean of the three replicates. With overlapping 95% confidence intervals, average inactivation was statistically identical at concentrations of 3 x 10^4 and 1.5 x 10^6 oocysts per mL in the petri dishes.

The statistical validity of the data was further evaluated using two-factor ANOVA to determine whether there was significant difference between the mean inactivation achieved in the two concentrations at the two UV dose levels. Two-way ANOVA was used since it combines all the data at both UV doses investigated for greater statistical power. The ANOVA was performed using 95% confidence level to decide whether to reject the null hypotheses. The null hypotheses here stated that the effect of the main factors, oocyst concentration and UV dose, the interaction of oocyst concentration and UV dose were not statistically significant. The results of the ANOVA are summarized in Tables 5-4 and A-4 of Appendix A. From the ANOVA output, the main effects, UV dose (p = 0.84) and oocyst concentration (p = 0.31), were found to be not statistically significant. The interaction effect of UV dose and oocyst concentration was also not statistically significant (p = 0.12). These indicated that the null hypotheses were true (Neter *et al.*, 1989; Helsel and Hirsch, 1992) and that there was no evidence for a concentration effect.

5.3 EFFECT OF TURBIDITY ON UV DOSE – RESPONSE OF *C. PARVUM*

5.3.1 Experimental Design

The impact of naturally-occurring suspended solids on UV disinfection efficiency was investigated at four levels of turbidity and two levels of UV dose. The planning matrix and summary of the results of the trials are shown in Table 5-5. The range of turbidity was chosen to represent expected conditions at Kelowna, when water quality is good in lake Okanagan at the water intake (0.25 NTU), at the regulated limit for turbidity (5.0 NTU) of unfiltered systems (USEPA, 2001) and

during times of extreme lake conditions (10.0 and 20.0 NTU). Mean turbidity of 0.5 NTU and a maximum turbidity of 3.50 NTU were recorded at the City of Kelowna according to a four-year turbidity (1998 to 2001) historical data (Figures D-2 to D-5, Appendix D).

Particle size analysis of the lake suspended solids showed that they were composed primarily of particles within the range of 2 to 15 μm (Figure 5-3). Data on the particle size distribution are provided in Tables D-1 and D-2 of Appendix D. Addition of suspended solids to the raw lake water samples increased the turbidity and decreased the UV transmittance and hence the average germicidal irradiance in the water sample. In order to maintain a constant UV dose at each turbidity level, the exposure time was increased accordingly. Table 5-6 provides typical data that shows the effect of turbidity on the percent transmittance, the average irradiance and the exposure time required for target UV doses of 5 and 40 mJ/cm^2. The 8 experimental conditions of UV dose and turbidity were replicated for a total of 24 trials. Two additional trials were conducted at turbidities of 0.25 and 20.0 NTU and UV dose of 40 mJ/cm^2 to improve upon the statistical power of the linear regression at this dose. Replication provided a measure of experimental error and enabled statistical interpretation of the results.

5.3.2 Effect of Turbidity and UV dose

In 23 out of the 26 trials, the results were within the detection limits of mouse infectivity assay. In 3 trials, no infections were observed in any mice inoculated with UV exposed oocysts meaning the inactivations were greater than the detection limit

of 4.4 log-units for neonatal CD-1 mouse assay. For the purposes of data representation and statistical analysis, the values of the experimental outcomes for these trials were set at the value of the detection limit (i.e. 4.4 log-units). Two of these results were recorded in trials involving a high UV dose (40 mJ/cm^2) and a low turbidity (0.25 NTU).

The data (Figures 5-4 and 5-5) from the trials showed a linear relationship between inactivation and turbidity. Simple least-squares linear regression was, therefore, applied to model inactivation as a function of turbidity. The basic linear regression was expressed as follows:

$$\text{Inactivation} = \beta_1(\text{Turbidity}) + \beta_o \qquad \text{Equation 5-1}$$

Where β_o and β_1 are the least-squares best-fit parameters of the linear model. The best-fit parameters of the model at UV dose of 5.0, with corresponding 95% confidence intervals of the model parameters enclosed in brackets, were:

$$\beta_1 = -0.040\ (-0.079, -0.002) \text{ and } \beta_o = 3.9\ (3.4, 4.3)$$

At a dose of 40.0 mJ/cm^2, the model parameters were:

$$\beta_1 = -0.041\ (-0.063, -0.019) \text{ and } \beta_o = 4.5\ (4.2, 4.7).$$

Confidence intervals quantify the uncertainty associated with the mean response in regression analysis (Helsel and Hirsch, 1992). Figures 5-4 and 5-5 present the data plots, predicted models, including 95% confidence intervals and associated *p*-values of the slopes.

From Figures 5-4 and 5-5, at 0.25 NTU, mean inactivation in replicated experiments was 3.9 and 4.5 log-units at UV doses of approximately 5.0 mJ/cm^2 and 40.0 mJ/cm^2, respectively. At 20.0 NTU, mean inactivation declined to 3.1 and 3.7 log-units for the same delivered UV dose. With replicated trials, the data were analyzed using regression and interpreted based on 95% confidence level. The results showed statistically significant decrease in inactivation at UV doses of 5 mJ/cm^2 (p = 0.039) and 40 mJ/cm^2 (p = 0.002) with increased turbidity (from 0.25 to 20.0 NTU).

Normal probability plots of inactivation residuals (Figures 5-6 and 5-7) indicated that the residuals are normally distributed. The residual plots (Figures 5-8 and 5-9) show a band around zero (zero mean). The lack of constant bands around zero indicate unequal variance and this may be due to the limited number of data. A lack of fit test was also employed to determine whether the data supports linear models (Sample calculations are found in Tables B-3 and B-4 of Appendix B). Since $F_{calculated}$ was found to be less than $F_{critical}$ or $MS_{Lack\ of\ Fit}$ was less than $MS_{Pure\ Error}$, the hypothesis assumption that there was a lack of fit for the proposed lines for the set of data at UV doses of 5.0 and 40.0 mJ/cm^2 was rejected.

To improve upon the statistical power of the data analyses and also to define a relationship between inactivation, turbidity and UV dose, all the outcomes were used

to develop a multiple linear regression model. The multiple linear regression of the following form was used and the results interpreted at the 95% confidence level.

$$\text{Inactivation} = \beta_o + \beta_1(\text{Turbidity}) + \beta_2\ (\text{UV Dose}) + \beta_{12}\ (\text{Turbidity})(\text{UV Dose}) \qquad \text{Equation 5-2}$$

Where β_o, β_1, β_2 and β_{12} are parameters of the multiple linear regression model. From the summary output (Table 5-7), turbidity ($p = 0.022$) and UV dose ($p = 0.012$) were found to be statistically significant but the interaction of turbidity and UV dose ($p = 0.99$) was not. Turbidity and UV dose were found to impact inactivation. According to the summary output (Table 5-7), turbidity impacted inactivation negatively and UV dose, positively. The predicted model from the multiple linear regression is:

$$\text{Inactivation} = 3.8 - 0.040\ (\text{Turbidity}) + 0.017\ (\text{UV Dose}) \qquad \text{Equation 5-3}$$

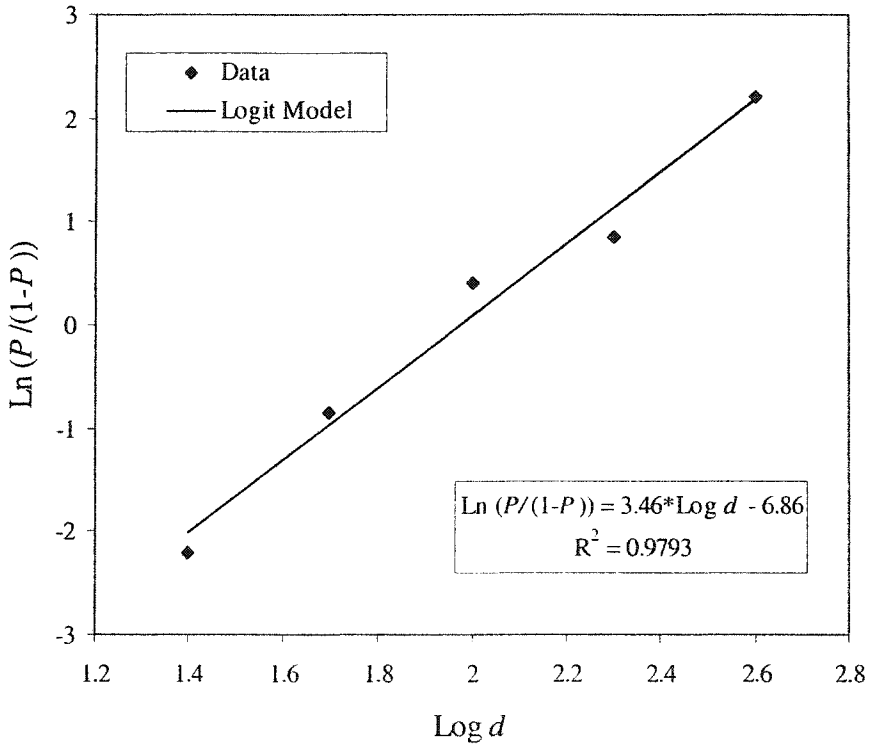

Figure 5-1 Dose-response of the batch of *C. parvum* oocysts used in the experimental trials and the predicted logit model. Best fit parameters of the logit model and their 95% confidence intervals enclosed in brackets are: slope, β_1 = 3.46 (3.3, 3.6) and intercept, β_o = -6.86 (-7.2, -6.6).

Table 5-1 Factors and level-settings used to investigate the effect of oocyst concentration on UV dose inactivation of *C. parvum*

Number	Factors	Level Setting	
		Low (-)	High (+)
A	Oocysts Concentration (Oocysts/mL)	3.0×10^4	1.5×10^6
B	UV Dose (mJ/cm^2)	5.0	40.0

Table 5-2 Design matrix and results of effect of oocyst concentration on UV dose inactivation of *C. parvum*

Run	Factors		Interactions	Yield (Log Inactivation)		
	A	B	AB	1	2	3
1	-	-	+	3.5	4.1	3.6
2	+	-	-	3.6	4.4	3.6
3	-	+	-	4.4	4.4	3.9
4	+	+	+	3.7	3.9	3.6

Table 5-3 Summary of analyses of the data from the effect of oocyst concentration on UV dose response of *C. parvum* experiments

Oocyst Concentration (Oocysts/mL)	UV Dose (mJ/cm^2)	Mean Inactivation (Log-units)	STDEV	SE	±95% C.I.	95% C.I.	
						L.C.I	U.C.I.
3.0×10^4	5.0	3.8	0.3251	0.1877	0.8077	2.99	4.60
3.0×10^4	40.0	4.3	0.3258	0.1881	0.8094	3.49	5.11
1.5×10^6	5.0	3.9	0.4619	0.2667	1.1575	2.74	5.06
1.5×10^6	40.0	3.7	0.1514	0.0874	0.3762	3.32	4.08

Table 5-4 Summary output of Two-factor analysis of variance of data from the effect of oocyst concentration on UV dose response of *C. parvum* experiments

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*Fcrit*
UV Dose	0.0936	1	0.0936	0.8358	0.3873	5.3176
Oocyst concentration	0.1323	1	0.1323	1.1810	0.3088	5.3176
Interaction	0.3400	1	0.3400	3.0353	0.1196	5.3176
Error	0.8962	8	0.1120			
Total	1.4622	11				

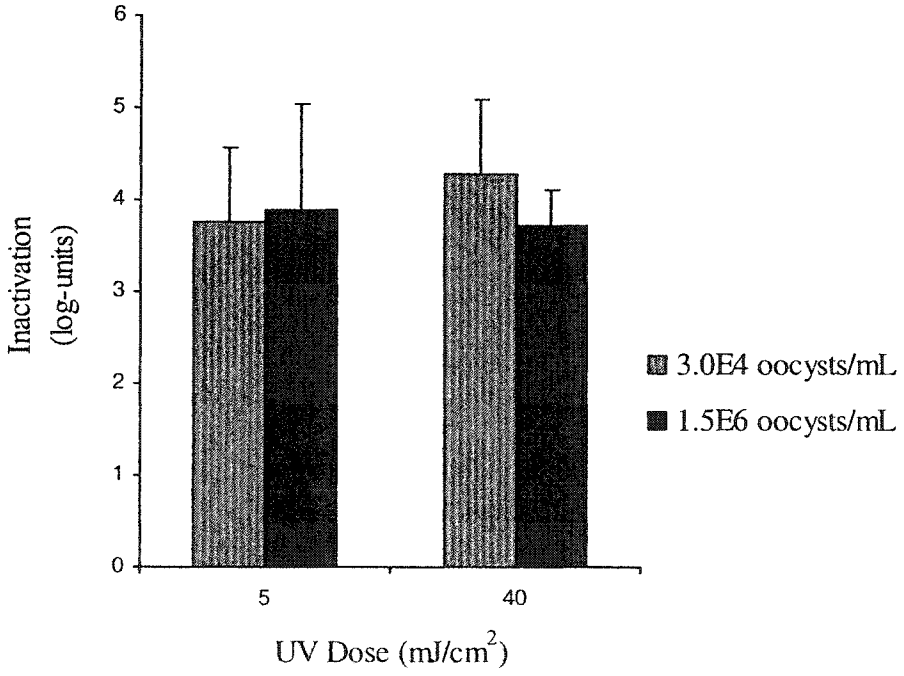

Figure 5-2 Effect of oocyst concentration on UV Dose inactivation of *C. parvum*. The error bars represent the 95% confidence intervals on the mean inactivation.

Table 5-5 Planning matrix and summary of results of UV turbidity experiments with *C. parvum*

Run #	UV Dose (mJ/cm^2)	Turbidity (NTU)	Inactivation (Log units)				
			1	2	3	4	Mean
1	5.0	0.25	3.9	>4.4	3.2		3.8
2	5.0	5.0	3.1	3.9	4.1		3.7
3	40.0	0.25	>4.4	4.4	>4.4	4.4	4.4
4	40.0	5.0	4.1	4.4	4.1		4.2
5	5.0	10.0	3.1	3.5	3.4		3.3
6	5.0	20.0	2.9	2.8	3.5		3.1
7	40.0	10.0	4.3	3.8	4.1		4.1
8	40.0	20.0	2.9	3.7	4.0	4.0	3.7

> The inactivation in these trials were beyond detection, but were set at the maximum detection limit (i.e. 4.4 log-units) for the purpose of statistical analyses

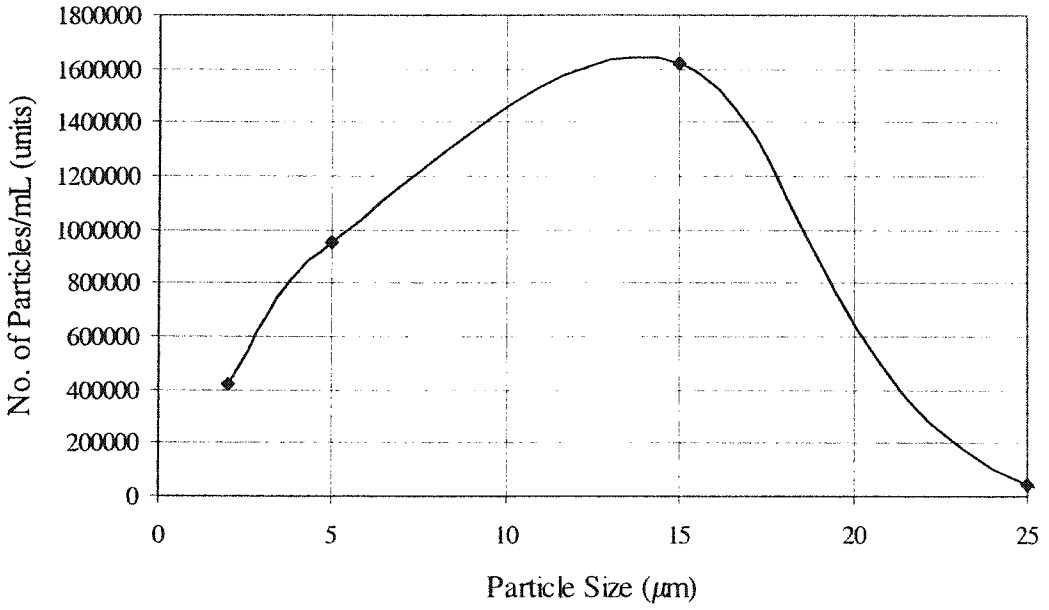

Figure 5-3 Particle size distribution of the lake suspended solids' suspension

Table 5-6 Typical effect of turbidity on transmittance, average germicidal irradiance and exposure times required to maintain a constant UV dose

Turbidity (NTU)	UV Dose = 5 mJ/cm^2			UV Dose = 40 mJ/cm^2		
	%T @254 nm (1 cm)	Average Germicidal Irradiance (mW/cm^2)	Exposure Time (s)	%T @254 nm (1 cm)	Average Germicidal Irradiance (mW/cm^2)	Exposure Time (s)
0.25*	86	0.40	12.4	86	0.39	102
5.0	78	0.39	12.8	77	0.37	108
10.0	71	0.36	13.9	71	0.35	115
20.0	59	0.33	15.0	58	0.32	125

