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Comparison of chlorine and chlorine dioxide as disinfectants for surface waters

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COMPARISON OF CHLORINE AND CHLORINE DIOXIDE AS DISINFECTANTS FOR SURFACE WATERS

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ABSTRACT

COMPARISON OF CHLORINE AND CHLORINE DIOXIDE AS DISINFECTANTS FOR SURFACE WATERS

by

Kristen S. Tilton
University of New Hampshire, September, 1995

Safe supplies of drinking water free of pathogenic agents such as viruses and protozoa are essential for humankind. The evaluation of current treatment practices for the removal of pathogens has become increasingly important with the occurrence of waterborne disease outbreaks. Shortcomings associated with chlorine have prompted the need to assess alternative disinfectants.

The overall objective of this research is to compare chlorine and chlorine dioxide as disinfectants for surface waters using *Giardia muris* cysts, bacteriophage MS2, total and fecal coliforms, and heterotrophic bacteria. Concentration x time (CT) values for the inactivation of *Giardia muris* cysts and MS2 bacteriophage as a function of pH and temperature by chlorine and chlorine dioxide at the bench and pilot scale are established.

Results indicate that chlorine dioxide is more effective against *G. muris* and MS2 than chlorine at the bench scale. Chlorine dioxide is more virucidal at pH 9.0 than at 6.0 and 7.0 and chlorine at all pH values tested. Chlorine, however, is more virucidal at pH 6.0 than at higher pH values. For both disinfectants, *G. muris* log reductions are not significantly different among any of the pH values tested. The effect of temperature on inactivation rates at the bench scale was inconclusive.

Pilot studies indicate chlorine dioxide is not as effective as chlorine in inactivating *G. muris* and MS2. However, the variation and small number of data points limits the statistical power of analysis of pilot results.

Differences between bench and pilot scale studies for the log reduction of both organisms by chlorine and chlorine dioxide are observed. Results indicate that *G. muris* cysts are approximately 30 times more resistant than MS2 to chlorine and 45 times more resistant to chlorine dioxide. Low levels of turbidity, total and fecal coliforms, and heterotrophic bacteria do not affect the disinfectants' ability to inactivate *G. muris* and MS2.

Results of this study indicate that chlorine dioxide exhibits strong disinfecting capabilities. It should be considered as a viable alternative to chlorine.

CHAPTER I

Introduction

Providing safe water for drinking, recreational, and other uses is a fundamental necessity for humankind. A safe, continuous supply of drinking water requires protection of our water sources from industrial, domestic, and agricultural runoff. Fecal contamination of potable water supplies from untreated or inadequately treated sewage effluents entering municipal water supplies such as lakes, rivers, or groundwaters creates conditions for the rapid dissemination of pathogens. Protection of our water sources through sewage treatment and natural barriers in addition to disinfection and filtration of surface waters are critical in preventing waterborne disease outbreaks.

Pathogenic Organisms and Potable Water

Sources of Surface Water Contamination

There are several potential sources of surface water contamination. These sources are typically classified into point and nonpoint sources. Discharges of municipal raw wastewaters, sewage, or treated effluents at specific point source locations into receiving waters such as rivers and lakes

are typical examples of point sources. Nonpoint sources such as stormwater runoff, cattle feedlot drainage, agricultural runoff, and other forms of urban runoff result in elevations of suspended solids, organic materials, and microorganisms in the watershed (Clark, 1990).

Another factor that can influence surface water quality is the recirculation of microorganisms that may have settled in bottom sediments in lakes, resevoirs, or streams. This condition is usually a result of spring thaws and major storms. Pathogens can also be introduced into our source waters via nontraditional methods of waste disposal such as land application of wastewaters that have been minimally treated or sanitary landfills. The latter poses a risk if the landfill liner is punctured, resulting in the leakage of solid waste leachates into the encompassing groundwater aquifer or surface waters (Laws, 1993).

Waterborne Disease Outbreaks

Water as a vehicle for infectious disease transmission has been documented by the Centers for Disease Control and Prevention (CDC) and the United States Environmental Protection Agency (USEPA) through a rigorous surveillance program since 1971. These agencies are responsible for collecting and periodically reporting data on the occurrence and causes of waterborne disease outbreaks associated with water intended for drinking and recreational water (CDC, 1993). The data that is gathered provides

information for assessing the currently used water treatment processes for producing safe drinking water and recreational waters (Moore, 1994).

The average annual number of documented waterborne disease outbreaks has increased four-fold since the 1960's (Gerba and Rose, 1990). Most outbreaks are a result of fecal contamination in source waters and insufficiently treated water supplies. The most common infectious agents can be classified within four broad groups: bacteria, viruses, protozoa, and helminths. Especially numerous and of great importance to health are viruses and parasites that infect the gastrointestinal tract of humans, and then are excreted in very high numbers in the feces of infected individuals (Sobsey, 1989).

For the two year period 1991-1992, 17 states and territories reported 34 outbreaks associated with water processed for drinking purposes (CDC, 1993). The outbreaks caused illness in an estimated 17,464 people. Thirty-one (91%) of the outbreaks caused gastroenteritis, resulting primarily from fecal contamination of enteric viruses and protozoal parasites such as *Giardia lamblia* and *Cryptosporidium parvum* (Table A). From 1991 to 1992, no etiologic agent was identified in 23 (68%) disease outbreaks associated with water intended for drinking. Fourteen of these outbreaks of acute gastrointestinal illness were suspected to be of viral etiology based on the symptom complex, incubation period, and duration of illness (CDC, 1993).

Viruses in Source Waters

The viruses that are typically transmitted by water belong to a group of small viruses (diameter 15-100 nm) called the enteric viruses. Any surface water that is exposed to contamination by human fecal wastes is at risk of harboring enteric viruses. At least 140 human enteric virus types are known, including 72 seroptyes of enteroviruses, adenoviruses, reoviruses, rotaviruses, Norwalk virus, and others (Hurst, 1991). In general, these viruses consist of a nucleic acid core wrapped in a protein capsid that is quite resistant to adverse environmental conditions and disinfection. It has been shown that viruses are more resistant to inactivation and removal by treatment processes and can survive for longer periods of time in the environment than bacteria (Gerba and Rose, 1990). Their durability is one reason why they are able to pass through the acidic medium in the stomach and subsequently reach the small intestines where most of them infect epithelial cells and replicate.

Most of the enteric viruses are responsible for causing gastroenteritis or hepatitis, but some can affect the central nervous system and respiratory system (Table B). The characteristic symptoms of viral gastroenteritis include sudden gastrointestinal pain, vomiting, and diarrhea, resulting in severe loss of body fluids and dehydration. Asymptomatic infections are particularly common among some of the enteric viruses. Individuals who are asymptomatic spread the virus unknowingly to others, thereby increasing the

potential of infection. This situation can overwhelm the treatment facility processes and eventually lead to outbreaks. The majority of viral infections are not fatal, but the socio-economic impact is alarming in terms of discomfort, inability to work, medical costs, and implications for the tourist and food industries.

In 1966, the first report of enteric viruses in drinking water was accredited to insufficient treatment (Gerba and Rose, 1990). It has since been shown that viruses can be recovered from treated drinking water. Akin (1984) reported that approximately 53% of reported isolations came from water with complete treatment (coagulation-sedimentation, filtration, and disinfection), 26% came from water which was only disinfected, and 15% from untreated water. More recently, of the 34 waterborne disease outbreaks reported from 1991-1992, 26 (76%) occurred in systems using well water. The water was untreated in 12 (46%) of these wells. In another 12 (46%) of the 26 wells, inadequate or interrupted disinfection (chlorine or ultraviolet (UV) light) was determined to be the cause (CDC, 1993).

From 1946 to 1980, 50% of documented waterborne disease outbreaks in the United States resulting in acute gastroenteritis were of unknown etiology (Lippy and Waltrip, 1984). It was later identified that viruses were the cause of 12% of the total number of outbreaks during this 34 year period. Improvements in recovering and detecting enteric viruses from environmental specimens have increased knowledge on the viral agents

reponsible for causing outbreaks. For example, rotavirus diarrhea is estimated to result in 500,000 physician visits and 67,000 hospitalizations each year (Matsumoto, 1989). Others have implicated adenoviruses in as many as 32% of the cases of hospitalized children with acute gastroenteritis in this country (Richmond, 1979).

Identifying the etiological agent of a waterborne outbreak is difficult because of the limitations in isolating several of the enteric viruses from the environment and from clinical samples. This factor alone leads to the underestimation of the incidence of virally contaminated water. As new and improved techniques for the detection of enteric viruses or their nucleic acid from the environment are developed, enteric virus contamination of drinking water will be more accurately documented.