* This was the turbidity of the natural lake water

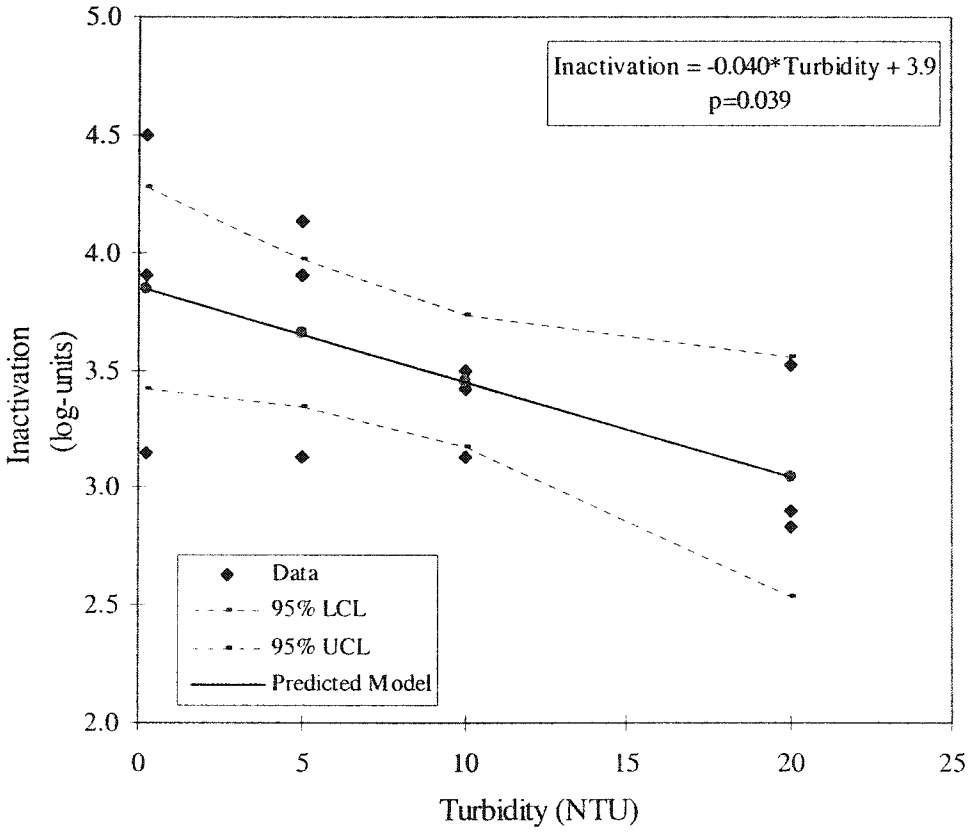

Figure 5-4 Effect of turbidity on inactivation of *C. parvum* oocysts at UV Dose of 5.0 mJ/cm^2. Best fit parameters of the linear model and their 95% confidence intervals enclosed in brackets were: slope, β_1 = -0.040 (-0.079, -0.002) and intercept, β_o = 3.9 (3.4, 4.3).

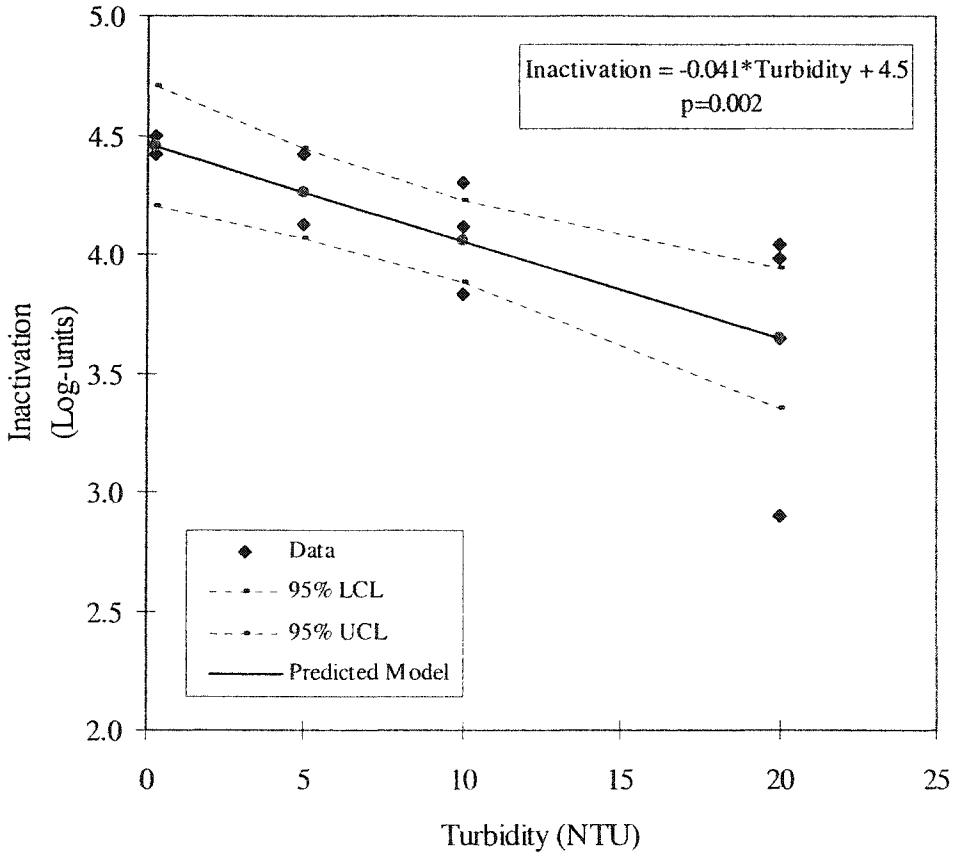

Figure 5-5 Effect of turbidity on inactivation of *C. parvum* oocysts at UV Dose of 40.0 mJ/cm^2. Best fit parameters of the linear model and their 95% confidence intervals enclosed in brackets were: slope, β_1 = -0.041 (-0.063, -0.019) and intercept, β_o = 4.5 (4.2, 4.7).

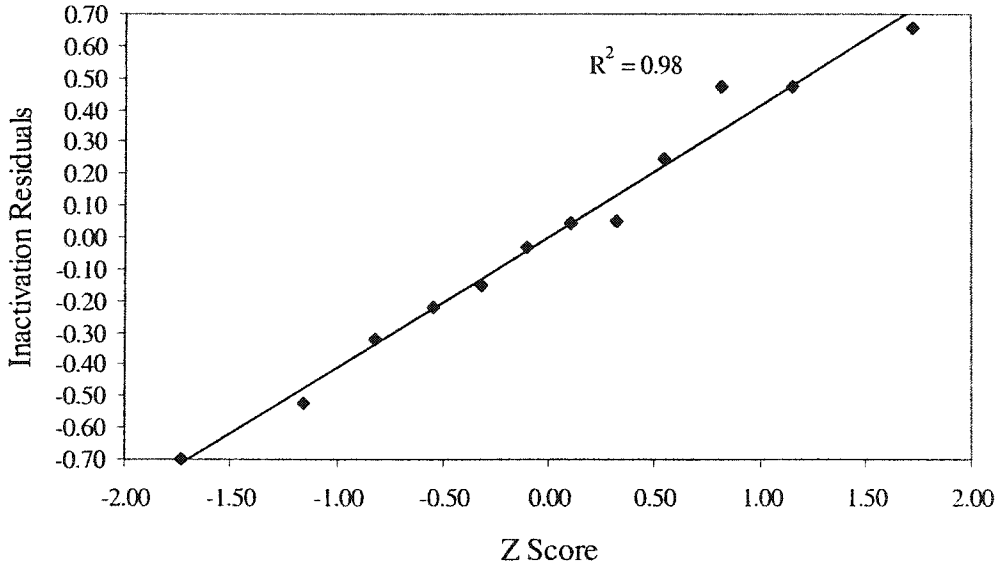

Figure 5-6 Normal probability plot of inactivation residuals at UV dose of 5.0 mJ/cm^2

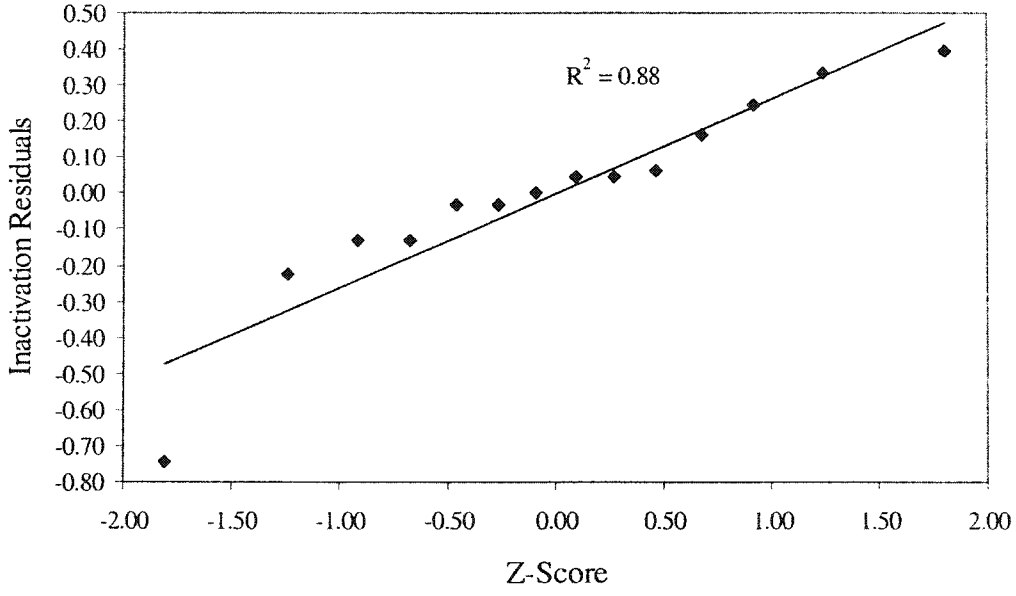

Figure 5-7 Normal probability plot of inactivation residuals at UV dose of 40.0 mJ/cm^2

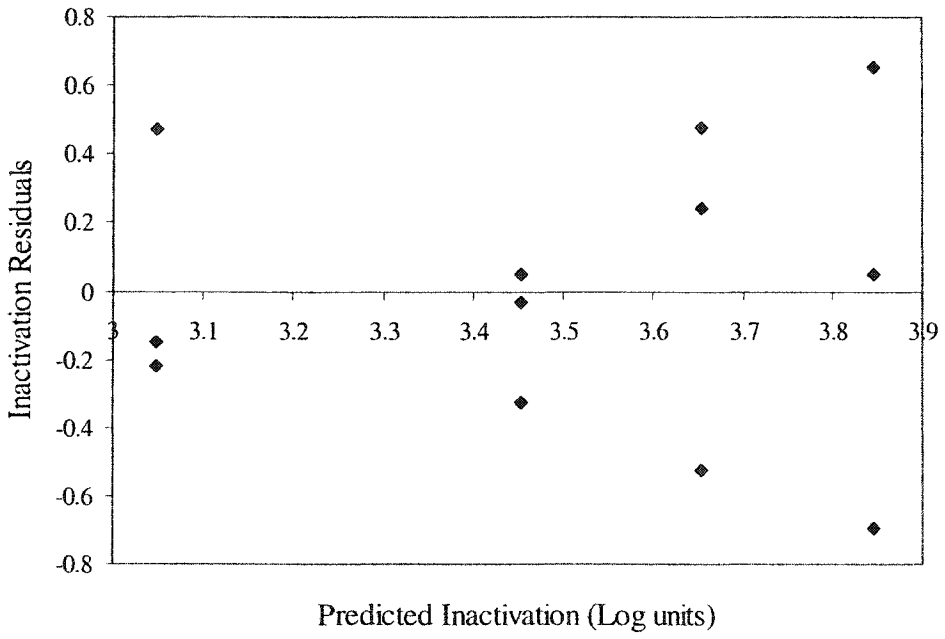

Figure 5-8 Residuals plot of inactivation data at UV dose of 5.0 mJ/cm^2

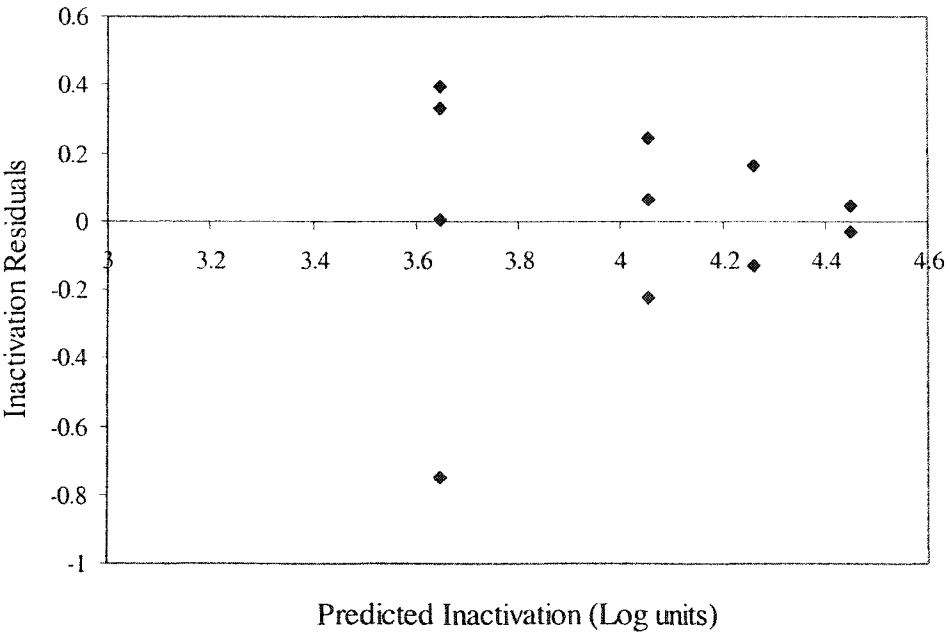

Figure 5-9 Residuals plot of inactivation data at UV dose of 40.0 mJ/cm^2

Table 5-7 Summary output of multiple linear regression of the results of UV turbidity experiments with *C. parvum*

SUMMARY OUTPUT

Regression Statistics	
Multiple R	0.7886
R Square	0.6220
Adjusted R Square	0.5704
Standard Error	0.3618
Observations	26

ANOVA

	df	*SS*	*MS*	*F*	*Sig. F*
Regression	3	4.7381	1.5794	12.0647	0.0001
Residual	22	2.8800	0.1309		
Total	25	7.6181			

	Coefficients	*SE*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*
Intercept	3.7709	0.1880	20.0595	0.0000	3.3810	4.1608
Turbidity	-0.0404	0.0164	-2.4642	0.0220	-0.0744	-0.0064
UV Dose	0.0173	0.0063	2.7437	0.0119	0.0042	0.0304
Turbidity*UV Dose	0.0000	0.0005	-0.0166	0.9869	-0.0011	0.0011

6 UV INACTIVATION OF *GIARDIA MURIS*

6.1 DOSE-RESPONSE IN C3H/HeN MICE

A dose-response experiment was conducted to characterize the cysts used in this work. It was also used to compare the viability of cysts purified with sucrose, followed by Percoll-Percoll gradients centrifugation to viability of cysts purified using sucrose only. Sucrose flotation used in isolation and purification of cysts used in previous studies (Belosevic *et al.*, 1984; Labatiuk *et al.*, 1991; Craik *et al.*, 2000), yielded cysts which were not completely from fecal debris. The Percoll-Percoll centrifugation following sucrose flotation produced cysts virtually free from fecal debris and intestinal flora as determined by microscopic examination. Percoll was chosen as a gradient medium because of its minimal contribution to osmolarity of the solution, its low toxicity to cells and its low viscosity (Pertoft *et al.*, 1978; Price, 1982).

The patterns of cyst shedding in C3H/HeN mice infected with different oral inoculums of fresh cysts produced using the two methods above are shown in Figure 6-1. The pattern of cyst shedding in C3H/HeN mice infected with cysts purified using the two methods of purification above were similar. A similar pattern of cyst shedding has been observed in previous studies (Belosevic *et al.*, 1984; Labatiuk *et al.*, 1991; Craik *et al.*, 2000). The pattern of cyst shedding was characterized by a latent period, in which no cysts were observed. The latent period was followed by a rapid increase in cyst output until the output was stable at about 10^6 cysts per gram feces regardless of the infective inoculum. The latent period and the position in time

of the rapid increase phase increased with decreasing cyst inoculum. Data on average cyst output in a cohort (number of cysts per gram feces and logarithm of number of cysts per gram feces) and observed latency period are provided in Tables C-1 to C-3 of Appendix C.

The linear model (Equation 4-6) was fitted to the data in Figure 6-2, using the method of least squares. The model parameters are shown with their standard errors in brackets.

$$\text{Log}(d) = (-11.4 \pm 0.8)\,\text{Log}(LP) + (9.4 \pm 0.4) \qquad \text{Equation 6-1}$$

The residual plot, Figure 6-4, showed data points scattered around zero, indicating a mean residual of zero.

The data and predicted latent period model developed from previous studies (Craik *et al.*, 2000) were superimposed on the ones from this work and are found in Figure 6-3. This was to allow for easy comparison of the results from the two studies. The predicted linear model from this work is nearly parallel to the one from Craik *et al.* (2000), with a little lower Log (*d*) intercept value.

6.2 EFFECT OF TURBIDITY ON UV DOSE-RESPONSE OF *G. MURIS*

6.2.1 Experimental Design

The effect of turbidity on UV dose-response of *G. muris* was investigated using a 2^2 factorial experimental design approach. The two factors were UV dose and

turbidity. The factors and their level-settings are found in Table 6-1. UV exposures were carried out at selected combinations of germicidal UV doses of 5 and 40 mJ/cm^2 and turbidities of 0.25 and 20.0 NTU. The 2^2 factorial design resulted in 4 experimental conditions. The planning matrix and results are presented in Table 6-2. Three replicates were performed for each combination of UV dose and turbidity for a total of 12 trials. The replicates were carried out on separate days. For each trial, cohorts of five C3H/HeN mice were infected with 5.0×10^4 UV exposed cysts concentrated from the UV exposed samples and the controls (samples which were not exposed to UV irradiation).

6.2.2 Effect of Turbidity and UV Dose

Typical patterns of cyst shedding in the feces of C3H/HeN mice infected with cysts concentrated from experimental trials are shown in Figure 6-5. They were based on the results from one set of replicates. Each point represented the average cyst output per gram of wet feces for a cohort of five mice on successive days post infection. The latent period and the position in time of the rapid increase phase increased with increasing UV dose. It increased from 0 UV dose (control) through 5 mJ/cm^2 to 40 mJ/cm^2. At each of the UV dose levels, the latent period increased from trials with high turbidity (20.0 NTU) to those with low turbidity (0.25 NTU). Inactivation, therefore, increased with increasing UV dose and decreased with increasing turbidity. Data on average cysts output in a cohort (number of cysts per gram feces and logarithm of number of cysts per gram feces) and observed latency

period from all three sets of replicates are provided in Tables C-4 to C-12 of Appendix C.

Table 6-3 provides a summary of statistical analyses of the individual experimental outcomes. The summary is illustrated in Figure 6-6. The error bars represent the 95% confidence intervals on the mean inactivation. From Figure 6-6, at UV dose of 5.0 mJ/cm^2, mean inactivation in replicated experiments was 3.0 and 2.6 log-units at turbidities of 0.25 NTU and 20.0 NTU, respectively. The mean inactivations were 4.4 and 4.0 log-units for the same levels of turbidity at UV dose of 40.0 mJ/cm^2. A minimal decrease in inactivation with increasing turbidity was, therefore, observed at both UV doses.

A two-factor ANOVA was used to determine the statistical significance of the observed trend. The ANOVA was performed using a 95% confidence level to decide whether to reject the null hypotheses of no difference in the mean inactivations achieved. The results of the ANOVA are summarized in Tables 6-4 and C-13 (Appendix C). The computed p-values in the ANOVA indicated that the effect of UV dose ($p = 0.00$) was statistically significant. The effect of turbidity ($p = 0.059$) was statistically significant only at the 94% confidence level. The interaction effect of UV dose and turbidity was not statistically significant ($p = 0.65$).

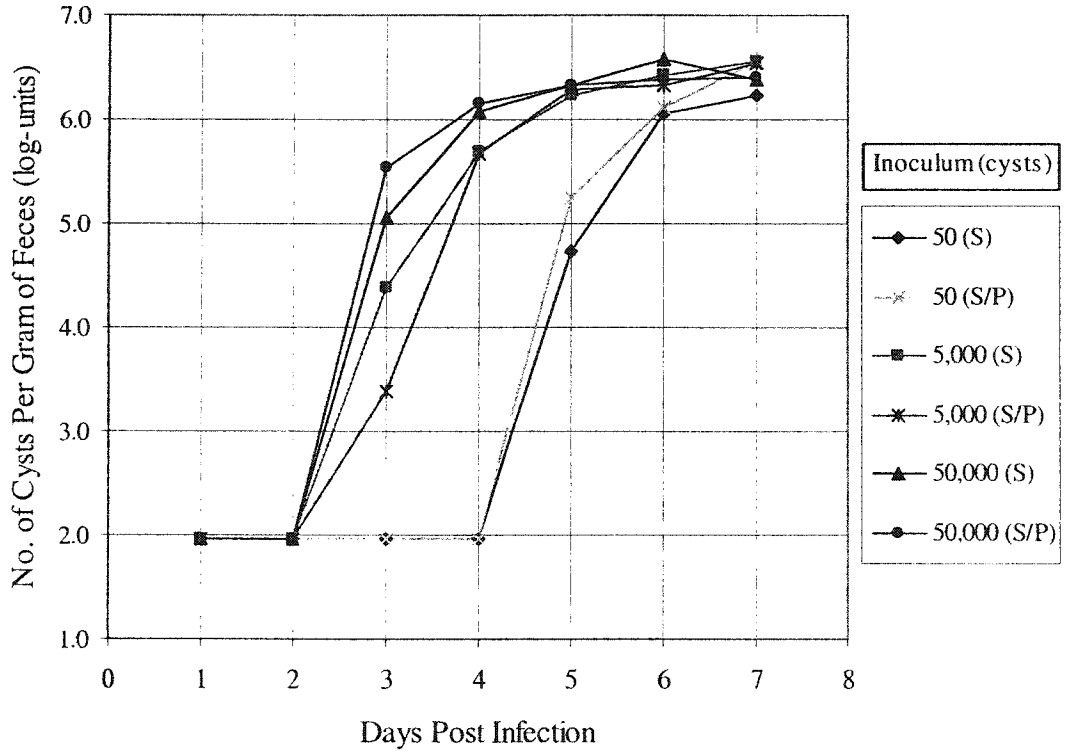

Figure 6-1 Pattern of *G. muris* cysts shedding in C3H/HeN mice in a dose-response experiment. S - indicates cysts purified using sucrose centrifugation only and S/P – cysts purified using sucrose followed by Percoll-Percoll gradients. For days on which no cysts were detected, cyst production was set at one-half the detection limit (i.e. 2.0 log-units).

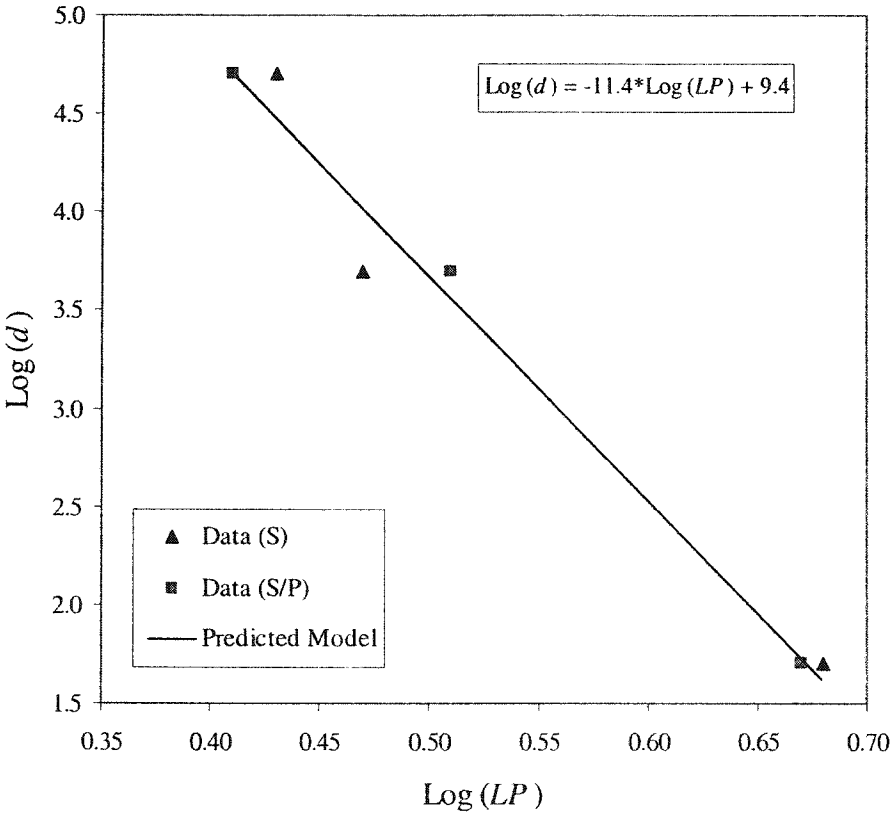

Figure 6-2 Latent period, *LP*, in C3H/HeN mice as a function of the number of fresh *G. muris* cysts in the oral inoculum, *d*. The best-fit parameters of the predicted linear model with their standard errors enclosed in brackets are: slope, $\beta_1 = -11.4$ (± 0.8) and intercept, $\beta_o = 9.4$ (± 0.4).

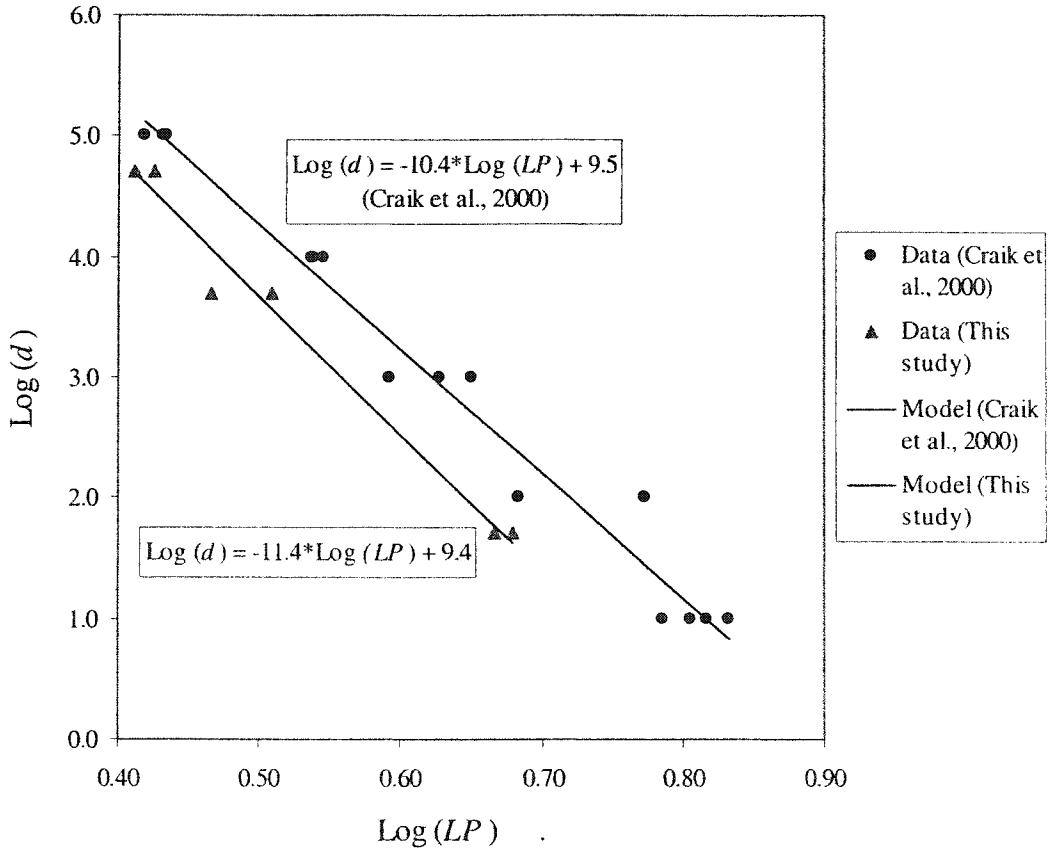

Figure 6-3 Latent Period, *LP*, in C3H/HeN mice as a function of the number of fresh *G. muris* cysts in oral inoculum, *d*, from this study and Craik *et al.* (2000).

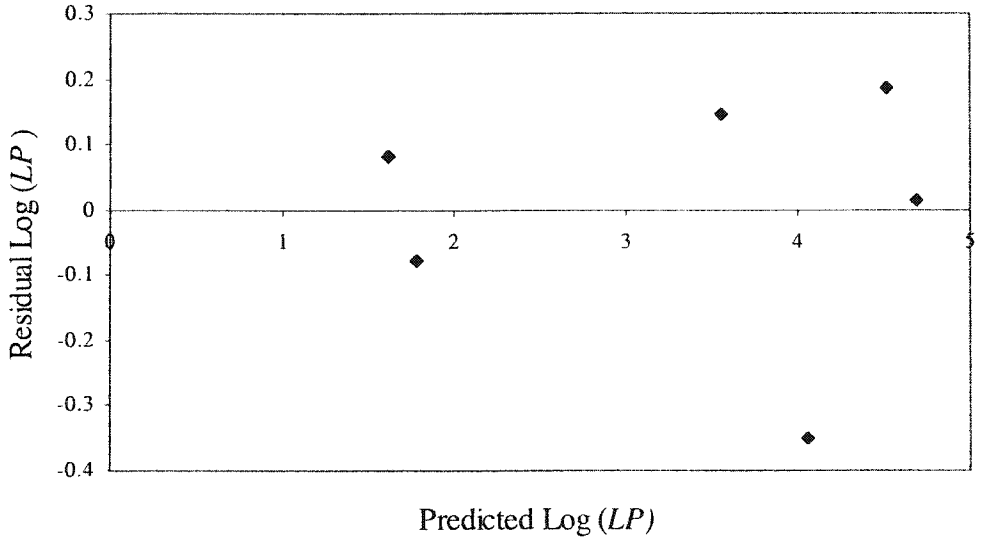

Figure 6-4 Plot of residuals against the predicted logarithm of latent period from the dose response experiment with C3H/HeN mice

Table 6-1 Factors and level-settings used to investigate the effect of turbidity on UV dose inactivation of *G. muris*

Number	Factors	Level Setting	
		Low (-)	High (+)
A	Turbidity (NTU)	0.25	20.0
B	UV Dose (mJ/cm^2)	5.0	40.0

Table 6-2 Planning matrix and results of effect of turbidity on UV dose inactivation of *G. muris*

Expt. Condition	UV Dose (mJ/cm^2)	Turbidity (NTU)	Inactivation (Log units)			
			1	2	3	Mean
1	5.0	0.25	2.8	3.2	3.1	3.0
2	5.0	20.0	2.8	2.6	2.3	2.6
3	40.0	0.25	3.9	4.7	4.4	4.4
4	40.0	20.0	4.2	3.8	4.2	4.0

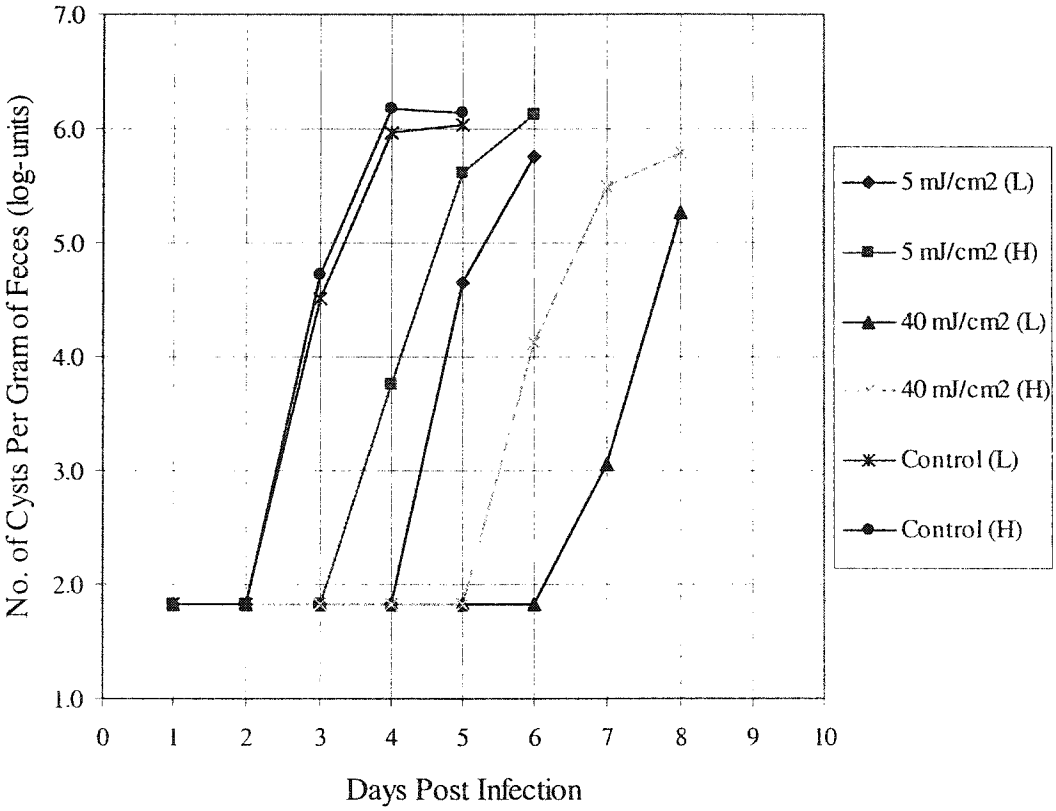

Figure 6-5 Typical pattern of cyst shedding in the feces of C3H/HeN mice infected with *G. muris* cysts from UV – Turbidity experimental trials. The figure was drawn using data from one set of replicate groups. L and H indicate the low (0.25 NTU) and high (20.0 NTU) level settings of turbidity, respectively, used in the experiments. For days on which no cysts were detected, cyst production was set at one-half the detection limit (i.e. 1.9 log-units).