Protozoa in Source Waters

Protozoal parasites are the most frequently identified etiologic agents of waterborne disease outbreaks. From 1978-1991, *Giardia* was the most commonly implicated pathogen (Moore, 1994), although the same numbers of outbreaks of giardiasis and cryptosporidiosis were reported in 1992. During March and April of 1993, the largest documented waterborne outbreak in United States history occurred in Milwaukee, Wisconsin. *Cryptosporidium* was identified as the etiologic agent (Gradus et al., 1994). A study by LeChevallier reported that *Giardia* or *Cryptosporidium* spp. or both were detected in 97% of the surface water supplies tested in the United States and

one Canadian province (LeChevallier et al., 1991). Both *Giardia* cysts and *Cryptosporidium* oocysts are spread through the fecal-oral pattern similar to virus transmission.

The causative agent of giardiasis in humans is the protozoan parasite *Giardia lamblia*. *Giardia* belongs to the phylum Sarcomastigophora and the class Zoomastigophorea. Three species of *Giardia* have been identified based on morphological criteria. *Giardia agilis* infects amphibians such as toads and frogs; *G. muris* infects rodents, birds, and reptiles; and *G. lamblia* (also called *G. duodenalis* and *G. intestinalis*) causes disease in humans, but can also be carried by aquatic mammals such as beavers and muskrats (Adam, 1991). The life cycle of *Giardia* is relatively simple, consisting of a vegetative trophozoite stage and infective cyst stage. In general, after the cyst (12-15 um long and 6-10 um wide) has been ingested, it excysts in the small intestine and forms two to four trophozoites. The trophozoites (10-12 um long and 5-7 um wide) divide by binary fission in the small intestine and are later induced to encyst in the descending colon. The cycle is completed when the cysts are excreted in the feces and are ingested by another host.

The clinical manifestations of giardiasis begin approximately 7 to 21 days after exposure, but asymptomatic infection is not uncommon. Symptoms include diarrhea (often sudden in onset), abdominal cramping, bloating, excessive flatulence, foul-smelling stools, nausea, fever, and vomiting. These acute symptoms usually last between 1 and 4 weeks, and are

followed by a subacute or chronic phase. Manifestations of this stage are intermittent bouts of soft stools, flatulence, weight loss, and general malaise (Adam, 1991). If the disease remains untreated, the individual may adjust to the parasite and virtually no symptoms will remain. The trophozoites will still continue to reproduce and cysts will be excreted in the environment.

Despite the increasing attention that *Giardia* has received over the past decade, the precise effect that the organisms have upon the human body is not well understood. It is known that infection results in altered levels of intestinal peptidases, decreased vitamin B12 absorption, disaccharidase deficiency with lactose intolerance, and intestinal malabsorption of fats and carbohydrates (Behnke, 1990). The mechanisms that are responsible for these effects are not well understood, but there are several possibilities which include damage to the fuzzy coat of microvilli by the trophozoite's sucking disks, mechanical blockade of intestinal mucosa by the trophozoites, organism-induced deconjugation of bile salts, altered intestinal motility, accelerated turnover of mucosal epithelium, and mucosal invasion (Adam, 1991 and Behnke, 1990).

Giardia and *Cryptosporidium* are relatively resistant to the external environment, gastric acid in the stomach of the infected individual, as well as to disinfection processes. Ozonation and sand filtration are the most effective means of cyst and oocyst inactivation and removal, but they are not routinely susceptible to the typical levels of chlorine recommended for drinking water

(Sobsey, 1989). Therefore, outbreaks of giardiasis and cryptosporidiosis can occur from water that has an acceptable low level of coliform organisms, the conventional indicators of fecal pollution in drinking water (Hurst, 1991).

Detection Methods for Monitoring Drinking Water

Direct Detection Methods

The direct detection of pathogenic organisms in water supplies is not routinely practiced. There are several factors that make the monitoring of pathogens impractical. Firstly, there are simply too many pathogens to test for. Monitoring the concentration of only a few pathogens would not provide an accurate measure of disease potential (Laws, 1993). This problem is further complicated by the fact that several enteric viruses are not detectable by conventional cell culture systems, a technique commonly used to test for a wide spectrum of gastroenteritis viruses that are waterborne. Secondly, enteric pathogens have varying levels of resistance to standard water treatment methods and environmental stresses. Subsequently, pathogen concentrations are likely to exhibit substantial temporal and spatial variations which makes monitoring erroneous. Another problem in testing for pathogens directly is the cost of setting up and operating a laboratory capable of assaying for a large variety of organisms. Techniques for determining the presence of viral and protozoan pathogens are expensive, time-consuming, and require highly-trained operators to perform the assays. Lastly, because

human enteric pathogens originate from the intestinal tracts of only a small percentage of the population, their discharge into the environment is usually sporadic and inconsistent. Consequently, the general approach for monitoring the microbiological quality of water is through the use of indicator organisms.

Indicator Organisms

The presence of an indicator organism in a water supply suggests the possible presence of fecal pollution and therefore the potential of pathogenic organisms also being present. Indicator organisms should have the following characteristics (Bitton, 1980):

1. Should be associated with the source of the pathogen and should be absent in unpolluted areas.
2. Should occur in greater numbers than the pathogen.
3. Should not multiply in water and in other environments.
4. Should be at least equally resistant to environmental stresses and to disinfection as the pathogen.
5. Should be detectable by means of easy, rapid, and inexpensive methods.
6. Should not be pathogenic.

Originally defined in 1914 by the U.S. Public Health Service, the indicator used for bacteriological drinking water standards has been the coliform group. Total coliforms are aerobic and facultative anaerobic gram-negative, nonspore-forming, rod-shaped bacteria that ferment lactose at 37°C and produce gas within 48 hours. It was later discovered that several coliforms were associated with soils and vegetation, rather than solely the gastroentestinal tract. (Hoadley and Dutka, 1977). As a result, a subgroup of

coliforms which are able to ferment lactose at 44.5°C was denoted as the fecal coliform group.

Although total and fecal coliforms continue to be used as the indicators of fecal pollution, there is poor correlation between the incidence of gastrointestinal illness and total and fecal coliform concentrations (Sobsey, 1989). This may be due in part by the finding that coliform bacteria can colonize on the interior surfaces of water mains in a distribution system (van der Wende and Characklis, 1990). These colonies, which are difficult to eliminate, shed bacteria into the distribution system when there is a rapid change in the flow of water. Although not related to health effects, their colonization results in violations of the standards.

Another shortcoming of the coliforms as indicators of fecal pollution is that viruses and protozoan cysts are more resistant to environmental stresses and disinfection than bacteria (Bitton, 1980). Microbiological examination of water has shown that water that has met all bacteriological criteria has later been shown to be contaminated with viruses. The 1955-1956 Hepatitis A epidemic in Delhi, India, dramatically illustrated that coliform counts of <2/100 mL can indeed be associated with an unsafe level of at least one enteric viral pathogen (Viswanathan, 1957). More recently, the Milwaukee, Wisconsin, *Cryptosporidium* outbreak has certainly demonstrated that water treatment may not be reliably assessed based on the routinely used indicators (Gradus, 1994).

Disinfection

History

In 1785, chlorine's first practical application was discovered in the textile industry where its bleaching property was exploited. Shortly thereafter, the disinfecting and deodorizing properties of chloride of lime were recognized (White, 1986). As early as 1854, chlorinated lime was applied in the treatment of sewage in London and also for disinfection and deodorization of hospital wards. Of landmark importance was the demonstration by Koch in 1881 that pure cultures of bacteria were destroyed by the use of hypochlorites (Lawrence and Block, 1968). During World War I, sodium hypochlorite solutions were widely used for disinfection of open and infected wounds.

The use of chloride of lime for the purification of water was first introduced in North America by Johnson in 1908 at Bubbly Creek (Chicago) and the Jersey City Water Company (Haas, 1990). The use of chlorine or chlorine compounds as disinfectants for water and wastewater was widely accepted. By 1918, more than 1000 cities were utilizing chlorine as a disinfectant (Race, 1918).

Chlorine Disinfection

Chlorine can be used as a disinfectant in the form of compressed gas under pressure or in water solutions, solutions of sodium hypochlorite, or solid calcium hypochlorite (Haas, 1990). When chlorine is dissolved in water,

it reaches the following equilibriums (Bitton, 1980):

$$Cl_2 + H_2O \text{ <----> } HOCl + H^+ + Cl^-$$

$$HOCl \text{ <----> } H^+ + OCl^-$$

The dissociation of hypochlorous acid depends on the pH of the water. At pH < 6.0, hypochlorous acid is present in higher concentrations than hypochlorite ion, whereas hypochlorite ion is the dominant species at pH > 9.0. Water that has a pH value between 6.0 and 9.0 is composed of both chlorine species.

Both HOCl and OCl^- are commonly referred to as free available chlorine. In chlorination of water, a certain portion of this chlorine is consumed by impurities in the water. Any unconsumed chlorine remains as residual available chlorine. The difference between the chlorine applied and the chlorine remaining in the water is referred to as the "chlorine demand" of the water. As a result of its electronic configuration, chlorine has an affinity for acquiring electrons, making chlorine a strong oxidizing agent. Chlorine readily combines with ammonia and other nitrogenous compounds present in the water, resulting in the formation of chloramines or N-chloro compounds. This combination is referred to as "combined" available chlorine (Haas, 1990). "Breakpoint chlorination" refers to the amount of chlorine that must be added to a water before a stable free residual can be obtained.