Table 6-3 Summary of data analyses of the effect of turbidity on UV dose response of *G. muris*

Turbidity (NTU)	UV Dose (mJ/cm^2)	Mean Inactivation (Log-units)	STDEV	SE	±95% C.I.	95% C.I.	
						L.C.I	U.C.I.
0.25	5.0	3.0	0.2065	0.1192	0.5130	2.49	3.51
0.25	40.0	4.4	0.4409	0.2546	1.0955	3.30	5.50
20.0	5.0	2.6	0.2359	0.1362	0.5860	2.00	3.19
20.0	40.0	4.1	0.2157	0.1245	0.5359	3.46	4.54

Table 6-4 Summary output of Two-factor analysis of variance of data from the effect of turbidity on UV dose response of *G. muris* experiments

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*Fcrit*
UV Dose	5.7963	1	5.7963	68.35	0.0000	5.3176
Turbidity	0.4107	1	0.4107	4.84	0.0589	5.3176
Interaction	0.0192	1	0.0192	0.23	0.6469	5.3176
Error	0.6785	8	0.0848			
Total	6.9047	11				

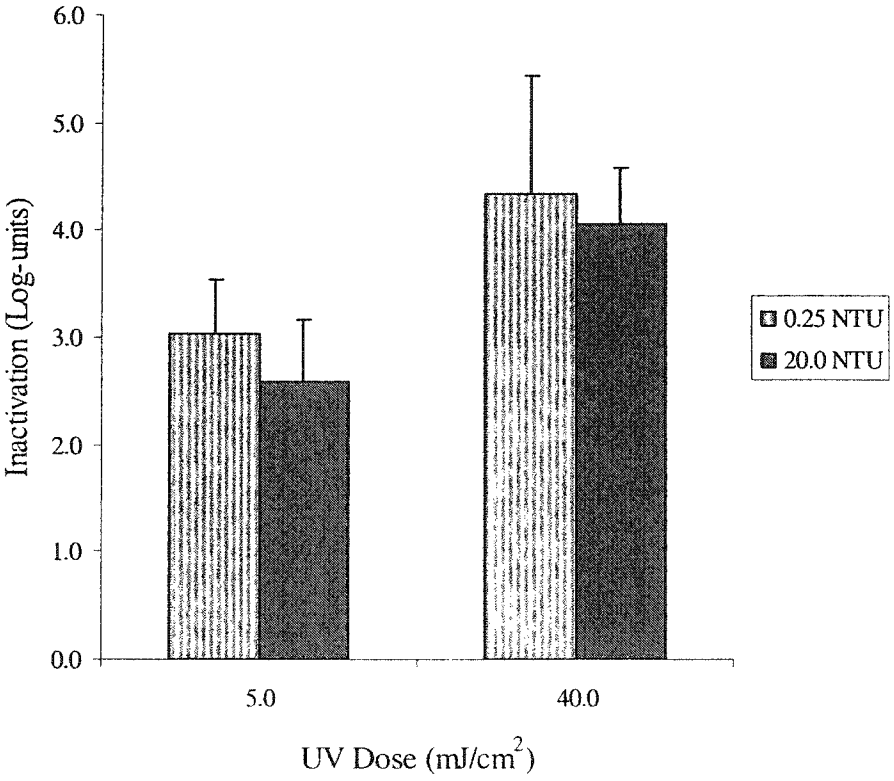

Figure 6-6 Effect of turbidity on inactivation of *G. muris* cysts in collimated beam experiments. The error bars indicate 95% confidence intervals on the mean inactivation.

7 DISCUSSION OF RESULTS

7.1 UV INACTIVATION OF *CRYPTOSPORIDIUM PARVUM*

7.1.1 Logistic Dose-Response in CD-1 Mice

The estimated oocyst dose required for 50% infection in the mice (ID_{50}) was calculated from the logit model parameters to be 96 oocysts. ID_{50}s ranging from 60 to 87 oocysts (Korich *et al.*, 2000) and 53 to 113 oocysts (Craik *et al.*, 2001) have been reported. Animal infectivity studies also indicated an ID_{50} ranging between 79 to 82 oocysts (Clancy *et al.*, 1998) and approximately 79 oocysts (Finch *et al.*, 1993a). The ID_{50} determined in this study compared well with ID_{50} values estimated in previous studies (Korich *et al.*, 2000; Craik *et al.*, 2001), an indication that the oocysts used showed typical viability.

7.1.2 Effect of Oocyst concentration on UV Dose – response of *C. parvum*

Mean inactivation (log-units) of 3.8 and 4.3 were achieved at UV doses of 5.0 and 40.0 mJ/cm^2, respectively. Similar levels of inactivation have been reported under similar conditions and using similar infectivity and UV exposure procedures (Bukhari *et al.*, 1999; Clancy *et al.*, 2000; Craik *et al.*, 2001).

Parasite inactivation was unaffected by the modified collimated beam protocol and 50-fold reduction in oocyst concentration provided that the same UV dose was delivered to the suspension. The use of animal infectivity assays to detect high levels of inactivation (greater than 4 log-units) requires the use of large number of parasites in experimental trials (Craik *et al.*, 2001). These suspensions, 20 mL (19.5 mL of

water sample and 30 x 10^6 oocysts in 0.5 mL deionized water) in volume, usually had turbidities of approximately 5.0 NTU and above resulting from the parasites themselves. Using the modified collimated beam protocol the turbidities averaged 0.5 NTU and reflected real water treatment conditions. The only drawback of the low concentration protocol was the fact that it involved 50 multiple UV exposures and was therefore time consuming.

There was no statistically significant increase in inactivation when the UV dose was increased from 5.0 to 40.0 mJ/cm^2 and this indicated the presence of non-linear inactivation kinetics and a 'tailing' effect even at the lower oocyst concentrations. Cerf (1977) reviewed tailing of survival curves and reported that people who have observed tails or who have considered the question either accepted them as facts or rejected them as artefacts. Tailing might be interpreted by assuming that it is a normal feature bound to the mechanism of inactivation or resistance and/or an artefact independent from the mechanism of inactivation. In a study to look at the treatment of municipal wastewater effluents with ultraviolet light, Severin (1980) observed a plateau or tail in the survival curve of fecal coliforms where increasing the UV dose had no further effect on inactivation but did not investigate the causes. More recently, tailing was observed in experiments with UV light using *G. muris* (Craik *et al.*, 2000) and *C. parvum* (Craik *et al.*, 2001). They found no improvement in inactivation at low oocyst concentration and suggested that the tailing may have been caused by the presence of a small sub-population of oocysts that are far more resistant to the effects of UV radiation than the bulk population due to genetic or morphological differences. The observation of tailing at low concentration in this

work confirmed the observation that tailing may not be due to high oocyst concentration and supports the hypothesis of the presence of a resistant sub-population.

7.1.3 Effect of Turbidity on UV Dose -Response of *C. parvum*

On average, increasing dose from 5.0 to 40.0 mJ/cm^2 resulted in a 0.6 log-unit increase in inactivation, while increasing turbidity from 0.25 to 20.0 NTU resulted in a 0.8 log-unit decrease. These results are based on the multiple linear regression model. This model (Eqn. 5-3) was preferred to the simple linear regression models for it used twice as much data and therefore had more statistical powering in its data analysis than in the individual linear regression models (Eqn. 5-1). The larger the data size, the smaller the standard error and hence the more precise the confidence interval on the mean inactivation determined. The decline in inactivation with increasing turbidity may appear to be unimportant practically but was found to be statistically significant. Statistical significance suggested that the naturally occurring suspended solids in Lake Okanagan, Kelowna, BC were impacting inactivation beyond simple absorbance, which was accounted for in the UV dose determinations.

The decline in mean inactivation was found to vary linearly with turbidity in the ranges investigated. There is limited literature on how naturally occurring suspended solids impacts UV inactivation of *C. parvum* in water treatment. Linear relationship between suspended solids and the surviving fecal coliforms have been reported (Stover *et al.*, 1986; Whitby and Palmateer, 1993). The ability of medium pressure UV to inactivate oocysts suspended in recycled backwash water supernatant

was investigated by Clancy *et al.* (2000). They found UV efficiency unaffected by the presence of the suspended solids and reported achieving high levels of oocyst inactivation in water with turbidity greater than 11 NTU. The particle size distribution and chemical composition of the suspended solids were not reported. These observations may have been due to the fact that the suspended solids were mainly made up of particles smaller than the size of oocysts (Hoff, 1978) and/or were mainly inorganic in nature (Qualls *et al.*, 1983).

The mechanism for the statistically significant decline in inactivation with increasing turbidity was not investigated. The solid suspension was composed primarily of particles ranging from 2 to 15 μm. The suspended particles may have provided some level of shielding of the parasites, averaging 3 μm in diameter, from the UV irradiation. Wastewater particles 10 μm in diameter and larger have been found capable of completely shielding organisms embedded within the particles from UV light (Qualls *et al.*, 1983; Chang *et al.*, 1985). The suspended solids could have blocked the UV irradiation from reaching the parasites (Qualls *et al.*, 1983). There could have been particle – parasite association and thus parasites shielded from the delivered UV radiation (Loge *et al.*, 1999). Sixty-seven percent of the total suspended solids were found to be volatile (organic). Organic particles and not inorganic surfaces such as clay could protect organisms from disinfectants (Qualls *et al.*, 1983). A combination of the above cases could also have resulted in providing some form of protection of the oocysts from UV irradiation.

It should be noted that in the reported studies on effect of suspended solids on UV inactivation of parasites, fecal coliforms and wastewater samples were used.

These are very much different from the unfiltered drinking water and *C. parvum* oocysts and *G. muris* cysts used in this study. Wastewater flocs provide substrates for the growth and multiplication of fecal bacteria. The oocysts and cysts are dormant stages of the parasites and do not undergo any growth or multiplication inside the suspended solids in the unfiltered drinking water. They are, therefore, less likely to interact with the suspended solids from Lake Okanagan in the same way that fecal coliforms have been observed to interact with wastewater particles.

7.2 UV INACTIVATION OF *GIARDIA MURIS*

7.2.1 Dose-Response in C3H/HeN Mice

The cyst shedding characteristics of mice infected with cysts purified using sucrose flotation were similar to those of mice infected with cysts produced using Percoll-Percoll gradients following sucrose flotation (Figure 6-1, Chapter 6). This meant that purifying cysts beyond sucrose flotation with Percoll-Percoll gradients did not reduce the cyst infectivity. The Percoll-purified cysts were as infectious as cysts produced using sucrose flotation alone but were much more free of fecal debris. This enabled meaningful turbidity experiments to be conducted with *G. muris*.

The parameters of the linear latent period model developed in this study, though with fewer data, compared well with parameters in a similar model developed by Craik *et al.* (2000) (Figure 6-3, Chapter 6). This was an indication that the dose response relationship was reproducible and was independent of the batch of cysts and the method of purification (Craik *et al.*, 2000). Their model parameters with

parameters from this work (Figure 6-3, Chapter 6) enclosed in brackets are: Slope, β_1 = -10.4 (-11.4) and intercept, β_o = 9.5 (9.4). Their corresponding standard errors are: ± 0.5 (±0.8) and ±0.3 (±0.4). The slight differences in the slopes and corresponding standard errors may be due to fewer data used in this work.

7.2.2 Effect of Turbidity and UV Dose

The mean inactivation of *G. muris* measured at a UV dose of 40 mJ/cm^2 in this work was 4.4 log-units. This value is more than 1 log-unit greater than previously reported for *G. muris* (Craik *et al.*, 2000) The main difference between this study and the previous study is the additional purification step using Percoll-Percoll gradients, which produced cysts free from fecal debris. This may have rendered the cysts more susceptible to the effects of UV radiation. For instance, the Percoll-Percoll gradients might have made the cysts more transparent to UV radiation by removing fine debris from the surfaces of the cysts' walls. Alternatively, the increase in inactivation achieved in this study over previous studies could have also been due to selection of a subpopulation of cysts by the Percoll-Percoll gradients that are relatively more sensitive to UV irradiation.

On average, increasing dose from 5 to 40 mJ/cm^2 resulted in a 1.4 log-unit increase in inactivation, while increasing turbidity from 0.25 to 20.0 NTU resulted in a 0.4 log-unit decrease in inactivation. The interaction of UV dose and turbidity was not significant. This meant that the turbidity effect was statistically similar at doses of 5 mJ/cm^2 and 40 mJ/cm^2.

7.3 COMPARISON OF UV INACTIVATION OF *C. PARVUM* AND *G. MURIS*

C. parvum oocysts and *G. muris* cysts were sensitive to UV irradiation, a finding which supports recently published studies (Clancy *et al.*, 1998; Bukhari *et al.*,1999; Clancy *et al.*, 2000; Craik *et al.*, 2000; Shin *et al.*, 2001; Craik *et al.* 2001; Mofidi *et al.*, 2002). Approximately 4.5 log-units of inactivation were achieved in both parasites at UV dose of 40 mJ/cm^2 and turbidity of 0.25 NTU. Generally, higher levels of inactivation of *C. parvum* oocysts were achieved than *G. muris* cysts at UV doses of 5 and 40 mJ/cm^2. Data from this study and previous studies (Craik *et al.*, 2000; Craik *et al.*, 2001; Campbell *et al.*, 2002) indicate that the *G. muris* cysts are more resistant than *C. parvum* oocysts. The apparent difference in resistance between the two parasites may be due to genetic or morphological differences (Campbell *et al.*, 2002). The experimental conditions were controlled in the laboratory but the inactivations were determined from biological models. The variability inherent in animal models may have also influenced the results (Finch *et al.*, 1993; Clancy *et al.*, 2002).

ANOVA, simple and multiple linear regression analyses were performed and decisions made at the 95% confidence level. *P*-values of less than or equal to 0.05 meant statistical significance. The interaction effect of UV dose and turbidity was not statistically significant in either experiment. The effect of UV dose on inactivation was found to be statistically significant in turbidity experiments with *C. parvum* (p = 0.012) and *G. muris* (p = 0.000). The effect of UV dose was found to be not statistically significant in preliminary experiments with oocyst concentration. The

results realized in the oocyst concentration experiments might have been due to the use of fewer data and variability inherent in the *C. parvum*/neonatal CD-1 mice assay (Clancy *et al.*, 1998).

The effect of turbidity was statistically significant in *C. parvum* experiments (p = 0.022) but was only statistically significant at the 94% confidence level in *G. muris* experiments (p = 0.059). This observation may be due to the relative sizes of *C. parvum* oocyst and *G. muris* cyst. The sizes of the suspended solids used ranged from 2 to 15 μm, *C. parvum* oocyst, averages 4 μm in diameter and *G. muris* cyst, averages 10 μm long by 8 μm wide. This meant that the particles could have effectively shielded the smaller *C. parvum* oocysts than the larger *G. muris* cysts. Alternatively, the lower significance level of the *G. muris* experiment may simply have been due to statistical limitations associated with sample size. One more replicate of the experimental trials would have rendered the reduction in inactivation of *G. muris* cysts with increased turbidity statistically significant at the 95% confidence level. This was based on the assumption that the level of inactivation achieved in these trials would be the same as the mean inactivation from previous trials. If this condition was met, the p-value for turbidity from a two-factor ANOVA with replication would have been 0.008.

High levels of inactivation of *C. parvum* and *G. muris* were achieved at a UV dose of 40 mJ/cm^2 and turbidity, 20.0 NTU, which are practically important. Turbidity of 20.0 NTU or greater is likely to occur in Lake Okanagan only during a very heavy rainstorm. A heavy rainstorm may also lead to an increase in the concentration of the parasites in the lake due to farm manure and other animal

droppings in the lake's watershed being washed into it. Increase in turbidity has been correlated with increase in the concentration of *C. parvum* oocysts and *Giardia* spp. (LeChevallier and Norton, 1992). The ability of UV light to achieve high levels of inactivation of *C. parvum* oocysts and *Giardia* spp. during plant upsets is very promising for using UV in unfiltered systems.

8 CONCLUSIONS AND RECOMMENDATIONS

8.1 CONCLUSIONS

The results are promising for using UV as a disinfectant for unfiltered drinking water supplies at the City of Kelowna. The main conclusions are:

1. Medium-pressure UV was very effective against *C. parvum* and *G. muris* (oo)cysts suspended in unfiltered water from Kelowna at a turbidity of 20.0 NTU and UV dose of 40 mJ/cm^2 in bench-scale collimated beam experiments.
2. The increase in inactivation of *C. parvum* oocysts and *G. muris* cysts when germicidal UV dose was increased from 5 to 40 mJ/cm^2 was statistically significant at the 95% confidence level.
3. The reduction in inactivation of *C. parvum* oocysts with increased turbidity (0.25 to 20.0 NTU) was statistically significant at the 95% confidence level at the two UV doses investigated. The reduction in inactivation of *G. muris* cysts with increased turbidity was, however, not statistically significant at the 95% confidence level but was significant at the 94% level. The mechanism that accounted for the reduction in mean inactivation with increased turbidity was not investigated.
4. The effect of turbidity on parasite inactivation was independent of UV dose. In other words, the reduction in mean inactivation with turbidity was the same at UV doses of 5 and 40 mJ/cm^2.
5. Oocysts inactivation was unaffected by a 50-fold decrease in oocyst concentration. This suggests that tailing observed in earlier collimated beam

studies was not an experimental artefact created by high parasite concentration.

8.2 RECOMMENDATIONS

1. Further research should be directed towards investigating the mechanism that accounted for the reduction of inactivation with increased turbidity. Parasite suspensions were stirred for one hour before UV exposures, the question is, was the time long enough for the occurrence of particle - parasite associations? Experimental designs, which allow parasite suspensions to stand for a day or more to allow more time for particle – parasite associations should be explored.
2. This study was conducted at bench-scale. There is the need to conduct on-site pilot plant testing at the City of Kelowna, in addition, to be able to determine the efficiency and adequacy of UV disinfection for the quality of water from Lake Okanagan. The efficiency test should involve injecting select microorganisms into influent water and sampling effluent water to determine survival rates. Fortunately, this has been done as a parallel study; in which challenge tests were done using MS2 colliphage and *Bacillus subtilis* spores but its findings are yet to be published.
3. It is worth exploring how UV inactivation of *C. parvum* and *Giardia* spp. is affected by different types of turbidity or particles from various sources such as other surface waters, soil sediments, wastewater flocs, etc.

4. In the determination of germicidal UV dose, UV absorbance measurements were done using a standard spectrophotometer. This has been reported to overestimate UV absorbance in wastewater due to scattering of light by particles (Qualls *et al.*, 1983, Linden and Darby, 1998). Further research should consider employing integrating sphere spectroscopy, which has been found most suitable for measuring UV absorbance of scattering suspensions (Linden and Darby, 1998).

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APPENDIX A DATA AND INFORMATION FROM DOSE – RESPONSE AND OOCYST CONCENTRATION EXPERIMENTS (CHAPTER 5)

Table A-1 Dose response experiments with *C. parvum* oocysts using neonatal CD-1 mice

Experiment No	Inoculum Size *d*	Number of Mice in Cohort	Number of Mice Infected
1	25	10	1
	50	10	5
	100	10	7
	200	10	7
2	25	10	0
	50	10	1
	100	10	7
	200	10	9
3	25	10	0
	50	10	3
	100	10	6
	200	10	7
4	50	10	2
	100	10	6
	200	10	6
	400	10	7
5	50	10	1
	100	10	5
	200	10	3
	400	10	8
6	50	10	1
	100	10	1
	200	10	5
	400	10	7

Table A-2 Summary of UV exposure parameters and log inactivation of the effect of oocyst concentration experiment

Experimental condition	Oocyst Concentration (Oocyst/mL)	Germicidal UV Irradiance (mW/cm^2)	Exposure Time (s)	Germicidal UV Dose (mJ/cm^2)	Inactivation (Log-units)
1	3.0×10^4	0.39	13.0	5.1	3.52
Control				0.0	-0.40
2	1.5×10^6	0.32	15.5	5.0	3.62
Control				0.0	-0.17
3	3.0×10^4	0.39	102.0	40.0	nd
Control				0.0	0.07
4	1.5×10^6	0.35	115.0	39.9	nd
Control				0.0	-0.40
5	3.0×10^4	0.45	11.0	5.0	nd
Control				0.0	nd
6	1.5×10^6	0.40	12.5	5.0	4.50
Control				0.0	0.12
7	3.0×10^4	0.45	88.0	39.9	4.42
Control				0.0	-0.17
8	1.5×10^6	0.40	100.0	39.9	3.90
Control				0.0	-0.17
9	3.0×10^4	0.45	11.0	4.9	3.63
Control				0.0	nd
10	1.5×10^6	0.38	13.0	4.9	3.62
Control				0.0	0.12
11	3.0×10^4	0.43	92.0	40.0	3.90
Control				0.0	-0.17
12	1.5×10^6	0.37	107.0	39.9	3.62
Control				0.0	0.83
13	3.0×10^4	0.45	11.0	4.9	3.63
Control				0.0	nd
14	1.5×10^6	0.38	13.0	4.9	3.62
Control				0.0	0.12
15	3.0×10^4	0.43	92.0	40.0	3.90
Control				0.0	nd
16	1.5×10^6	0.37	107.0	39.9	3.62
Control				0.0	0.83

Table A-3 Results of infectivity analysis in oocyst concentration experiments with *C. parvum* using neonatal CD-1 mice

Expt. Cond.	Sample	UV Dose (mJ/cm^2)	d_o	Mice in Cohort	Mice Infected	P	d	Inactivation - Log (d/d_o)	
								Obs.	Mean
1	Treated[a]	5.0	10,000	5	0	0.0	<38	>2.42	
			100,000	5	1	0.2	38	3.42	3.52
			1,000,000	5	4	0.8	242	3.62	
	Control[a]	0.0	50	5	3	0.6	126	-0.40	-0.40
			500	5	5	1.0	>242	<0.32	
2	Treated[b]	5.0	10,000	5	0	0.0	<38	>2.42	3.62
			100,000	5	0	0.0	<38	>3.42	
			1,000,000	5	4	0.8	242	3.62	
	Control[b]	0.0	50	5	2	0.4	73	-0.17	-0.17
			500	5	5	1.0	>242	<0.32	
3	Treated[a]	40.0	10,000	5	0	0.0	<38	>2.42	nd
			100,000	5	0	0.0	<38	>3.42	
			1,000,000	5	0	0.0	<38	>4.42	
	Control[a]	0.0	50	5	2	0.4	74	-0.17	0.07
			500	5	4	0.8	242	0.32	
4	Treated[b]	40.0	10,000	5	0	0.0	<38	>2.42	nd
			100,000	5	0	0.0	<38	>3.42	
			1,000,000	5	0	0.0	<38	>4.42	
	Control[b]	0.0	50	5	3	0.6	126	-0.40	-0.40
			500	5	5	1.0	>242	<0.32	

Table Notes:

[a] oocyst concentration was 3.0×10^4/mL

[b] oocyst concentration was 1.5×10^6/mL

d_o Inoculum as determined by hemacytometer count

d Infectious concentration determined from dose response experiment

P Rate of infection (i.e. fraction of mice infected in a cohort)

Obs. observed log inactivation

nd not done

Table A-3 Results of infectivity analysis in oocyst concentration experiments with *C. parvum* using neonatal CD-1 mice

Expt. Cond.	Sample	UV Dose (mJ/cm^2)	d_o	Mice in Cohort	Mice Infected	P	d	Inactivation - Log (d/d_o)	
								Obs.	Mean
5	Treated[a]	5.0	10,000	5	0	0.0	<38	>2.42	
			100,000	5	0	0.0	<38	>3.42	nd
			1,000,000	5	0	0.0	<38	>4.42	
	Control[a]	0.0	50	5	0	0.0	<38	>0.12	
			500	5	5	1.0	>242	<0.32	
6	Treated[b]	5.0	10,000	5	0	0.0	<38	>2.42	nd
			100,000	5	0	0.0	<38	>3.42	
			1,000,000	5	0	0.0	<38	>4.42	
	Control[b]	0.0	50	5	2	0.0	<38	>0.12	
			500	5	5	1.0	>242	<0.32	
7	Treated[a]	40.0	10,000	5	0	0.0	<38	>2.42	4.42
			100,000	5	0	0.0	<38	>3.42	
			1,000,000	5	1	0.2	38	4.42	
	Control[a]	0.0	50	4	0	0.0	<46	>0.03	<0.40
			500	4	4	1.0	>200	<0.40	
8	Treated[b]	40.0	10,000	5	0	0.0	<38	>2.42	3.90
			100,000	5	0	0.0	<38	>3.42	
			1,000,000	5	3	0.6	126	3.90	
	Control[b]	0.0	50	5	2	0.4	73	-0.17	-0.17
			500	5	5	1.0	>242	<0.32	

Table A-3 Results of infectivity analysis in oocyst concentration experiments with *C. parvum* using neonatal CD-1 mice

Expt. Cond.	Sample	UV Dose (mJ/cm^2)	d_o	Mice in Cohort	Mice Infected	P	d	Inactivation - Log (d/d_o)	
								Obs.	Mean
9	Treated[a]	5.0	10,000	5	0	0.0	<38		
			100,000	5	2	0.4	73	3.42	3.52
			1,000,000	5	2	0.4	73	3.62	
	Control[a]	0.0	50	5	0	0.0	<38	>0.12	nd
			500	5	5	1.0	>242	<0.32	
10	Treated[b]	5.0	10,000	5	0	0.0	<38	>2.42	3.62
			100,000	5	0	0.0	<38	>3.42	
			1,000,000	5	4	0.8	242	3.62	
	Control[b]	0.0	50	5	1	0.2	38	0.12	0.12
			500	5	5	1.0	>242	<0.32	
11	Treated[a]	40.0	10,000	5	0	0.0	<38	>2.42	3.90
			100,000	10	0	0.0	<22	>3.62	
			1,000,000	5	3	0.6	126	3.90	
	Control[a]	0.0	50	5	0	0.0	<38	>0.12	nd
			500	5	5	1.0	>242	<0.32	
12	Treated[b]	40.0	10,000	5	0	0.0	<38	>2.42	3.62
			100,000	5	0	0.0	<38	>3.42	
			1,000,000	5	4	0.8	242	3.62	
	Control[b]	0.0	50	5	0	0.0	<38	>0.12	0.83
			500	5	2	0.4	73	0.83	

Table A-3 Results of infectivity analysis in oocyst concentration experiments with *C. parvum* using neonatal CD-1 mice

Expt. Cond.	Sample	UV Dose (mJ/cm^2)	d_o	Mice in Cohort	Mice Infected	P	d	Inactivation - Log (d/d_o)	
								Obs.	Mean
13	Treated[a]	5.0	10,000	5	0	0.0	<38		
			100,000	5	2	0.4	73	3.42	3.52
			1,000,000	5	2	0.4	73	3.62	
	Control[a]	0.0	50	5	0	0.0	<38	>0.12	nd
			500	5	5	1.0	>242	<0.32	
14	Treated[b]	5.0	10,000	5	0	0.0	<38	>2.42	3.62
			100,000	5	0	0.0	<38	>3.42	
			1,000,000	5	4	0.8	242	3.62	
	Control[b]	0.0	50	5	1	0.2	38	0.12	0.12
			500	5	5	1.0	>242	<0.32	
15	Treated[a]	40.0	10,000	5	0	0.0	<38	>2.42	3.90
			100,000	10	0	0.0	<22	>3.62	
			1,000,000	5	3	0.6	126	3.90	
	Control[a]	0.0	50	5	0	0.0	<38	>0.12	nd
			500	5	5	1.0	>242	<0.32	
16	Treated[b]	40.0	10,000	5	0	0.0	<38	>2.42	3.62
			100,000	5	0	0.0	<38	>3.42	
			1,000,000	5	4	0.8	242	3.62	
	Control[b]	0.0	50	5	0	0.0	<38	>0.12	0.83
			500	5	2	0.4	73	0.83	

Table A-4 Summary output of ANOVA test of results of oocyst concentration experimental trials

UV Dose (mJ/cm^2)	Concentration (Oocysts/mL)	
	3.0E+04	1.5E+06
5.0	3.52	3.62
	4.13	4.42
	3.63	3.62
40.0	4.5	3.66
	4.42	3.9
	3.9	3.62

Anova: Two-Factor With Replication

SUMMARY	30000	1500000	Total
5			
Count	3	3	6
Sum	11.28	11.66	22.94
Average	3.76	3.89	3.82
Variance	0.1057	0.2133	0.1324
40			
Count	3	3	6
Sum	12.82	11.18	24
Average	4.27	3.73	4.00
Variance	0.1061	0.0229	0.1413
Total			
Count	6	6	
Sum	24.1	22.84	
Average	4.02	3.81	
Variance	0.1638	0.1022	

ANOVA

Source of Variation	*SS*	*df*	MS	F	*P-value*	*F crit*
Sample	0.0936	1	0.0936	0.8358	0.3873	5.317645
Columns	0.1323	1	0.1323	1.1810	0.3088	5.317645
Interaction	0.3400	1	0.3400	3.0353	0.1196	5.317645
Within	0.8962	8	0.1120			
Total	1.4622	11				

APPENDIX B DATA AND INFORMATION FROM *C. PARVUM* TURBIDITY EXPERIMENTS (CHAPTER 5)

Table B-1 Summary of results of infectivity analysis in UV Turbidity experiments with *C. parvum* using neonatal CD-1 mice

Experimental Condition	Measured Turbidity (NTU)	Germicidal UV Irradiance (mW/cm^2)	Exposure Time (s)	Germicidal UV Dose (mJ/cm^2)	Inactivation (Log-units)
1	0.24	0.41	12.5	5.1	3.90
Control	0.24	-	-	-	-0.17
2	4.9	0.38	13.0	5.0	3.13
Control	4.9	-	-	-	0.12
3	0.28	0.39	102.0	40.1	>4.42
Control	0.28	-	-	-	0.32
4	5.1	0.37	108.0	40.2	4.13
Control	5.1	-	-	-	-0.40
5	9.9	0.36	14.0	5.0	3.13
Control	9.9	-	-	-	0.32
6	19.6	0.33	15.0	5.0	2.90
Control	19.6	-	-	-	-0.17
7	9.6	0.35	115.0	40.1	3.52
Control	9.6	-	-	-	-0.17
8	20.4	0.32	125.0	40.0	2.90
Control	20.4	-	-	-	-0.17
9	0.20	0.36	14.0	5.1	>4.42
Control	0.20	-	-	-	0.48
10	5.6	0.33	15.0	5.0	3.90
Control	5.6	-	-	-	-0.04
11	0.28	0.36	111.0	40.0	4.42
Control	0.28	-	-	-	0.60
12	4.8	0.34	117.0	40.0	4.42
Control	4.8	-	-	-	0.22
13	9.8	0.33	15.0	4.9	3.50
Control	9.8	-	-	-	0.32
14	19.9	0.30	16.0	4.8	2.83
Control	19.9	-	-	-	0.32
15	9.9	0.31	130.0	40.0	3.83
Control	9.9	-	-	-	0.83
16	20.8	0.29	139.0	39.9	3.65
Control	20.8	-	-	-	0.32.