Chlorine has been the most widely used disinfectant in the United States (Oliveri, 1986), although the use of chloramines is increasing (van der Wende and Characklis, 1990). It provides the most cost effective and simplest means of providing water that may be used for drinking or recreation. While its most popular application is to reduce the number of microorganisms in water and wastewater, there are several other qualities of chlorine that are useful in water treatment. It has been shown to be effective in preventing algal growth, removing iron and manganese, maintaining clean filter media, controlling slime growth in the distribution system, preserving pipeline capacity, and removing color by bleaching certain chromogenic compounds (White, 1986).

In 1948, Knox et al. confirmed the mechanism by which chlorine inactivates bacteria. The bactericidal effect of chlorine is produced by the inhibition of certain enzyme systems essential to life. More specifically, the action of chlorine on the SH groups of vital enzymes sensitive to oxidation results in the inhibition of essential cytoplasmic metabolic reactions. They demonstrated a correlation of inhibition of glucose oxidation and bacterial death for several bacterial species that had been treated with chlorine. Friberg (1957), using radioactive phosphorus (^{32}P), illustrated that chlorine results in a destructive permeability change in the bacterial wall. This conclusion was based on demonstrating the leakage of ^{32}P from nucleoproteins of bacterial cells.

Chlorine has also been shown to react with nucleic acids. Dennis et al (1979) assessed the inactivation of bacteriophage f2 by HOCl and OCl^-. They determined that the incorporation of chlorine into viral RNA was the cause of inactivation. Experiments with poliovirus, however, indicated that the protein coat, and not the the nucleic acid, is the crucial site for inactivation by chlorine (Tenno, 1980).

Some viruses are more resistant to chlorine than other viruses. Harakeh (1984) reported that of six viruses tested, Coxsackie B5 is the most resistant, with 99.99% inactivation achieved at a dose of 18.0 mg/L of chlorine after five minutes of contact, whereas simian rotavirus was the most sensitive, with a dose of 5.0 mg/L of chlorine required to obtain 99.99% inactivation. Protozoan cysts are even more resistant than viruses. Leahy et al. (1987) showed that 2.80 mg/L of chlorine achieves a 99% inactivation of *Giardia muris* cysts in 16 minutes of contact time at pH 7.0 and 25°C. Studies by Korich et al. (1990) demonstrated that 80.0 mg/L of chlorine requires approximately 90 minutes for 90% inactivation of *Cryptosporidium parvum* oocysts.

Chlorine has been widely accepted as a disinfectant for water and wastewater for several decades. Recently, however, it has been shown that the interaction of free chlorine with selected precursors (ie: humic substances and organics) in drinking water produce a group of halogen-substituted single-carbon compounds referred to as total trihalomethanes (TTHMs)

(Craun, 1988). These THMs are carcinogenic and pose a health risk to the public. In 1979, the USEPA set a maximum contaminant level (MCL) of 100 ug/L for TTHMS in drinking water (USEPA, 1979). Factors that determine the concentration of THMs include the presence and concentration of humic substances, chlorine dosage, pH, temperature, and contact time. Approaches to control THM formation are the removal of organic precursors that react with the chlorine, removal of THMs from finished water, and the use of alternative disinfectants (Stetler, 1984).

Chlorine Dioxide Disinfection

There are several applications of chlorine dioxide in the United States including taste and odor control, iron and manganese oxidation, disinfection, color control, organic oxidation-enhanced coagulation-filtration, and in-plant control of micro- and macro-biofouling. Its utilization in water treatment first occurred at Niagara Falls, NY, in 1944 to control taste and odors from algae and decayed vegetation as well as phenolic tastes and odors from industrial wastes (Katz, 1980).

Of the alternative disinfectants being studied, chlorine dioxide appears to be one of the most promising. Most notably, chlorine dioxide does not react with hydrocarbons to form carcinogens and it remains effective in the presence of ammonia. It is five times more soluble and has more than twice the oxidizing capability of chlorine (Oliveri, 1986). It is also easy to maintain a residual throughout the distribution system and it is relatively cost effective.

Hettche and Ehlbeck (1953) demonstrated that chlorine dioxide is more virucidal than chlorine and ozone.

Despite the advantages of using chlorine dioxide as an alternative disinfectant, there are also some drawbacks. During the treatment of water and wastewater, a part of the chlorine dioxide is reduced to chlorite ions which are suspected of being toxic (Katz et al., 1994). Chlorite has been associated with hemolytic anemia at levels as low as 50 mg/L (Abdul-Rahman et al., 1980). Studies with rats indicated a decrease in sperm motility at levels above 100 mg/L (Carlton and Smith, 1986). The production of chlorite ions led the USEPA to set a MCL of residual chlorine dioxide together with its inorganic byproducts, chlorite and chlorate ions, at 1.0 mg/L in drinking water (Werdehoff, 1987).

Chlorine dioxide can be generated by many different methods, but there are two that are generally used for water treatment operations. One process uses chlorine, in either gas or liquid state, and sodium chlorite to produce chlorine dioxide. The other uses chlorine, a mineral acid (ie: hydrochloric acid), and sodium chlorite to produce the chlorine dioxide (White, 1986). Chlorine dioxide is a more effective oxidant when applied to relatively clean waters because it readily reacts with suspended solids and dissolved organic matter. Consequently, chlorine dioxide is usually applied as a postdisinfection step or as a preventative disinfectant in untreated groundwaters (Glaze, 1990).

The physiological mode of inactivation of bacteria by chlorine dioxide has been shown to be the disruption of protein synthesis. The mechanism by which chlorine dioxide inactivates viruses appears to be similar to that of chlorine. In 1982, Alvarez and O'Brien suggested that chlorine dioxide is virucidal to poliovirus by targeting the viral nucleic acid and impairing the viral genome to act as a template for RNA synthesis. Shortly thereafter, this hypothesis was disputed by Hauchman et al. (1986) who demonstrated that the infectivity and physical integrity of RNA within chlorine dioxide treated viruses was retained despite several log units of viral inactivation. In 1986, disinfection studies conducted with bacteriophage f2 by Noss et al. showed that f2 was inactivated by chlorine dioxide reacting with tyrosine residues on the capsid protein and/or the A protein, resulting in the inhibition of viral adsorption to host bacteria.

As with chlorine, bacteria are more susceptible to chlorine dioxide treatment than both viruses and protozoan cysts. In a comparative disinfection study of *Escherichia coli* and poliovirus, chlorine dioxide dosages of 1.80 mg/L achieved a >5.0 log reduction of *E. coli* and a 1.8 log reduction of poliovirus after one minute of contact (Fujioka, 1986). Korich et al. (1990) demonstrated that exposure to 1.30 mg/L of chlorine dioxide yields 90% inactivation of *C. parvum* oocysts after 60 minutes of contact.

Alternative Disinfectants

Both ozone and ultraviolet radiation as disinfectants for surface waters have received greater attention in recent years. Ozone is an oxidant that is moderately soluble in water. It is produced by gas-phase electrolytic oxidation of oxygen by using very dry air or pure oxygen. This gaseous phase is then contacted with water in a bubble contactor or in a countercurrent tower contactor (Haas, 1990). When ozone is added to water, it reacts with hydroxide ions to form hydroxyl radicals and organic radicals. These radicals increase the decomposition of ozone and result in a half-life on the order of seconds to minutes. Ozone's effectiveness is also impeded because it dissipates quickly when it reacts with humic material in water (Glaze, 1990). The inability to maintain a residual following ozone treatment requires the application of a terminal disinfection process such as chlorination or chloramination. To date the health significance of byproducts remains unresolved, but there are several classes of potential ozone byproducts. These include aldehydes, ketones, carboxylic acids, epoxides, peroxides, quinones, phenols, and brominated organics (Jacangelo et al., 1989).

Over a thousand drinking water systems in Europe currently use ozone at concentrations of 2.0-3.0 mg/L (Tate and Arnold, 1990). In 1987, five water treatment facilities in the United States were using ozone for taste and odor control or for removal of trihalomethane precursors (Haas, 1990). The capital costs to install ozonation equipment is higher than other disinfectants,

however, the operating costs are relatively low. In many cases, preozonation saves chemical costs and allows a faster filtration rate (Rice, 1986).

The mode of inactivation of microorganisms by ozone is not well understood due to the difficulties in measuring low concentrations of dissolved ozone. Kim et al. (1980) demonstrated that ozone breaks the protein capsid of phage f2 into several subunits which liberates the viral RNA and disrupts the adsorption to the host pili.

Ultraviolet (UV) radiation is also an effective biocide. UV radiation is produced by the use of mercury vapor lamps. To ensure effective inactivation of microorganisms, the radiation must be in direct contact with the organism(s) for a sufficient amount of time. Therefore, the presence of biological or chemical fouling materials (ie: high concentrations of suspended solids) can decrease the intensity of UV and therefore decrease it's disinfecting action. UV light is effective at destroying phenols, thereby improving the taste and odor of water. Currently, there is no known harmful byproduct resulting from UV radiation (White, 1986).

The mode of viral inactivation has been shown to be the formation of thymine dimers in nucleic acid, and intense UV can disrupt the virion structure (Battigelli et al., 1993). In vegetative organisms, the nucleic acid may be repaired by light-inactivated or dark repair enzymes. UV has been approved for treating potable water supplies on passenger ships and is currently used in wastewater treatment (Haas, 1990). Like ozone, however, it

is more expensive than chlorine and it leaves no residual for distribution protection. Therefore, the application of another disinfectant is necessary to ensure safe potable water.

There are a variety of other disinfecting agents that may be used to inactivate microorganisms. Alternatives include heat, extremes in pH, potassium permanganate, surfactants, and metals (silver, copper). These agents provide some degree of microbial inactivation, but are not usually economical or sufficient as a sole disinfectant.

Governmental Regulation for Public Water Supplies

History

The Interstate Quarantine Act of 1893 was the first set of governmental standards for drinking water systems. The Act authorized the Surgeon General of the U.S. Public Health Service "to make and enforce such regulations as in his judgment are necessary to prevent the introduction, transmission, or spread of communicable disease from foreign countries into the states or possessions, or from any state or possession into any other state or possession." (McDermott, 1973). In 1974, the Safe Drinking Water Act (SDWA) was signed into law which directed the EPA to identify substances in drinking water "which in the judgment of the administrator may have any adverse effect" on public health (Larson, 1989). As amended in 1986, the EPA was also required to establish National Primary Drinking Water Regulations

(NPDWRs) which apply to all public water systems. NPDWRs specify maximum contaminant levels (MCLs) or treatment techniques for drinking water contaminants (Cotruvo and Regelski, 1989). Factors such as source water quality, treatment practices (such as disinfection and length of water storage) and watershed management are considered in establishing these criteria.

Surface Water Treatment Rule (SWTR)

The United States Environmental Protection Agency (USEPA) mandated two regulations that specifically undertake the threat of pathogens in public water supplies: the Total Coliform Rule and the Surface Water Treatment Rule (SWTR). The Total Coliform Rule, which applies to all public water supplies, requires a maximum contaminant level goal (MCLG) of zero for total coliforms, including both fecal coliforms and *Escherichia coli*. This rule indicates the maximum contaminant levels (MCLs) for selected contaminants and describes monitoring and analytical requirements.

The SWTR which became effective on December 31, 1990, was developed on the assumption that all surface water or groundwater under the direct influence of surface water (GWUDI) are at risk from contamination by pathogens such as *Giardia lamblia* or other protozoa, viruses, and pathogenic bacteria. The primary purpose of the regulation is to protect the public, as much as possible, from waterborne diseases by removing or inactivating pathogenic microorganisms (USEPA, 1989). Major provisions of the SWTR

include that all public water supplies disinfect and all such systems to adopt filtration unless certain water quality source and site-specific requirements are met. Since the SWTR specifies several strict requirements for public water systems that use surface water, only the main provisions are discussed here.

Pathogenic microorganisms such as *Legionella pneumophila, Giardia lamblia,* and viruses are difficult to monitor in source waters because assays to detect their presence are impractical due to costs, time, and labor consumptions. These pathogens exist in small numbers in the environment, their entry into the water supplies occurs sporadically, and as a result their presence is not frequently reported (USEPA, 1979, Regli, 1991). Results of these limitations in detecting pathogens in the environment motivated the SWTR's emphasis on treatment technique requirements as the condition for compliance instead of MCLs for microorganisms. These microorganisms can be removed by filtration and/or inactivated by disinfection. Each water system is intended to choose the best method of treatment for its particular situation.

The SWTR specifies that systems using surface water must treat water to remove/inactivate at least 99.9% (3 logs) of *Giardia lamblia* cysts and at least 99.99% (4 logs) of viruses. Microbiological examination of water has shown that viruses and protozoan cysts are more resistant to inactivation by adverse environments and disinfection than coliforms, the standard bacterial indicators of fecal pollution in drinking water (Moore, 1994). Therefore, a

treatment facility operates on the assumption that if they disinfect adequately for viruses and cysts, they have treated sufficiently for bacteria, which are more susceptible to disinfection. Treatment facilities are not required to monitor source waters or drinking waters for viruses or cysts (USEPA, 1989).

The SWTR indicates the acceptable microbial risk levels associated with providing safe drinking water that ensures less than one case of microbiologically caused illness per year per 10,000 people. The risk analysis performed to generate the specified log removal/inactivation of *Giardia* cysts was based on the cyst concentrations found in different source waters of varying quality (USEPA, 1989).

The strength of the disinfection process to achieve the specified reductions in microorganisms is measured by the contact time (CT) value. These values are provided by the EPA in a series of tables in the SWTR *Guidance Manual for Compliance With the Filtration and Disinfection Requirements for Public Water Systems Using Surface Water Sources* (USEPA, 1991). To generate the CT value, the disinfectant residual concentration (C, measured in mg/L) is multiplied by the contact time (T, measured in minutes). Compliance with the disinfection requisites of the SWTR requires that the operators determine the CT value for their system and compare it to the tables in the SWTR. CT values for both finished water and water in the distribution system muct be achieved for the system to be in compliance. Effectiveness of different disinfectants varies, resulting in

different CT values required for the disinfectant employed. The state primacy agency is responsible for determining the level of disinfection needed to meet the required log reductions (Von Huben, 1990). The stringency of the states' requirements may indicate a greater removal or inactivation to ensure that the water is properly treated for source waters that are of relatively poor quality.

Research Approach

Objectives

This research project, which is supported by the USEPA, is to compare chlorine and chlorine dioxide as disinfectants for surface waters. The primary objective is to establish concentration x time (CT) values for the inactivation of *G. muris* cysts and bacteriophage MS2 as a function of ambient temperature and pH for chlorine and chlorine dioxide in batch and field trials. The estalishment of disinfection efficacies of chlorine and chlorine dioxide using *Giardia muris* cysts, MS2 bacteriophage, total and fecal coliforms, and total heterotrophic bacteria is also a main goal of this research. The project is highly relevant to the USEPA's Drinking Water Program. Results will be used by communities who must comply with the SWTR and regulatory agencies who require technical data to support their policies.

Phase 1 - Batch, Bench-scale Laboratory Studies

The primary goal of bench scale laboratory experiments is to provide

information concerning disinfection in controlled settings. This project design allowed the careful control of experimental variables including water quality, pH and temperature. Experiments were performed to identify the required disinfectant doses to inactivate 3 logs of *Giardia* cysts and 4 logs of viruses. The data generated was compared to CT values provided by the USEPA in the SWTR *Guidance Manual for Compliance With the Filtration and Disinfection Requirements for Public Water Systems Using Surface Water Sources*. Results of Phase 1 studies dictated disinfectant dosages and contact times to be used during Phase 2 pilot studies.

Phase 2 - Continuous, Pilot-scale Field Studies

Disinfection experiments are typically conducted with pure cultures of batch-grown microorganisms suspended in particle-free, chlorine demand-free test solutions (Hoff and Geldreich, 1981). Research has shown, however, that traditional laboratory experiments do not reliably predict the effectiveness of disinfectants in drinking water systems. Wolfe and Olson (1986) identified five factors that limit the usefulness of results from laboratory-derived disinfection studies: 1) differences in tolerance between naturally occurring and subcultured organisms; 2) differences in inactivation kinetics among pure cultures and mixed populations; 3) differences in the variability and reproducibility of inactivation rates; 4) differences in the techniques used to recover microorganisms exposed to disinfectants; and 5) the presence of interfering compounds (ie: nitrogenous organic compounds)

that can alter the microbicidal activity of the disinfectant. Their research strongly suggested that results obtained in laboratory studies are inappropriate for predicting performance of a disinfectant under field conditions. Therefore, to increase the usefulness and of the data and to insure application of the results generated from this project, Phase 2 pilot-scale studies were conducted.

Table A - Outbreaks Associated with Water Intended for Drinking, by Etiologic Agent and Type of Water System - United States, 1991-1992 (N=34) *

	Type of water system							
	Community		Noncommunity		Individual		Total	
Agent	Outbreaks	Cases	Outbreaks	Cases	Outbreaks	Cases	Outbreaks	Cases
AGI**	3	10,077	19	3,252	1	38	23	13,367
Giardia	2	95	2	28	0	0	4	123
Crypto-sporidium	2	3,000	1	551	0	0	3	3,551
HAV	0	0	0	0	1	10	1	10
S. sonnei	0	0	1	150	0	0	1	150
Nitrate	0	0	0	0	1	1	1	1
Fluoride	1	262	0	0	0	0	1	262
Total	**8**	**13,434**	**23**	**3,981**	**3**	**49**	**34**	**17,464**
(Percent)	(24)	(77)	(68)	(23)	(9)	(<1)	(100)	(100)

*MMWR, Vol.42, November 19, 1993
**AGI = acute gastrointestinal illness of unknown etiology.