Table B-1 Summary of results of infectivity analysis in UV Turbidity experiments with *C. parvum* using neonatal CD-1 mice

Experimental Condition	Measured Turbidity (NTU)	Germicidal UV Irradiance (mW/cm^2)	Exposure Time (s)	Germicidal UV Dose (mJ/cm^2)	Inactivation (Log-units)
17	0.31	0.33	15.0	5.0	3.15
Control	0.31	-	-	-	-0.04
18	5.0	0.32	16.0	5.1	4.13
Control	5.0	-	-	-	0.22
19	0.34	0.32	123.0	39.9	>4.42
Control	0.34	-	-	-	0.36
20	4.8	0.31	131.0	40.1	4.13
Control	4.8	-	-	-	0.22
21	9.9	0.30	17.0	5.1	3.42
Control	9.9	-	-	-	0.22
22	19.9	0.28	18.0	5.0	3.52
Control	19.9	-	-	-	0.22
23	9.9	0.29	137.0	39.9	4.12
Control	9.9	-	-	-	0.36
24	19.8	0.27	146.0	39.8	3.98
Control	19.8	-	-	-	0.48
25	0.27	0.29	137.5	39.9	4.42
Control	0.27	-	-	-	0.36
26	20.0	0.24	167.0	40.0	4.04
Control	20.0	-	-	-	0.48

- not determined

Table B-2 Results of Infectivity Analysis in UV Turbidity Experiments with *C. parvum* using Neonatal CD-1 mice

Expt. Cond.	Turbidity (NTU)	UV Dose (mJ/cm^2)	d_o	Mice in Cohort	Mice Infected	P	d	Inactivation - Log (d/d_o)	
								Obs.	Mean
1	0.25	5.0	10,000	5	0	0.0	<38	>2.42	3.90
			100,000	5	0	0.0	<38	>3.42	
			1,000,000	5	3	0.6	126	3.90	
	0.25	0.0	50	5	2	0.4	73	-0.17	-0.17
			500	5	5	1.0	>242	<0.32	
2	5.0	5.0	10,000	5	0	0.0	<38	>2.42	3.13
			100,000	5	2	0.4	73	3.13	
			1,000,000	5	5	1.0	>242	<3.62	
	5.0	0.0	50	5	1	0.2	38	0.12	0.12
			500	5	5	1.0	>242	<0.32	
3	0.25	40.0	10,000	5	0	0.0	<38	>2.42	>4.42
			100,000	5	0	0.0	<38	>3.42	
			1,000,000	5	0	0.0	<38	>4.42	
	0.25	0.0	50	5	0	0.0	<38	>0.12	0.32
			500	5	4	0.8	242	0.32	
4	5.0	40.0	10,000	5	0	0.0	<38	>2.42	4.13
			100,000	5	0	0.0	<38	>3.42	
			1,000,000	5	2	0.4	73	4.13	
	5.0	0.0	50	5	3	0.6	126	-0.40	-0.40
			500	5	5	1.0	>242	<0.32	

Table Notes:

- Oocyst concentration used in all trials was 3.0×10^4/mL

d_o Inoculum as determined by hemacytometer count

d Infectious concentration determined from dose response experiment

P Rate of infection (i.e. fraction of mice infected in a cohort)

Obs. Observed log inactivation

Ave. Average log inactivation

Expt. cond. Experimental condition

Table B-2 Results of Infectivity Analysis in UV Turbidity Experiments with *C. parvum* using Neonatal CD-1 mice

Expt. Cond.	Turbidity (NTU)	UV Dose (mJ/cm^2)	d_o	Mice in Cohort	Mice Infected	P	d	Inactivation - Log (d/d_o)	
								Obs.	Mean
5	10.0	5.0	10,000	5	0	0.0	<38	>2.42	
			100,000	5	2	0.4	73	3.13	3.13
			1,000,000	5	5	1.0	>242	<3.62	
	10.0	0.0	50	5	0	0.0	<38	>0.12	0.32
			500	5	4	0.8	242	0.32	
6	20.0	5.0	10,000	5	0	0.0	<38	>2.42	2.90
			100,000	5	3	0.6	126	2.90	
			1,000,000	5	5	1.0	>242	<3.62	
	20.0	0.0	50	5	2	0.4	73	-0.17	-0.17
			500	5	5	1.0	>242	<0.32	
7	10.0	40.0	10,000	5	0	0.0	<38	>2.42	
			100,000	5	0	0.0	<38	>3.42	3.52
			1,000,000	5	5	1.0	>242	<3.62	
	10.0	0.0	50	5	1	0.2	38	0.12	0.22
			500	5	4	0.8	242	0.32	
8	20.0	40.0	10,000	5	0	0.0	<38	>2.42	2.90
			100,000	5	3	0.6	126	2.90	
			1,000,000	5	5	1.0	>242	<3.62	
	20.0	0.0	50	5	2	0.4	73	-0.17	-0.17
			500	4	4	1.0	>200	<0.40	

Table B-2 Results of Infectivity Analysis in UV Turbidity Experiments with *C. parvum* using Neonatal CD-1 mice

Expt. Cond.	Turbidity (NTU)	UV Dose (mJ/cm^2)	d_o	Mice in Cohort	Mice Infected	P	d	Inactivation - Log (d/d_o)	
								Obs.	Mean
9	0.25	5.0	5,000	5	0	0.0	<38	>2.42	
			50,000	5	0	0.0	<38	>3.42	>4.42
			500,000	5	0	0.0	<38	>4.42	
	0.25	0.0	50	5	1	0.2	38	0.12	0.48
			500	5	2	0.4	73	0.83	
10	5.0	5.0	10,000	5	0	0.0	<38	>2.42	3.90
			100,000	5	0	0.0	<38	>3.42	
			1,000,000	5	3	0.6	126	3.90	
	5.0	0.0	50	5	3	0.6	126	-0.40	-0.04
			500	5	4	1.8	242	0.32	
11	0.25	40.0	10,000	5	0	0.0	<38	>2.42	4.42
			100,000	5	0	0.0	<38	>3.62	
			1,000,000	5	1	0.2	38	4.42	
	0.25	0.0	50	5	0	0.0	<38	>0.12	0.60
			500	5	3	0.6	126	0.60	
12	5.0	40.0	10,000	5	0	0.0	<38	>2.42	4.42
			100,000	5	0	0.0	<38	>3.42	
			1,000,000	5	1	0.2	38	4.42	
	5.0	0.0	50	5	1	0.2	38	0.12	0.22
			500	5	4	0.8	242	0.32	

Table B-2 Results of Infectivity Analysis in UV Turbidity Experiments with *C. parvum* using Neonatal CD-1 mice

Expt. Cond.	Turbidity (NTU)	UV Dose (mJ/cm^2)	d_o	Mice in Cohort	Mice Infected	P	d	Inactivation - Log (d/d_o)	
								Obs.	Mean
13	10.0	5.0	10,000	5	0	0.0	<38	>2.42	
			100,000	5	0	0.0	<38	>3.42	>3.52
			1,000,000	5	5	1.0	>242	<3.62	
	10.0	0.0	50	5	0	0.0	<38	>0.12	0.32
			500	5	4	0.8	242	0.32	
14	20.0	5.0	5,000	5	0	0.0	<38	>2.12	2.83
			50,000	5	2	0.4	73	2.83	
			500,000	5	5	1.0	>242	<3.32	
	20.0	0.0	50	5	0	0.0	<38	>0.12	0.32
			500	5	4	0.8	242	0.32	
15	10.0	40.0	5,000	5	0	0.0	<38	>2.12	3.83
			50,000	5	0	0.0	<38	>3.12	
			500,000	5	2	0.4	73	3.83	
	10.0	0.0	50	5	0	0.0	<38	>0.12	0.83
			500	5	2	0.4	73	0.83	
16	20.0	40.0	9,000	5	0	0.0	<38	>2.37	3.65
			90,000	5	0	0.0	<38	>3.37	
			900,000	4	3	0.75	200	3.65	
	20.0	0.0	50	5	0	0.0	<38	>0.12	0.32
			500	5	4	0.8	242	0.32	

Table B-2 Results of Infectivity Analysis in UV Turbidity Experiments with *C. parvum* using Neonatal CD-1 mice

Expt. Cond.	Turbidity (NTU)	UV Dose (mJ/cm^2)	d_o	Mice in Cohort	Mice Infected	P	d	Inactivation - Log (d/d_o)	
								Obs.	Mean
17	0.25	5.0	10,000	5	1	0.2	38	2.42	3.15
			100,000	5	1	0.2	38	3.42	
			1,000,000	5	4	0.8	242	3.62	
	0.25	0.0	50	5	3	0.6	126	-0.40	-0.04
			500	5	4	0.8	242	0.32	
18	5.0	5.0	10,000	5	0	0.0	<38	>2.42	4.13
			100,000	5	0	0.0	<38	>3.42	
			1,000,000	5	2	0.4	73	4.13	
	5.0	0.0	50	5	1	0.2	38	0.12	0.22
			500	5	4	0.8	242	0.32	
19	0.25	40.0	10,000	5	0	0.0	<38	>2.42	
			100,000	5	0	0.0	<38	>3.42	>4.42
			1,000,000	5	0	0.0	<38	>4.42	
	0.25	0.0	50	5	1	0.0	38	0.12	0.36
			500	5	3	0.6	126	0.60	
20	5.0	40.0	10,000	5	0	0.0	<38	>2.42	4.13
			100,000	5	0	0.0	<38	>3.42	
			1,000,000	5	2	0.4	73	4.13	
	5.0	0.0	50	5	1	0.2	38	0.12	0.22
			500	5	4	0.8	242	0.32	

Table B-2 Results of Infectivity Analysis in UV Turbidity Experiments with *C. parvum* using Neonatal CD-1 mice

Expt. Cond.	Turbidity (NTU)	UV Dose (mJ/cm^2)	d_o	Mice in Cohort	Mice Infected	P	d	Inactivation - Log (d/d_o)	
								Obs.	Mean
21	10.0	5.0	10,000	5	0	0.0	<38	>2.42	
			100,000	5	1	0.2	38	3.42	3.42
			1,000,000	5	5	1.0	>242	<3.62	
	10.0	0.0	50	5	1	0.2	38	0.12	0.22
			500	5	4	0.8	242	0.32	
22	20.0	5.0	10,000	5	0	0.0	<38	>2.42	3.52
			100,000	5	1	0.2	38	3.42	
			1,000,000	5	4	0.8	242	3.62	
	20.0	0.0	50	5	0	0.0	<38	>0.12	0.83
			500	5	2	0.4	73	0.83	
23	10.0	40.0	5,000	5	0	0.0	<38	>2.42	3.90
			50,000	5	0	0.0	<38	>3.42	
			500,000	5	1	0.2	38	4.12	
	10.0	0.0	50	5	1	0.2	38	0.12	0.36
			500	5	3	0.6	126	0.6	
24	20.0	40.0	7,000	5	0	0.0	<38	>2.42	3.98
			70,000	5	0	0.0	<38	>3.42	
			700,000	5	2	0.4	73	3.98	
	20.0	0.0	50	5	1	0.2	38	0.12	0.48
			500	5	2	0.4	73	0.83	
25	0.25	40.0	10,000	5	0	0.0	<38	>2.42	4.42
			100,000	5	0	0.0	<38	>3.42	
			1,000,000	5	1	0.2	38	4.42	
	0.25	0.0	50	5	1	0.2	38	0.12	0.36
			500	5	3	0.6	126	0.60	
26	20.0	40.0	8,000	5	0	0.0	<38	>2.42	4.04
			80,000	5	0	0.0	<38	>3.42	
			800,000	5	2	0.4	73	4.04	
	20.0	0.0	50	5	1	0.2	38	0.12	0.48
			500	5	2	0.4	73	0.83	

Table B-3 Summary of Test of Lack of Fit for the linear model at UV dose of 5.0 mJ/cm^2

Data Input for Regression

Turbidity (NTU) x	Inactivation (log-units) y
0.25	3.90
0.25	4.50
0.25	3.15
5	3.13
5	3.90
5	4.13
10	3.13
10	3.50
10	3.42
20	2.90
20	2.83
20	3.52

Data Input for ANOVA

Turbidity (NTU)	Inactivation (log units)		
0.25	3.90	4.50	3.15
5.0	3.13	3.90	4.13
10.0	3.13	3.50	3.42
20.0	2.90	2.83	3.52

REGRESSION SUMMARY OUTPUT

Regression Statistics	
Multiple R	0.5996
R Square	0.3595
Adjusted R Square	0.2955
Standard Error	0.4331
Observations	12

ANOVA

	df	*SS*	*MS*	*F*	*Sig. F*
Regression	1	1.0530	1.0530	5.6130	0.0393
Residual	10	1.8759	0.1876		
Total	11	2.9289			

	Coefficients	*Std Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*
Intercept	3.8574	0.1957	19.7153	0.0000	3.4214	4.2933
Turbidity (NTU)	-0.0405	0.0171	-2.3692	0.0393	-0.0785	-0.0024

SS Lack of fit (SS_{LOF}) = SS ANOVA (SS_A) – SS Regression (SS_R)

SS_{LOF} = SS_A - SS_R
= 1.1010 – 1.0530
= 0.0481

DF_{LOF} = k – 2 = 4 – 2 = 2

MS_{LOF} = $\frac{SS_{LOF}}{DF_{LOF}} = \frac{0.0481}{2}$

= 0.0240

$SS_{Pure\ Error}$ = 1.8279

$DF_{Pure\ Error}$ = N – k = 12 – 4 = 8

$MS_{Pure\ Error}$ = $\frac{SS_{Pure\ Error}}{DF_{Pure\ Error}} = \frac{1.8279}{8}$

= 0.2285

$F = \frac{MS_{LOF}}{MS_{Pure\ Error}} = \frac{0.0240}{0.2285} = 0.1052$

$F_{0.5(1)2.8} = 4.46$

Table B-4 Summary of Test of Lack of Fit for the linear model at UV dose of 40.0 mJ/cm^2

Data Input for Regression

Turbidity (NTU) x	Inactivation (log-units) y
0.25	4.50
0.25	4.42
0.25	4.50
0.25	4.42
5	4.13
5	4.42
5	4.13
10	4.30
10	3.83
10	4.12
20	2.90
20	3.65
20	3.98
20	4.04

Data Input for ANOVA

Turbidity (NTU)	Inactivation (Log units)			
0.25	4.50	4.42	4.50	4.42
5.0	4.13	4.42	4.13	-
10.0	4.30	3.83	4.12	-
20.0	2.90	3.65	3.98	4.04

REGRESSION SUMMARY OUTPUT

Regression Statistics	
Multiple R	0.7629
R Square	0.5821
Adjusted R Square	0.5473
Standard Error	0.2893
Observations	14

ANOVA

	df	*SS*	*MS*	*F*	*Sig. F*
Regression	1	1.3985	1.3985	16.7145	0.0015
Residual	12	1.0040	0.0837		
Total	13	2.4025			

	Coefficients	*Std Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*
Intercept	4.4627	0.1185	37.6726	0.0000	4.2046	4.7208
Turbidity (NTU)	-0.0408	0.0100	-4.0883	0.0015	-0.0625	-0.0190

ANOVA: SINGLE FACTOR

SUMMARY

Groups (Turbidity, NTU)	*Count*	*Sum*	*Average*	*Variance*
0.25	4	17.84	4.4600	0.0021
5.0	3	12.68	4.2267	0.0280
10.0	3	12.25	4.0833	0.0562
20.0	4	14.57	3.6425	0.2744

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	1.4043	3	0.4681	4.6895	0.0271	3.7083
Within Groups	0.9982	10	0.0998			
Total	2.4025	13				

SS Lack of fit (SS_{LOF}) = SS ANOVA (SS_A) – SS Regression (SS_R)

$SS_{LOF} = SS_A - SS_R$

$= 1.4043 - 1.3985$

$= 0.0058$

$DF_{LOF} = k - 2 = 4 - 2 = 2$

$$MS_{LOF} = \frac{SS_{LOF}}{DF_{LOF}} = \frac{0.0058}{2}$$

$$= \underline{0.0029}$$

$SS_{Pure\ Error} = 0.9982$

$DF_{Pure\ Error} = N - k = 14 - 4 = 10$

$$MS_{Pure\ Error} = \frac{SS_{Pure\ Error}}{DF_{Pure\ Error}} = \frac{0.9982}{10}$$

$= 0.0998$

$$F = \frac{MS_{LOF}}{MS_{Pure\ Error}} = \frac{0.0029}{0.0998} = 0.0291$$

$F_{0.5(1)2,10} = 4.1$

APPENDIX C DATA AND INFORMATION FROM *GIARDIA MURIS* EXPERIMENTS (CHAPTER 6)

Table C-1 Average *G. muris* cysts output in cohort (no. of cysts/g feces) of C3H/HeN mice in dose-response experiments

Expt. Cond.	Type of cyst Purification[1]	Cysts in Inoculum d_o	Day Post Infection (DPI)				
			3	4	5	6	7
1	S	50	0	0	54,008	1,151,615	1,697,472
2	S	5,000	24719	500199	1719351	2620972	3627135
3	S	50,000	112,459	1,170,948	2,136,488	3,753,327	2,384,322
4	S/P	50	0	0	181595	1309249	3561887
5	S/P	5,000	2,417	474,132	1,914,446	2,097,259	3,518,718
6	S/P	50,000	349,883	1,440,903	2,147,477	2,422,714	2,507,657

Table C-2 Average cyst output in cohort (logarithm of no. of cysts/g feces) in *G. muris* dose-response experiments

Expt. Cond.	Type of cyst Purification[1]	Cysts in Inoculum d_o	Day Post Infection (DPI)						
			1	2	3	4	5	6	7
1	S	50	2.0	2.0	2.0	2.0	4.7	6.1	6.2
2	S	5,000	2.0	2.0	4.4	5.7	6.2	6.4	6.6
3	S	50,000	2.0	2.0	5.1	6.1	6.3	6.6	6.4
4	S/P	50	2.0	2.0	2.0	2.0	5.3	6.1	6.6
5	S/P	5,000	2.0	2.0	3.4	5.7	6.3	6.3	6.5
6	S/P	50,000	2.0	2.0	5.5	6.2	6.3	6.4	6.4

[1] S – cysts purified using sucrose only and S/P – cysts purified using sucrose followed by percoll gradients

Table C-3 Summary of observed latent period in *G. muris* dose-response experiments

Expt. Cond.	Type of cyst Purification[1]	Cysts in Inoculum d_o	Observed Latency Period (Days)			
			10^3 cysts/g	10^4 cysts/g	10^5 cysts/g	Mean
1	Sucrose	50	2.0	2.0	2.0	2.0
2	Sucrose	5,000	2.0	2.0	4.4	5.7
3	Sucrose	50,000	2.0	2.0	5.1	6.1
4	Sucrose/Percoll	50	2.0	2.0	2.0	2.0
5	Sucrose/Percoll	5,000	2.0	2.0	3.4	5.7
6	Sucrose/Percoll	50,000	2.0	2.0	5.5	6.2

Table C-4 Average cysts output in cohort (no. of cysts/g feces) in *G. muris* UV turbidity experiments (First set of trials)

UV Dose (mJ/cm^2)	Turbidity (NTU)	Day Post Infection (DPI)					
		3	4	5	6	7	8
4.9	0.25	0	12,431	495,244	967,441	1,983,990	nd
4.9	20.0	0	354	165,322	573,883	1,033,152	nd
39.9	0.25	0	0	0	160,723	373,222	606,963
40.0	20.0	0	0	0	19,960	366,760	627,624
Control	0.25	240,053	1,378,997	2,067,340	3,255,528	nd	nd
Control	20.0	181,370	999,597	2,283,519	2,288,014	nd	nd

nd – not done

Table C-5 Average cysts output in cohort (logarithm of no. of cysts/g feces) in *G. muris* UV turbidity experiments (First set of trials)

UV Dose (mJ/cm^2)	Turbidity (NTU)	Day Post Infection (DPI)							
		1	2	3	4	5	6	7	8
4.9	0.25	1.9	1.9	1.9	1.9	5.7	6.0	6.3	nd
4.9	20.0	1.9	1.9	1.9	2.5	5.2	5.8	6.0	nd
39.9	0.25	1.9	1.9	1.9	1.9	1.9	5.2	5.6	5.8
40.0	20.0	1.9	1.9	1.9	1.9	1.9	4.3	5.6	5.8
Control	0.25	1.9	1.9	5.4	6.1	6.3	6.5	nd	nd
Control	20.0	1.9	1.9	5.3	6.0	6.4	6.4	nd	nd

Table C-6 Summary of observed latent period in *G. muris* UV turbidity experiments (First set of trials)

Turbidity (NTU)	UV Dose (mJ/cm^2)	d_o	Observed Latency Period (Days)				d	Log Kill (-Log d/d_o)
			10^3 cysts/g	10^4 cysts/g	10^5 cysts/g	Mean		
0.25	4.9	50,750	4.28	4.55	4.80	4.5	81	2.80
20.0	4.9	51,300	4.17	4.55	4.93	4.6	79	2.81
0.25	39.9	50,938	5.32	5.65	5.95	5.6	7	3.87
20.0	40.0	51,300	5.45	5.88	6.55	6.0	4	4.15
0.25	0.0	51,106	2.30	2.60	2.90	2.6	46699	0.04
20.0	0.0	50,119	2.30	2.60	2.90	2.6	46699	0.03

Table C-7 Average cysts output in cohort (no. of cysts/g feces) in *G. muris* UV turbidity experiments (Duplicate trials)

UV Dose (mJ/cm^2)	Turbidity (NTU)	Day Post Infection (DPI)					
		3	4	5	6	7	8
4.9	0.25	0	0	24,673	691,594	1,459,636	nd
4.9	20.0	0	981	454,043	915,726	1,172,668	nd
39.9	0.25	0	0	0	0	0	67,711
40.0	20.0	0	0	0	328,483	889,868	1,014,723
Control	0.25	1,033	437,980	1,247,329	880,885	nd	nd
Control	20.0	856	398,985	783,232	1,040,699	nd	nd

Table C-8 Average cysts output in cohort (logarithm of no. of cysts/g feces) in *G. muris* UV turbidity experiments (Duplicate trials)

UV Dose (mJ/cm^2)	Turbidity (NTU)	Day Post Infection (DPI)							
		1	2	3	4	5	6	7	8
4.9	0.25	1.8	1.8	1.8	1.8	4.4	5.8	6.2	nd
4.9	20.0	1.8	1.8	1.8	3.0	5.7	6.0	6.1	nd
39.9	0.25	1.8	1.8	1.8	1.8	1.8	1.8	1.8	4.8
40.0	20.0	1.8	1.8	1.8	1.8	1.8	5.5	5.9	6.0
Control	0.25	1.8	1.8	3.0	5.6	6.1	5.9	nd	nd
Control	20.0	1.8	1.8	2.9	5.6	5.9	6.0	nd	nd

Table C-9 Observed latent period in *G. muris* UV turbidity experiments (Duplicate trials)

Turbidity (NTU)	UV Dose (mJ/cm^2)	d_o	Observed Latency Period (Days)				d	Log Kill ($-\text{Log } d/d_o$)
			10^3 cysts/g	10^4 cysts/g	10^5 cysts/g	Mean		
0.25	4.9	51,000	4.45	4.85	5.40	4.9	34	3.18
20.0	4.9	50,325	4.00	4.38	4.75	4.4	123	2.61
0.25	39.9	55,062	7.40	7.73	8.45	7.9	1	4.74
20.0	40.0	50,119	5.32	5.58	5.85	5.6	8	3.81
0.25	0.0	52,119	3.00	3.40	3.80	3.4	2194	1.38
20.0	0.0	20,625	3.03	3.40	3.80	3.4	2121	0.99

Table C-10 Average cysts output in cohort (no. of cyst/g feces) in *G. muris* UV turbidity experiments (Triplicate trials)

UV Dose (mJ/cm^2)	Turbidity (NTU)	Day Post Infection (DPI)					
		3	4	5	6	7	8
4.9	0.25	0	843	43,748	561,578	nd	nd
4.9	20.0	0	5,621	407,945	1,319,833	nd	nd
39.9	0.25	0	0	0	0	1,137	186,567
40.0	20.0	0	0	0	13,050	314,904	615,235
Control	0.25	32,197	932,949	1,075,732	nd	nd	nd
Control	20.0	52,189	1,515,826	1,401,925	nd	nd	nd

Table C-11 Average cysts output in cohort (logarithm of no. of cysts/g feces) in *G. muris* UV turbidity experiments (Triplicate trials)

UV Dose (mJ/cm^2)	Turbidity (NTU)	Day Post Infection (DPI)							
		1	2	3	4	5	6	7	8
4.9	0.25	1.8	1.8	1.8	1.8	4.6	5.7	nd	nd
4.9	20.0	1.8	1.8	1.8	3.7	5.6	6.1	nd	nd
39.9	0.25	1.8	1.8	1.8	1.8	1.8	1.8	3.1	5.3
40.0	20.0	1.8	1.8	1.8	1.8	1.8	4.1	5.5	5.8
Control	0.25	1.8	1.8	4.5	6.0	6.0	nd	nd	nd
Control	20.0	1.8	1.8	4.7	6.2	6.1	nd	nd	nd

Table C-12 Summary of observed latent period in *G. muris* UV turbidity experiments (Triplicate trials)

Turbidity (NTU)	UV Dose (mJ/cm^2)	d_o	Observed Latency Period (Days)				d	Log Kill (-Log d/d_o)
			10^3 cysts/g	10^4 cysts/g	10^5 cysts/g	Mean		
0.25	4.9	53,212	4.42	4.77	5.32	4.8	39	3.13
20.0	4.9	50,225	3.60	4.15	4.68	4.1	230	2.34
0.25	39.9	26,688	6.95	7.44	7.87	7.4	1	4.43
20.0	40.0	51,300	5.50	5.94	6.65	6.0	3	4.21
0.25	0.0	52,588	2.44	2.80	3.34	2.9	15755	0.52
20.0	0.0	51,562	2.40	2.75	3.20	2.8	21476	0.38

Table C-13 Summary output of ANOVA test of *G. muris* UV turbidity experimental outcomes

UV Dose (mJ/cm^2)	Turbidity (NTU)	
	0.25	20
5	2.8	2.81
	3.18	2.61
	3.13	2.34
40	3.87	4.15
	4.74	3.81
	4.43	4.21

Anova: Two-Factor With Replication

SUMMARY	0.25	20	Total
5			
Count	3	3	6
Sum	9.11	7.76	16.87
Average	3.04	2.59	2.81
Variance	0.0426	0.0556	0.1001
40			
Count	3	3	6
Sum	13.04	12.17	25.21
Average	4.35	4.06	4.20
Variance	0.1944	0.0465	0.1216
Total			
Count	6	6	
Sum	22.15	19.93	
Average	3.69	3.32	
Variance	0.6097	0.6891	

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Sample	5.7963	1	5.7963	68.35	3.44E-05	5.3176
Columns	0.4107	1	0.4107	4.84	0.058938	5.3176
Interaction	0.0192	1	0.0192	0.23	0.646933	5.3176
Within	0.6785	8	0.0848			
Total	6.9047	11				

APPENDIX D INFORMATION AND DATA ON LAKE SUSPENDED SOLIDS' CONCENTRATES AND HISTORICAL TURBIDITY DATA FROM THE CITY OF KELOWNA, BC

Table D-1 Summary output of particle size distribution analysis of lake suspended solids sample (Trial 1)

Run #	2	5	15	25	50	100
	(Micrometers)					
1	15135	13055	8391	240	10	1
2	15246	13162	8474	247	7	0
3	15224	13186	8497	275	10	1
4	15239	13238	8419	237	8	1
5	15314	13230	8541	218	8	0
6	15254	13169	8493	257	10	1
7	15284	13249	8518	249	9	1
8	15249	13279	8548	242	9	0
9	15304	13193	8432	213	6	0
10	15458	13297	8340	173	4	0
No. of particles/200 mL	15271	13206	8465	235	8	1
No. of particles/mL	3054140	2641160	1693060	47020	1620	100
No. of particles in range	412980	948100	1646040	45400	1520	100

Table D-2 Summary output of particle size distribution analysis of lake suspended solids sample (Trial 2)

Run #	2	5	15	25	50	100
	(Micrometers)					
1	15153	12981	8234	222	6	1
2	14961	12917	8195	236	6	1
3	15072	13021	8296	219	8	1
4	15109	13035	8276	226	9	0
5	15080	13046	8243	234	9	0
6	14967	12903	8222	237	7	1
7	15055	12962	8251	199	7	1
8	15226	12993	8110	195	50	1
9	15141	12975	8124	185	3	0
10	15186	12995	8062	182	4	1
No. of particles/200mL	15095	12983	8201	214	11	1
No. of particles/mL	3019000	2596560	1640260	42700	2180	140
No. of p'cles in range	422440	956300	1597560	40520	2040	140

Table D-3 Summary of Tables D-1 and D-2

Particle Size (μm)	Number of particles in range, Trial number 1	Number of particles in range, Trial number 2	Mean number of particles
2	412980	422440	417710
5	948100	956300	952200
15	1646040	1597560	1621800
25	45400	40520	42960
50	1520	2040	1780
100	100	140	120

Table D-4 Data from the calibration of lake suspended solids sample

Trial #	Volume per 1000 mL Water Sample (mL)	Turbidity (NTU), Stirring Time (minutes), 30			60			90		
		1	2	3	1	2	3	1	2	3
1	1.00	6.23	6.73	6.4	5.25	5.85	5.75			
2	1.50	9.33	9.96	9.61	9.88	9.61	9.42	9.68	9.87	9.42
3	2.00	12.90	12.50	12.80	12.40	12.30	12.70	12.50	12.70	12.70
4	2.50	16.10	15.80	16.60	16.00	16.60	15.90	15.70	15.70	15.50
5	3.00	19.00	19.10	19.90	18.80	18.70	19.30	19.70	19.50	18.60

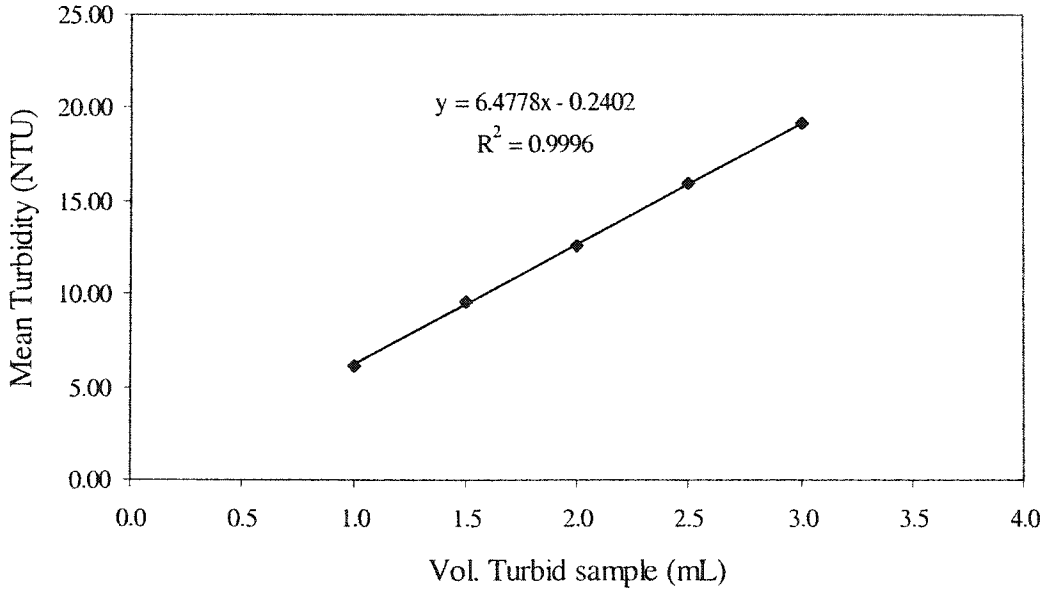

Figure D-1 Variation of turbidity with volume of lake suspended solids sample

Table D-5 Summary of observed transmittance at 254 nm (UV Dose of 5.0 mJ/cm^2)

Experimental Condition	Turbidity (NTU)	Average Germicidal Irradiance (mW/cm^2)	Time (s)	Percent Transmittance (%)
1	0.25	0.404	12.5	86
	5.0	0.388	13	78
	10.0	0.359	14	71
	20.0	0.333	15	59
2	0.25	0.366	14	86
	5.0	0.345	14.5	78
	10.0	0.328	15	73
	20.0	0.303	16	60
3	0.25	0.332	15	86
	5.0	0.317	16	79
	10.0	0.298	17	73
	20.	0.279	18	61

Table D-6 Summary of observed transmittance at 254 nm (UV Dose of 40.0 mJ/cm^2)

Experimental Condition	Turbidity (NTU)	Average Germicidal Irradiance (mW/cm^2)	Time (s)	Percent Transmittance (%)
1	0.25	0.393	102	86
	5.0	0.372	108	77
	10.0	0.349	115	71
	20.0	0.320	125	58
2	0.25	0.360	111	87
	5.0	0.342	117	79
	10.0	0.308	130	73
	20.0	0.287	139	59
3	0.25	0.324	123	86
	5.0	0.306	131	78
	10.0	0.291	137	74
	20.	0.273	146	61

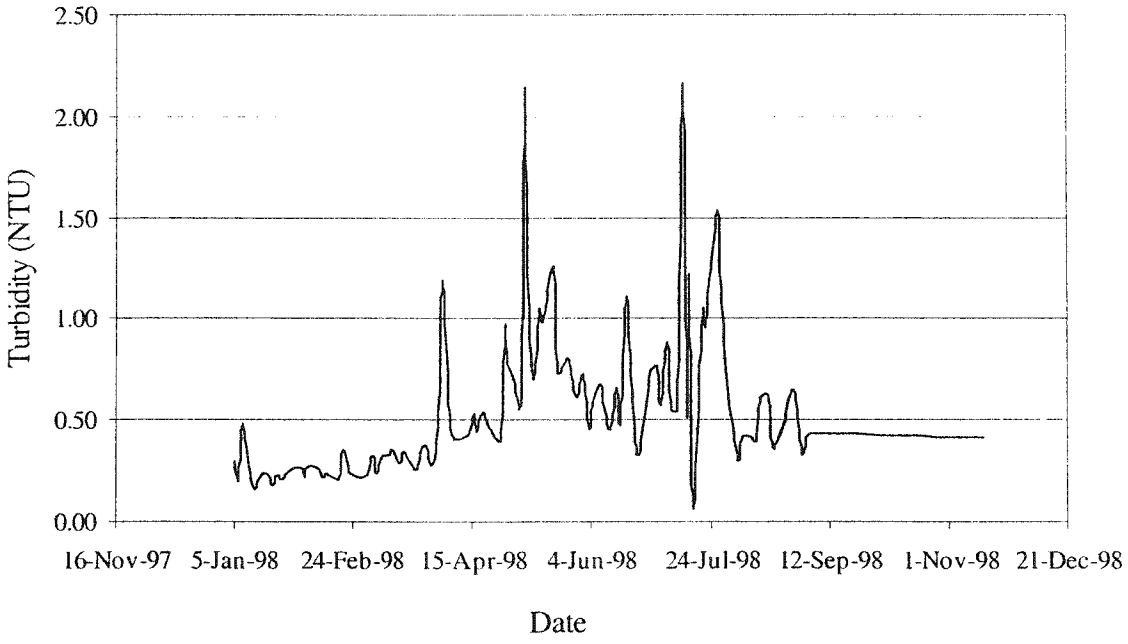

Figure D-2 Trends in measured turbidity of raw water at the El Dorado Pumping station at Kelowna, BC in 1998