Table B - Characteristics of Enteric Viruses*

Virus group	No. of types	Size (nm)	Type of nucleic acid	Diseases
Enterovirus				
Poliovirus	3	20-30	RNA	Paralysis Aseptic meningitis
Coxsackievirus				
A	24	20-30	RNA	Herpangia Aseptic meningitis Respiratory illness Paralysis fever
B	6	20-30	RNA	Pleurodynia Aseptic meningitis Pericarditis Myocarditis Nephritis
Echovirus	34	20-30	RNA	Respiratory infection Aseptic meningitis Diarrhea Pericarditis Myocarditis, rash
Reovirus	3	75-80	RNA	Respiratory disease Gastroenteritis
Adenovirus	41	68-85	DNA	Acute conjunctivitis Diarrhea Respiratory illness
Hepatitis A Virus	1	27	RNA	Infectious hepatitis
Rotavirus	4	70	RNA	Infantile gastroenteritis
Norwalk Agent	1?	27	RNA	Gastroenteritis
Astrovirus	4	28	RNA	Gastroenteritis
Calcivirus	1?		RNA	Gastroenteritis
Snow Mtn. Agent	1?		RNA?	Gastroenteritis
Norwalk-like Viruses	?		?	Gastroenteritis
Epidemic non A non B Hepatitis	1?		RNA?	Hepatitis

* Adapted from Gerba and Rose, 1990.

CHAPTER II

Methodology

Bench-Scale Studies

Preparation of Chlorine-Demand Free Water

Chlorine-demand free water was prepared in a five gallon carboy by spiking 18 M-ohm deionized (MilliQ) water with a 5% NaOCl solution to yield a residual of 5.0 mg/L of free chlorine. Free chlorine was detected by the AccuVac DPD Free Chlorine Reagent method (Hach, Loveland CO) which is measured on the DR100 Direct Reading Spectrophotometer (Hach). The remaining free chlorine was removed by immersing an ultraviolet light into the carboy and stirring the water until no free chlorine was detected.

Glassware Preparation

All glassware was made chlorine-demand free by washing with mild detergent, rinsing three times with hot tap water, followed by five rinses with deionized, distilled water. The glassware was then baked overnight at 170.0°C.

Preparation of Virus and Protozoa Stocks

Both MS2 bacteriophage and *Giardia muris* cyst stocks were prepared and diluted using chlorine-demand free water.

Predisinfection Experiments

Experiments were done to determine if the polypropylene tubes used in the bench-scale studies exerted any chlorine demand or affected the pH ofthe samples. These variables were tested by adding 50.0 mL of chlorine-demand free water to 50.0 mL polypropylene conical tubes, adjusting the pH with the buffers used in the disinfection experiments, and adding NaOCl to maintain a residual between 0.5 - 1.0 mg/L (for method, refer to next section). Samples were taken at 30 second intervals to measure the chlorine residual and the pH. To determine if the polypropylene tubes adversely affected the microbiological spikes, the same procedure was followed without the addition of NaOCl. Samples were taken for enumeration of the microbiological spikes (see procedures below). To determine if $Na_2S_2O_3$, a dechlorinating agent, affected the viability of the organisms, microbiological spikes were added to polypropylene tubes containing 0.5 mL of 0.05 M $Na_2S_2O_3$. After 30 minutes contact, samples were taken for enumeration of the microbiological spikes.

Chlorine Disinfection

Chlorine-demand free water or post-filtered water from the Portsmouth Water Treatment Facility (Portsmouth, NH) or Concord Water Treatment Facility (Concord, NH) was brought to a pH of 6.0 using the buffers KH_2PO_4, pH 4.4 and Na_2HPO_4, pH 9.9. The temperature was also recorded. The water was spiked with NaOCl to give a final residual ranging from 0.5

mg/L - 2.0 mg/L. Chlorine residuals were measured by removing a 10.0 mL aliquot of water and mixing it with an ampule of AccuVac DPD Free Chlorine Reagent. A 1.0 mL aliquot of a known concentration of MS2 bacteriophage was added to 49.0 mL of chlorinated water in a 50.0 mL conical polypropylene tube. The tube was inverted several times and the chlorine residual was measured. The initial spike of MS2 was diluted 1:250 in chlorine demand-free water to ensure that the residual did not fall below 0.2 mg/L when the virus was added to the chlorinated water.

Forty-nine milliliters of chlorine-spiked water was added to four individual polypropylene tubes. A 1.0 mL aliquot of the MS2 spike was added to the first tube. At 30 seconds, a 10.0 mL aliquot was withdrawn and added to a 15.0 mL conical polypropylene tube that contained 0.5 mL of 0.05 M $Na_2S_2O_3$. $Na_2S_2O_3$ is a dechlorinating agent and was used to quench the reaction. The sample was brought to pH 7.0 with KH_2PO_4. The chlorine residual was measured from the remaining sample in the 50.0 mL tube. The procedure was repeated for the 1.0, 1.5, and 2.0 minute time points (or as indicated in the Results and Discussion sections).

The entire procedure was repeated for pH 7.2 and 9.0. The samples performed at pH 9.0 were brought to pH 7.0 with Na_2HPO_4. The procedure was also performed at 4.0°C and 10.0°C to determine the effect of temperature on inactivation.

The same procedure was used for bench-scale disinfection experiments

using *Giardia muris* cysts (Cleveland State University). Prior to all experimental runs the cysts were enumerated and excysted to determine their concentration and viability (see procedure below). Any suspensions with less than 80% viability were not used in any experimentation as they may be more susceptible to disinfection and injury than "healthy" cysts. This was one measure taken to decrease sample to sample variation.

Chlorine Dioxide Disinfection

Disinfection experiments using chlorine dioxide were performed in a similar manner as those with chlorine. The chlorine dioxide was generated on-site by adding 1.0 gm of sodium chlorite to 1.0 L of MilliQ water. In a fume hood, 1.69 gm of acetic anhydride was added and the solution was brought to a pH of 7.0 with 5 M NaOH.

An initial dosage of 0.5 to 1.1 mg/L of chlorine dioxide was used for these sets of experiments. As with the chlorine trials, three temperatures (24.0°C, 10.0°C, and 4.0°C) and three pH values (6.0, 7.2, and 9.0) were assayed. Chlorine dioxide residuals were measured in a DR/2000 Direct Reading Spectrophotometer (Hach) using chlorine dioxide reagents (Hach).

Plaque Assay for Disinfected Viral Samples

A double-agar-layer method (Adams, 1959) to enumerate male-specific bacteriophages was used to quantify all viral samples. *Escherichia coli* C3000 was used as the bacterial host for all assays. Viral dilutions using 1X phosphate buffered saline (PBS, pH 7.2) were made from each time point

assayed. *E. coli* C3000 was grown up to mid-exponential phase in tryptic soy broth (TSB, Difco). One milliliter from a viral sample and 0.3 mL of *E.coli* C3000 were added to a tube that contained 5.0 mLs of soft overlay. The tube was mixed well and was poured on tryptic soy agar (TSA, Difco). The soft overlay was allowed to harden for 30 minutes. The plates were then inverted and incubated at 37.0°C. Plaques were counted after 18-24 hours. All assays were done in duplicate to ensure reliability.

Assays for Disinfected Protozoan Samples

Enumeration

Each 10.0 mL sample taken at a given time interval was transferred into an Oakridge tube and centrifuged at 10,000 RPM for 10 minutes (Beckman J26). All but 1.0 mL of the supernate was removed and the samples were resuspended in the remaining fluid. They were then transferred to separate conical microfuge tubes and were centrifuged at 10,000 RPM for 3 minutes (Centra 8). Each sample was carefully decanted and resuspended in 500 uL of 1X PBS (pH 7.2). 250 uL was removed from each and transferred into another microfuge tube for excystation. The remaining 250 uL was used for enumeration purposes.

To enumerate, the 250 uL sample was again centrifuged to pellet the cysts. The supernate was decanted and the pellet was resuspended in 50 uL of 1X PBS. A hemacytometer counting chamber was used to enumerate four 10 uL aliquots from each sample and the number of cysts/mL was calculated

using the following equation:

$$N = \frac{(n \times d)}{4} (10^4)$$

N = # cysts/mL
n = # cysts counted/square
d = dilution factor

Excystation

Employing the procedure from Rice and Schaefer (1981), the excystation procedure was performed separately on the remaining 250 uL samples. The samples were centrifuged at 10,000 RPM for 3 minutes to pellet the cysts. After decanting the samples, the following pre-warmed solutions were added to the pellet: 500 uL HCl (pH 2.0), 250 uL Hank's Balanced Salt Solution, and 250 uL 0.1 M $NaHCO_3$. The samples were vortexed and incubated for 30 minutes at 37.0°C. Following incubation, the cysts were again centrifuged, decanted and 500 uL of pre-warmed Tyrode's Salt Solution were added to the samples. These were vortexed and incubated at 37.0°C for 15 minutes. The samples were centrifuged, the supernate was decanted, and the pellet was resuspended in 50 uL of 1X PBS. A hemacytometer counting chamber was used to enumerate a minimum of four 10 uL aliquots from each sample. A minimum of 100 organisms was counted and the percent excystation was calculated using the following equation:

$$\% \text{ excystation} = \{ (TET) + (PET) \} / \{ (TET) + (PET) + (IC) \} \times 100$$

TET = totally excysted trophozoites
PET = partially excysted trophozoites
IC = intact cysts

Determination of Log Reduction - Standard Method

Log reductions were calculated for both MS2 and *Giardia muris* cysts using the following survival ratio:

$$\text{Log reduction} = \text{Log } N_t / N_0$$

N_t = number of organisms measured at given exposure time
N_0 = number of organisms measured at time = 0 (unexposed)

Optimization of *Giardia* Cyst Recoveries

G. lamblia cysts which were formalin inactivated were obtained from Biovir (Benicia, CA). Viable *G. lamblia* cysts were obtained from Parasitology Research Laboratories (Phoenix, AZ). Suspensions of viable, percoll-purified *G. muris* cysts were acquired from Cleveland State University.

A known concentration of cysts (either *G. muris* or *G. lamblia*) was seeded into 800.0 mL of 18 M-ohm deionized water (Millipore; Bedford, MA) [pH 6.6-6.8, 19.0-22.0 °C, residual chlorine <0.1 mg/L], mixed and allowed to stand for one hour. The seeded water was then aliquoted into 30.0 mL, 50.0 mL, or 200.0 mL polypropylene centrifuge containers and centrifuged at either 1300 x g (Beckman TJ-6) or 12,000 x g (Beckman J2-21M) for various lengths of times (Tables 1.0 and 1.5). The supernatant fluid was aspirated and set aside, the pellets were resuspended in 5.0 mL 1X PBS, recentrifuged and then resuspended to a final volume of 250 uL in PBS. The cysts were enumerated using a hemacytometer. For experiments with poor recovery, the supernatant fluid was examined for the presence of cysts by passing it through

a 0.2 um filter (Millipore), manually harvesting the cysts onto a slide, and viewing the slide under phase-contrast microscopy. This procedure was always positive for *Giardia* cysts, insuring that the cysts were intact and had not degraded during centrifugation.

Pilot-Scale Studies

Preparation of Bacteriophage MS2 Spike

One liter Fernbach flasks of sterile tryptic soy broth were inoculated with 20.0 mL of *E. coli* C3000 from overnight cultures. This liter culture was placed in a coffin shaker and grown to mid-exponential phase at 37.0°C (about 3.0 hours). The culture was then infected with 20.0 mL of a high-titer stock of MS2. Twenty milliliters of 1 M $MgCl_2$ was also added at this time to aid viral absorption to the host cells. The culture was mixed well and allowed to sit for 20 - 25 minutes to enhance viral absorption. The flask was then shaken for another 2.5 - 3.0 hours to let viral replication occur. The one liter sample was divided into six 250.0 mL sterile centrifuge bottles and 5.0 mL of chloroform was added to each to reduce viral clumping. These were well mixed and centrifuged at 10,000 RPM for 10 minutes to pellet the bacterial debris. The supernatant fluid was transferred into sterile 1.0 L polypropylene storage bottles and a plaque assay was performed to determine the viral titer. The sample was then refrigerated until further use.

For experimental trials at the pilot plant, four 1.0 L bottles of

bacteriophage MS2 were diluted 1:4 with MilliQ water and were stored at 4.0°C in a sterile jerrican until the experimental run.

Preparation of *Giardia muris* Spike

Suspensions of *Giardia muris* cysts for which the concentration and percent viability were known were diluted with 1X PBS to a final volume of 400.0 mL. This suspension was then divided in half and was transferred to two sterile polypropylene bottles. These suspensions represent the control trial and the treatment trial at the pilot plant.

Pilot Plant at Concord Water Treatment Facility

Pilot Plant

The entire pilot plant was housed in a trailer for ease of mobility (Figure 1). The plant provided a rapid mix region to evenly disperse all added materials (ie: *Giardia* cysts or viral spike, Cl_2, ClO_2), followed by a plug flow region which allowed variations in hydraulic detention time. A design flow of 10 gallons per minute (gpm) was chosen to be consistent with previous studies. The rapid mix region provided a 2 minute contact time and consisted of a ClO_2/Cl_2 diffuser combined with a static mixer. Water flowed from this region via gravity over inlet weirs and into a rectangular plug flow reactor with longitudinal baffles and turning vanes spaced 1 ft. apart. This arragement provided a serpentine flow and minimized short circuiting. The basin measured 4 ft x 24 ft x 4 ft (L x W x D) which included a 0.5 ft freeboard.

The total theoretical detention time was 240 minutes. Sample ports were installed which corresponded to theoretical contact times of 0.5, 1, 1.5, 2, 5, 10, 15, 30, 60, 90, 120, 150, 180, 210, and 240 minutes. System detention times were determined using step-dose tracer studies with rhodamine dye. Each tap was fitted with perforated piping to collect a composite sample spanning the tank depth.

Disinfectant Generation

The most cost effective approach to generate chlorine dioxide on-site was to purchase a laboratory scale ClO_2 generator which produces ClO_2 from the reaction of liquid sodium chlorite with hydrochloric acid (HCl) as follows:

$$5\ NaClO_2 + 4\ HCl \longrightarrow 4\ ClO_2 + 5\ NaCl + 2\ H_2O$$

Aieta and Berg (1986) reported that these generators yield ClO_2 solutions in excess of 95% with less than 5% excess chlorine. This generator produces chlorine dioxide solutions which are comparable to full-scale ClO_2 generators used at water treatment facilities.

In pilot plant experiments using chlorine, commercial grade sodium hypochlorite solutions were used. These solutions were chosen because they are commonly employed by drinking water plants and can easily be fed through the same diffuser system as ClO_2 via metering pumps.

Collection of Viral Samples

Bacteriophage MS2 was continually metered into the contact basin via a positive displacement pump. Fifty milliliter samples were taken at every

tap desired prior to the addition of disinfectant. Each tap in the contact basin represented an independent contact time. In obtaining the samples, the given tap was opened and one gallon of water was allowed to flow through. The samples were then collected in 50.0 mL tubes that contained 0.05 M $Na_2S_2O_3$ and the pH was adjusted to neutrality. These "pre-trial" samples represented the T=0 samples and were used to calculate the log reductions for the various contact times. Following these samples, the disinfectant was metered into the system until the desired residual was obtained. A second series of samples was collected from the same set of taps in the same manner as the "pre-trial" samples.

The viral samples were stored in a cooler until all samples were taken. They were then transported to the laboratory and analyzed within 24 hours following the assay previously mentioned.

During each MS2 trial at the pilot plant, temperature, pH, turbidity, and disinfectant residual was monitored. Additionally, samples were taken for heterotrophic bacteria and total and fecal coliforms.

Collection and Determination of Heterotrophic Bacteria

Prior to the addition of MS2 to the contact basin of the pilot plant, two 50.0 mL samples were collected from two different taps for determining the number of heterotrophic bacteria in the water. Samples were analyzed using the spread plate method as described in *Standard Methods for the Examination of Water and Wastewater* within 24 hours of collection. One

milliliter of each sample was plated on Plate Count Agar (Difco) and incubated at 37.0°C for 48 hours. Each sample was plated in duplicate.

Collection and Determination of Total and Fecal Coliforms

The two samples taken for the heterotrophic bacteria counts were also used for determining total and fecal coliforms. The multiple-tube fermentation technique as described in *Standard Methods for the Examination of Water and Wastewater* was the method used to analyze these samples. For the Presumptive Phase, Lauryl Tryptose Broth (LSB) fermentation tubes were arranged in 3 rows of 3 tubes each: first 3 tubes receive 10.0 mL each of sample, second 3 tubes receive 1.0 mL each of sample, and the third 3 tubes receive 0.1 mL each of sample. A positive control of *E. coli* C3000 and a negative control of 1% peptone were also prepared. Inoculated tubes were incubated at 35 $\pm$ 0.5°C for 24 hours, at which time they were shaken gently and examined for gas or acidic growth. If there was no growth, they were reincubated and reexamined at the end of 48 hours. The presence or absence of gas or acid production was recorded.

All LSB tubes showing gas or acid production were submitted within 24 hours to the Confirmed Phase. The positive LSB tubes were shaken to resuspend any growth. A loopful of culture was aseptically transferred to fermentation tubes with Brilliant Green Lactose Bile Broth (BGBB) and were incubated for 48 hours at 35 $\pm$ 0.5°C. Formation of gas in any amount within 48 hours was considered a positive result.

Collection of *Giardia muris* Cyst Samples

A prepared suspension of cysts was added to the system in a slug-dose using nitrogen to sparge the holding vessel. This sample run had no disinfectant added to the contact basin. The selected tap was opened at the pre-determined optimal time of collection, and the sample (308 gallons total) was collected at 3.5 gallons per minute (gpm) in 44 gallon barrels that contained approximately one gallon of 0.05 M $Na_2S_2O_3$. Temperature, pH, and disinfectant residual was measured every 15 minutes during the collection. The turbidity was also recorded. The entire sample was then pumped through a 1.0 um (nominal pore size) wound filter (Filterite) at 1.0 - 1.5 gpm.