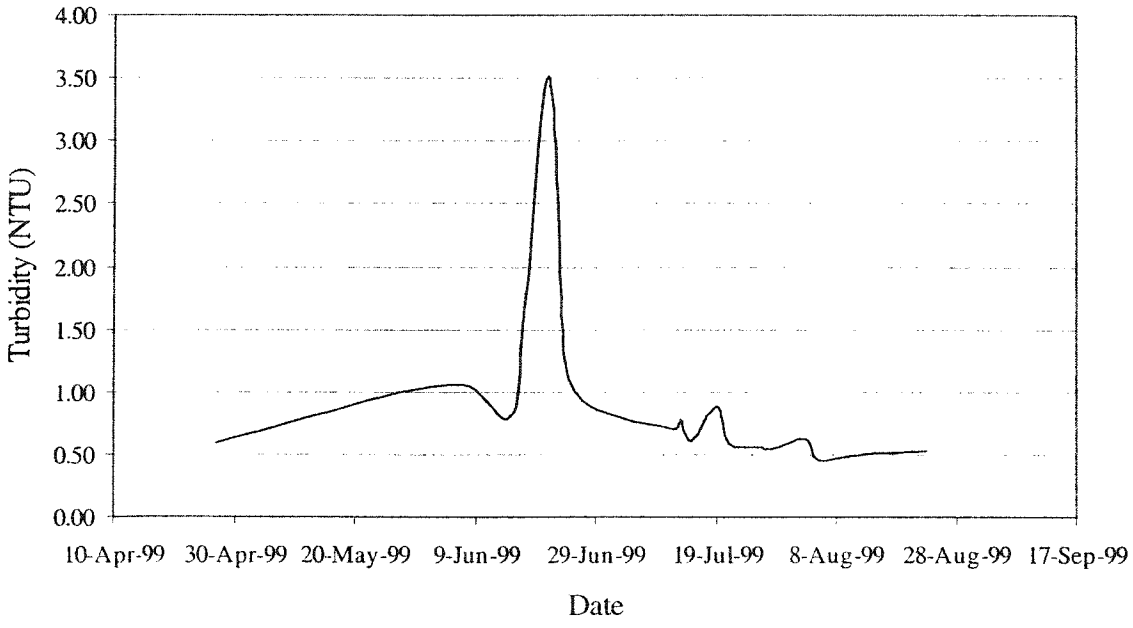

Figure D-3 Trends in measured turbidity of raw water at the El Dorado Pumping station at Kelowna, BC in 1999

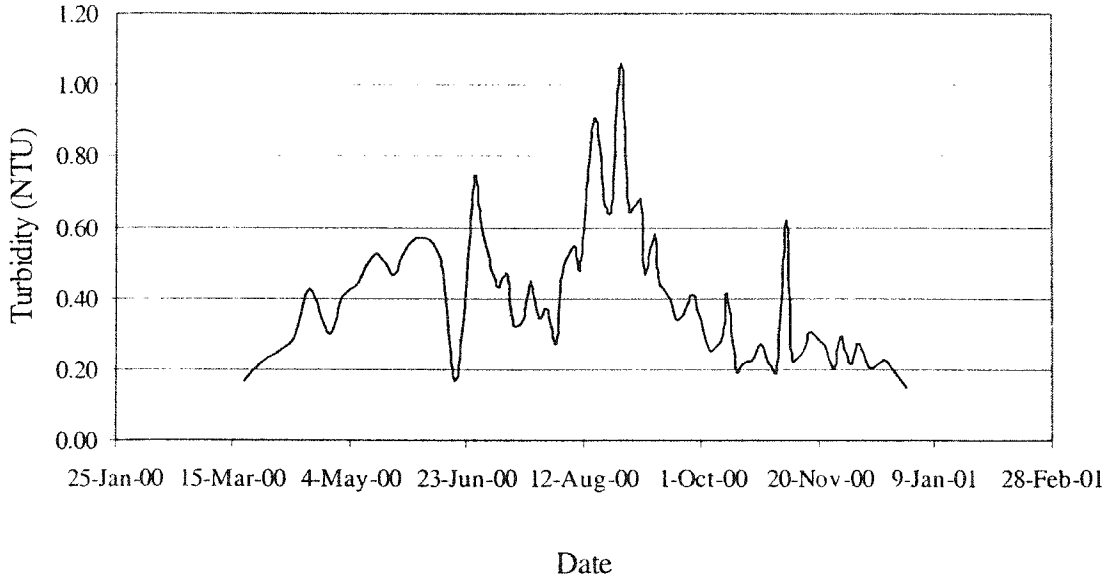

Figure D-4 Trends in measured turbidity of raw water at the El Dorado Pumping station at Kelowna, BC in 2000

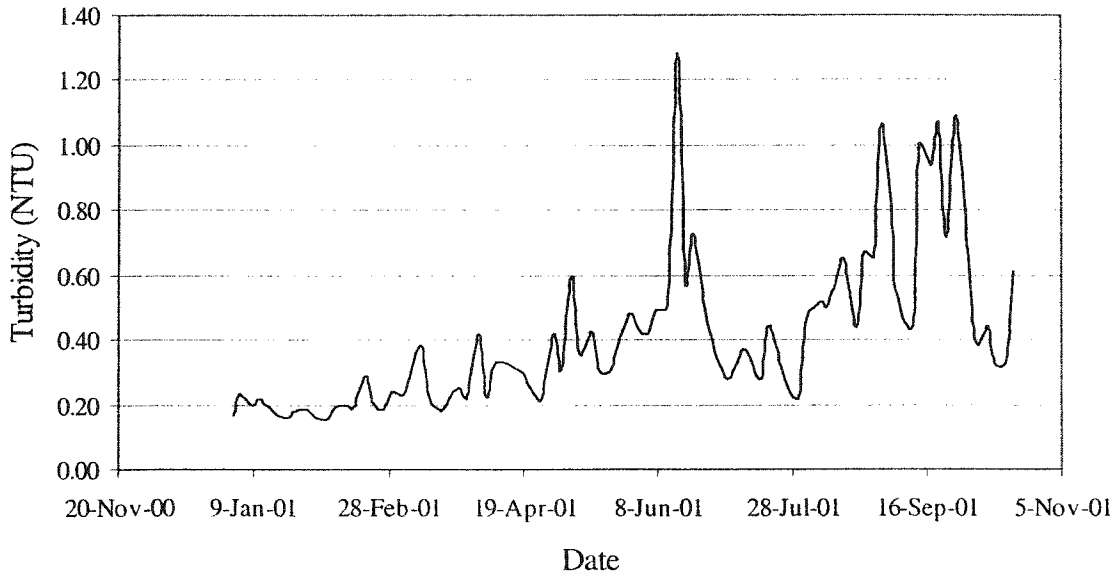

Figure D-5 Trends in measured turbidity of raw water at the El Dorado Pumping station at Kelowna, BC in 2001

Table D-7 Summary of gravimetric analysis of lake suspended solids sample

No.	W1 (g)	W2 (g)	W3 (g)	W4 (g)	W5 (g)	W6 (g)
1	1.0118	1.0113	1.1174	1.505	1.1196	1.1188
2	1.0078	1.0078	1.1139	1.4657	1.1169	1.1155
3	1.0123	1.0119	1.1180	1.6278	1.1211	1.1201
4	1.0156	1.0153	1.1214	1.5236	1.1255	1.1243
5	1.0136	1.0133	1.1194	1.3642	1.1218	1.1208
6	1.0147	1.0145	1.1206	1.4647	1.1242	1.1233
7	1.0168	1.0163	1.1224	1.3970	1.1273	1.1262
8	1.0082	1.0077	1.1138	1.6583	1.1177	1.1166

Table D-7 Continued

No.	W6 (g)	TSS (mg/L)	TFS (mg/L)	TVS (mg/L)	% Inorganic	% Organic
1	1.1188	55.0	20.0	35.0	36	64
2	1.1155	75.0	35.0	40.0	47	53
3	1.1201	77.5	25.0	52.5	32	68
4	1.1243	102.5	30.0	72.5	29	71
5	1.1208	60.0	25.0	35.0	42	58
6	1.1233	90.0	22.5	67.5	25	75
7	1.1262	122.5	27.5	95.0	22	78
8	1.1166	97.5	27.5	70.0	28	72
	Mean	85.0	26.6	58.4	33	67

Notes:

W1 = weight of aluminum drying pan

W2 = weight of pan after drying at 550°C

W3 = weight of pan and glass microfilter

W4 = weight of pan and wet sample before drying at 105°C

W5 = weight of pan and sample after drying at 105°C

W6 = weight of pan and sample after igniting at 550°C

Volume of turbid sample filtered (V)= 40.0 mL

Sample calculation:

$$\text{Total suspended solids (TSS)} = \frac{W5 - W3}{V} \text{ (mg/L)}$$

$$\text{Total volatile solids (TVS)} = \frac{W5 - W6}{V} \text{ (mg/L)}$$

Total fixed solids (TFS) = TSS – TFS

APPENDIX E PROCEDURE FOR THE PREPARATION OF WORKING SOLUTIONS OF PERCOLL

ONE STEP PROCEDURE FOR PREPARATION OF SOLUTIONS OF PERCOLL (Pharmacia, undated)

Percoll (from bottle) may be diluted directly to make a final working solution of known density by the following procedure. In a measuring cylinder add $^1/_{10}$ of the final desired volume, 1.5 M NaCl or 2.5 M sucrose (e.g. 10 mL for 100 mL working solution). To this add the required volume of percoll (from bottle). Calculate using the formula shown below.

$$V_o = V\left(\frac{\rho - 0.1\rho_{10} - 0.9}{\rho_o - 1}\right)$$

Where:

V_o = volume of percoll (from bottle), mL

V = Volume of the final working solution, g/mL

ρ = Desired density of the solution, g/mL

ρ_o = Density of percoll (from bottle), g/mL

ρ_{10} = density of 1.5 M NaCl = 1.058 g/mL (minor differences for other salts) or density of 2.5 M sucrose = 1.316 g/mL (minor differences for other additives)

Sample calculation

To produce 100 mL of working solution of percoll of density 1.07 g/mL in 0.15 M NaCl. To 10 mL of 1.5 M NaCl add

$$\text{Volume of percoll required} = 100x\left(\frac{1.07 - 0.1x1.06 - 0.9}{1.13 - 1}\right)$$

$$= 49.2 \text{ mL}$$

(If percoll density is 1.13 g/mL) and make up with distilled water

Sample Densities

1. Percoll (from bottle), $\rho_o = 1.130$ g/mL
2. 1.5 M NaCl, $\rho_{10} = 1.058$ g/mL
3. 2.5 M sucrose, $\rho_{10} = 1.316$ g/mL

Preparation of working solutions (of Percoll)

Volume of working solutions = 100 mL

a. Percoll of density, 1.05 g/mL

Working solution of percoll of density, 1.05 g/mL in 1.5 M NaCl

$$\text{Volume of percoll required, V} = 100\left(\frac{1.05 - 0.1x1.06 - 0.9}{1.130 - 1}\right)\text{mL}$$

$$= 33.85 \text{ mL}$$

Final solution:

10 mL 1.5M NaCl, 33.85 mL Percoll, 56.15 mL Distilled water

b. Percoll of density, 1.09 g/mL

Working solution of percoll of density, 1.09 g/mL in 1.5 M NaCl

$$\text{Volume of percoll required, V} = 100\left(\frac{1.09 - 0.1x1.06 - 0.9}{1.130 - 1}\right)\text{mL}$$

$$= 64.62 \text{ mL}$$

Final solution:

10 mL 1.5M NaCl, 64.62 mL Percoll (from bottle) and 25.38 mL Distilled water

c. Preparation of sodium chloride solution of Molarity 1.5 (1.5 M NaCl)

Volume of working solution = 100 mL

Molarity, M = 1.5

Molecular weight of NaCl = 58.44 grams

Required mass (of NaCl)

= Molarity (M) x Volume (L) x Molecular Weight (MW)

= 1.5 x 0.100 x 58.44 grams/100 mL

= 8.766 grams/100 mL

APPENDIX F DETERMINATION OF UV DOSE

The procedure for determining UV dose was introduced briefly in Section 4.4 of Chapter 4 (Materials and Methods). The detailed procedure is the following.

"Prior to each UV treatment, the UV irradiance at the surface of the parasite suspension and at the center of the UV beam was measured with a radiometer (International Light, Model IL1400A) equipped with a SED240 UV detector. The UV irradiance was also measured after each UV exposure. The irradiance at the center of the UV beam was multiplied by factors, which account for radial variation, reflection at the liquid surface, attenuation of the beam within the liquid, and variation in sensor sensitivity and lamp output with wavelength. The last two corrections were needed to account for the broad emission spectrum between 200 and 300 nm, which is a characteristic of medium-pressure lamps. To determine the radial variation, the UV irradiance at the liquid surface level was measured at 5 mm intervals along two perpendicular imaginary lines intersecting at the center of the UV beam. The average irradiance across the liquid surface was estimated to be 0.79 x the center point measurement. Reflection of radiation was estimated to be 2.5% based on refractive indices of water and air. The depth averaged total irradiance, E, was determined by using an integrated form of Beer's law:

$$\frac{E}{E_o} = \frac{\left(1 - 10^{-A}\right)}{\ln(10)A} \qquad \text{Equation 4-1}$$

Where E_o is the incident irradiance at the surface and A is the absorbance within the liquid. The 200 to 300 nm absorbance spectrum of a sample of suspension was measured in a 10 mm quartz cell using a UV-vis spectrophotometer (Hewlett Packard, Model HP 8452A). The attenuation (Equation 4-1) at each wavelength was multiplied by the normalized output of the lamp at each wavelength (provided by the lamp manufacturer), and these values were summed over the 200 to 300 nm wavelength range. This value was then multiplied by the sensor factor of 1.286 (weighted average of the sensor factor provided by the sensor manufacturer, weighted by the relative photon emission of the lamp), the reflection factor (0.975) and the radial factor to yield the total average irradiance, E. Germicidal average irradiance, E_g, was calculated in the same way as the total average irradiance except that the attenuation at each wavelength was multiplied by both the normalized output of the lamp and a germicidal factor for each wavelength. The germicidal factor in this work was based on the UV absorbance spectrum of DNA, which has a maximum at approximately 260 nm (von Sonntag, 1986)." The UV dose, either total (H) or germicidal (H_g) was then determined from:

UV Dose (H or H_g) = Irradiance (E or E_g) x Exposure time (t)

mJ/cm^2 = mW/cm^2 x s Equation 4-2

(Craik *et al.*, 2001).

APPENDIX G INFECTIVITY IN NEONATAL CD-1 MICE

The infectivity in neonatal CD-1 mice was described briefly in Section 4.5.3 of Chapter 4 (Materials and Methods). The detailed procedure is the following.

"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). They were housed in cages with covers fitted with 0.22 μm filter in a specific pathogen-free (P-2 level) animal facility being given food and water *ad libitum*. Five-day old mice were inoculated with oocysts suspended in 50 μL of deionized water using a ball-point neonate feeding needle (24 gauge syringe, Popper and Sons, Inc.).

The infectivity of the oocysts was determined 7 days after infection. The mice were killed by cervical dislocation and the large intestine (rectum to 300 mm anterior to the caecum) was removed and placed in 10 mL of Milli-Q® water. The intestine was homogenized for 40 s in a Sorvall Omni-Mixer and the homogenate placed in a 15 mL polypropylene test tube. The suspension was centrifuged for 15 minutes at 2000 x g and 4°C. The supernatant was then removed, the pellet re-suspended in 10 mL of deionized water containing 0.01% Tween 20, and centrifuged for 15 minutes at 2000 x g and 4°C. After centrifugation, the supernatant was discarded and 20 μL of the viscous pellet removed and placed into a 6 mL polystyrene flow cytometer test tube fitted with 35 μm sieve (Becton Dickinson). The intestinal homogenate was forced through the sieve by adding 400 μL of 1% bovine serum albumen (BSA) in phosphate buffered saline (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 microlitres of a 1:400 dilution of flourescein 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 (Neumann *et al.*, 2000a). Flow cytometry has been shown to be comparable to microscopic examination (Neumann *et al.*, 2000b). Settings for the flow were as follows: forward scatter – photodiode voltage equivalent to E00, AmpGain 4.00; side light scatter – photomultiplier voltage set to 402, AmpGain 4.00; FL1 – photomultiplier voltage set to 470. All flow cytometric analysis was done at 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 ≥ 1.25%. Values of 1.24±0.1 were verified using standard microscopic techniques (Finch *et al.*, 1993). Daily negative controls were run in which the infectivity was checked in a group of mice not exposed to oocysts. None of these mice were positive for infection" (Craik *et al.*, 2001).