Following the run with no disinfectant, the selected disinfectant was metered into the contact basin until the desired residual was obtained. The collection process was repeated and the samples were transported to the laboratory for analysis.

Filter Elution and Concentration of *Giardia muris* Cysts

Each filter was eluted individually no later than 24-48 hours following collection. The filters were sliced open using a scalpel, and were separated from the cores. They were placed in a prepared wash basin with 1.0 L of elution solution (800.0 mL 1X PBS, 200.0 mL 1% SDS, and 200.0 mL 1% Tween 80). The filter parts were massaged for 10.0 minutes and the elution solution was collected by wringing out the filter. The eluate was aliquoted into sterile

45.0 mL Oakridge tubes and centrifuged for 10 minutes at 10,000 RPM. All but 2.0 mLs was aspirated off and the supernate was discarded. The remaining fluid was used for resuspension and was combined into fresh Oakridge tubes. These were centrifuged again and the pellet, usually between 5.0 and 8.0 mLs, was collected.

Purification of *Giardia muris* Cysts

Giardia muris cyst pellets were purified using a percoll-sucrose gradient. A percoll-sucrose solution was prepared by combining 45.0 mL Percoll, 10.0 mL 2.5 M sucrose, and 45.0 mL distilled water on a stir plate. For each gradient, 30.0 mL of the percoll-sucrose solution was placed in a sterile 50.0 mL conical polypropylene tube. One milliliter of pelleted sample was resuspended in 20.0 mL of elution solution and was slowly layered on top of the percoll-sucrose solution. Each gradient was centifuged with no brake at 2500 RPM for 15 minutes. The upper layer was aspirated off and transferred to a 45.0 mL Oakridge tube. Elution solution was added to a final volume of 40.0 mL and these were centrifuged at 10,000 RPM for 10 minutes to pellet the cysts. The supernate was slowly aspirated off and the pellets were recombined. This procedure was repeated until the samples were relatively free of particulates. The samples were then divided in two and one aliquot was used for enumeration and the other for excystation procedures.

Determination of Log Reduction - Volumetric Method

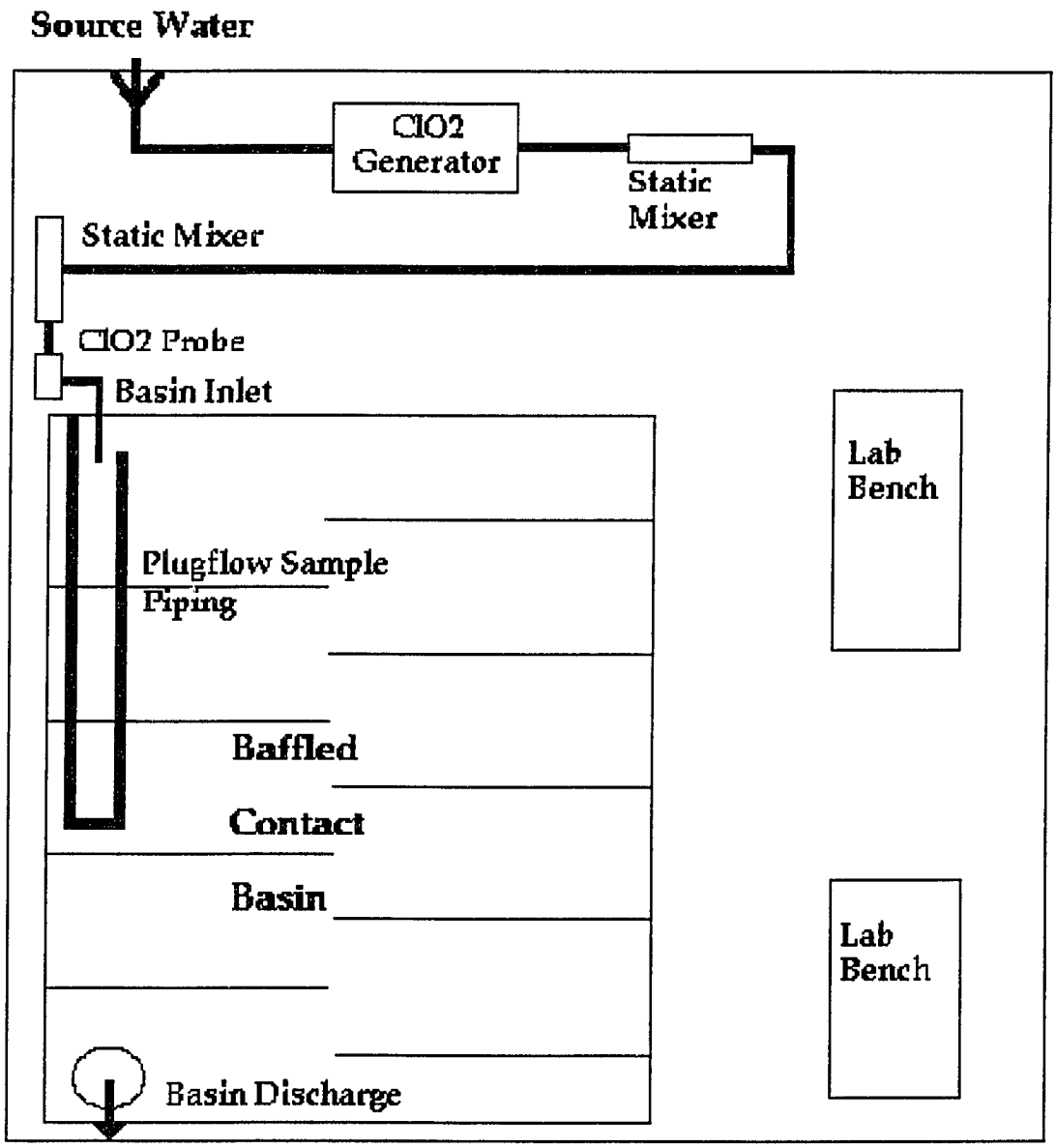
Due to the cost of preparing cyst suspensions of high concentration and low recovery efficiencies from the disinfection basin, the standard method for determining log reductions of *Giardia* was not possible (see Discussion). An alternative method, the volumetric method, was used to assess *Giardia* inactivation (Haas, 1994). In this method, the number of trophozoites in the control (nondisinfected) sample and the number of trophozoites in the disinfected sample was counted, and the volume of sample from which those trophozoites were enumerated was determined. A survival ratio was then calculated:

$$\text{Log reduction} = \text{Log}\left(TR_t / V_t\right) / \left(TR_0 / V_0\right)$$

TR_t = number of trophozoites from disinfected sample
V_t = volume of sample from which TR_t was counted
TR_0 = number of trophozoite from nondisinfected sample
V_0 = volume of sample from which TR_0 was counted

Figure 1.0

Pilot Treatment Plant



CHAPTER III

Results

Optimization of *Giardia* Cyst Recoveries

Experimental trials at the pilot plant required optimization for the recovery of *G. muris* cysts from the system. Initial trials were performed with suspensions of less expensive formalin inactivated *G. lamblia* cysts which yielded recovery rates between 80.0-90.0%. Subsequent trials with viable *G. muris* cysts yielded poor recovery of cysts (2.0-10.0%). Experiments to establish the cause of the loss of cysts demonstrated that the cysts were not sedimenting completely during the centrifugation steps. Ultimately, experiments to determine optimal cyst recovery were performed.

The recoveries of *G. lamblia* and *G. muris* cysts following different centrifugation schemes are presented in Tables 1.0 and 1.1. Results indicate better recovery of *G. lamblia* cysts - both formalin inactivated and viable - than *G. muris* cysts for every scheme tested. Viable *G. muris* cysts were optimally recovered at 12,000 x g for 10 minutes (48.0%), but differed substantially from the recovery of viable *G. lamblia* cysts (91.2%) at the same relative centrifugal force (RCF). Viable *G. muris* cysts were not pelleted at a RCF of 1,300 x g, resulting in 0% recoveries for all schemes examined (Table

1.1). Another notable difference in recoveries is seen between formalin inactivated *G. lamblia* cysts and viable *G. lamblia* cysts (Table 1.0). For each centrifugation scheme examined, viable *G. lamblia* cyst recoveries were between 88.2% and 100%, whereas formalin inactivated *G. lamblia* cyst recoveries ranged from 2.5% to 100%.

Chlorine vs. Chlorine Dioxide

The primary goal of this research is to compare chlorine and chlorine dioxide as disinfectants for surface waters. Inactivation rates of *Giardia muris* cysts and bacteriophage MS2 as a function of pH and temperature using chlorine and chlorine dioxide were determined for bench and pilot scale studies.

Table 2.0 indicates the average log reductions of *G. muris* cysts and MS2 by chlorine and chlorine dioxide at bench and pilot scales. The mean log reductions are always higher at the bench scale than at the pilot scale for both organisms.

Table 2.1 shows the CT values for *G. muris* and MS2 for both disinfectants at bench and pilot scales. At the bench scale, average CT values of chlorine dioxide with *G. muris* cysts (5.77) and MS2 (3.71) are much lower than the average CT values of chlorine with *G. muris* cysts (14.37) and MS2 (7.03). However, average log reductions are very similar for both disinfectants and both organisms. For chlorine dioxide, the average log reduction of *G.*

muris is 2.65 and of MS2 is 3.90. These values are not remarkably different than those of chlorine, which are 2.67 for *G. muris* cysts and 3.21 for MS2.

At the pilot scale, average CT values of chlorine dioxide with *G. muris* cysts (29.17) and MS2 (4.04) are similar to the average CT values of chlorine with *G. muris* cysts (24.29) and MS2 (4.62). However, the average log reductions are relatively higher with chlorine than with chlorine dioxide for both organisms. The average log reductions of *G. muris* (0.99) and MS2 (1.97) by chlorine dioxide are lower than those achieved with chlorine (1.20 for *G. muris* and 2.48 for MS2).

Chlorine vs. Chlorine Dioxide as a Function of pH

The average log reductions of *G. muris* and MS2 by chlorine and chlorine dioxide as a function of pH at bench and pilot studies are illustrated in Tables 3.0 and 4.0. Tables 5.0 - 7.0 are the results of ANOVA and post-hoc analysis (Scheffe's test) performed on the same data. Table 3.0 indicates that at the bench scale level, chlorine has a higher mean log reduction (of both organisms combined) at pH 6.0 than at pH values 7.0 and 9.0. Chlorine dioxide has a higher mean log reduction at the bench scale level at pH 9.0 than at 6.0 and 7.0. Pilot scale studies do not seem to follow the same trend as bench scale studies.

Table 4.0 indicates that at the bench scale, MS2 is more effectively inactivated by chlorine at pH 6.0 than at 7.0 and 9.0. The mean log reduction

of MS2 is higher with chlorine dioxide at pH 9.0 than at 6.0 and 7.0. *G. muris* cysts do not seem affected by pH at the bench scale as the mean log reductions do not follow the aforementioned trend. As mentioned above, pilot scale studies do not indicate that *G. muris* cysts and MS2 inactivation rates are influenced by pH.

Results from ANOVA and Scheffe's test for the log reduction of *G. muris* and MS2 by chlorine and chlorine dioxide as a function of pH appear in Tables 5.0 - 7.0. ANOVA Table 5.0 indicates that pH has a significant effect on the log reduction of MS2 by chlorine ($P<.0001$). Scheffe's Table 5.1 indicates that this significant difference is between pH values 6.0 and 7.0 ($P = .0012$) and also between pH values 6.0 and 9.0 ($P< .0001$). Tables 6.0 and 6.1 present results from ANOVA and Scheffe's test for the log reduction of MS2 by chlorine dioxide as a function of pH. As with the analysis of chlorine, pH has a significant effect on the log reduction of MS2 by chlorine dioxide ($P = .0006$). The difference in log reduction is significant between pH values 6.0 and 7.0 ($P = .03430$) and pH values 6.0 and 9.0 ($P = .0009$).

Chlorine vs. Chlorine Dioxide as a Function of Temperature

The average log reductions of *G. muris* and MS2 by chlorine and chlorine dioxide as a function of temperature at bench and pilot scale studies are shown in Tables 7.0 and 8.0. Table 7.0 indicates that at the bench scale level, higher log reductions are achieved by both disinfectants for both

organisms (combined) at 4.0°C and 10.0°C than at 24.0°C. However, at the pilot scale, chlorine appears more effective at 24.0°C and chlorine dioxide does not seem to be effected by temperature. Table 8.0 indicates that mean log reductions of both MS2 and *G. muris* cysts by chlorine are higher at 4.0°C and 10.0°C than at 24.0°C at the bench scale level. This same effect is seen with chlorine dioxide and *G. muris*, but not with MS2 at the bench scale. At the pilot scale, average log reductions for both organisms are higher at 24.0°C than at 4.0°C and 10°C with chlorine. Chlorine dioxide at the pilot scale indicates no effect of temperature on viral inactivation, but the log reduction of *G. muris* at 10°C is higher than at 4.0°C (no 24.0°C available).

Results from ANOVA and Scheffe's tests for the log reduction of MS2 by chlorine and chlorine dioxide as a function of temperature for bench scale studies **only** are presented in Tables 9.0 -11.0. For experiments with chlorine dioxide, significant differences in log reduction of MS2 are between 4.0°C and 10.0°C ($P = .0158$) and also between 10.0°C and 24.0°C ($P = .0140$) as indicated in Tables 9.0 and 9.1. For chlorine, Tables 10.0 and 10.1 demonstrate the significant differences are between 4.0°C and 24.0°C ($P < .0001$) and between 10.0°C and 24.0°C ($P = .0029$). No significant effects of temperature on the log reduction of *G. muris* cysts were indicated for either disinfectant at the bench scale.

ANOVA and Scheffe's test results for the log reduction of MS2 by chlorine and chlorine dioxide as a function of temperature for bench and

pilot studies combined are shown in Tables 11.0 - 13.0. For chlorine dioxide, results are similar to those generated for bench scale experiments only, as shown in Tables 11.0 and 11.1. More specifically, significant differences in log reduction of MS2 are between 4.0°C and 10.0°C (P = .0084) and also between 10.0°C and 24.0°C (P = .0281). Chlorine, however, indicates a significant difference in log reduction between 4.0°C and 24.0°C (P = .0003), but not for 10.0°C and 24.0°C as shown with bench scale experiments. No significant effects of temperature were seen with *G. muris* inactivation by chlorine or chlorine dioxide.

Bench vs. Pilot Scale Studies

Water treatment facilities are required to comply with the CT values provided by the USEPA in a series of tables in the SWTR *Guidance Manual for Compliance With the Filtration and Disinfection Requirements for Public Water Systems Using Surface Water Sources* (USEPA, 1991). These CT values are generated from disinfection studies performed at the bench scale level. Another goal of this research is to see if log reductions generated at the bench scale can be extrapolated to pilot scale studies.

Tables 13.0 - 16.1 show results from ANOVA and Scheffe's tests for the differences in bench and pilot scale studies in log reductions of *G. muris* cysts and MS2. As shown in Tables 13.0 - 15.0, significant differences were found between bench and pilot studies for inactivation of MS2 and *G. muris* cysts

using chlorine dioxide (P <.0001 and P = .0016, respectively). Although not significant at the 5% level, log reductions of MS2 and *G.muris* were different at the bench and pilot scale level with chlorine as indicated in Tables 15.0 - 16.1.

G. muris is More Resistant to Disinfection than MS2

It has been documented repeatedly that protozoan cysts are more resistant than viruses to adverse environments and disinfection. This conclusion has been based primarily on bench scale experiments. Another objective of this research is to establish how much more resistant *G. muris* cysts are than MS2 to chlorine and chlorine dioxide and to determine if this resistance is apparent at the field scale.

A comparison of average CT values and average log reductions for *G. muris* cysts and MS2 indicate that *G. muris* cysts are more resistant to disinfection than MS2 (Tables 2.0 and 2.1). Experiments at the bench scale with chlorine dioxide and *G. muris* cysts indicate that an average CT value of 5.77 yields an average log reduction of 2.67. With MS2 and chlorine dioxide, a relatively lower CT value of 3.71 results in a relatively higher log reduction of 3.21. Similar results occur with chlorine at the bench scale. An average CT value of 14.37 yields an average log reduction of 2.65 for *G. muris* cysts. For MS2, however, a relatively lower CT value of 7.03 achieves a relatively higher log reduction of 3.90.

Pilot scale studies also indicate that *G. muris* is more resistant than MS2. The average CT values of chlorine and chlorine dioxide combined for *G. muris* cysts (26.73) are higher than those for MS2 (4.33). However, the average log reductions for *G. muris* (1.10) are lower than those for MS2 (2.23).

Effects of Turbidity, Total and Fecal Coliforms or Heterotrophic Bacteria on Inactivation Rates

Two measures to determine source water quality include the coliform level and turbidity level. Both measures are required in the SWTR. The presence of particulate matter and bacteria associated with drinking water exert a chlorine demand, thereby decreasing the concentration of available chlorine to inactivate microorganisms. Maintaining low turbidity throughout the distribution system is essential to sustaining an adequate chlorine residual.

Turbidity of the water entering the pilot plant was measured during every pilot scale experimental run. Total and fecal coliform counts and heterotrophic plate counts were measured a minimum of two times during each season both prior to disinfection and following disinfection.

The turbidity of the water at the Concord Water Treatment Plant was relatively low, with the turbidity measurements ranging from 0.04 to 0.11 nephelometric turbidity units (NTUs). Total and fecal coliforms were also low, as they never exceeded 50/100 mL prior to disinfection and were always 0/100 mL following treatment. Similarly, heterotrophic plate counts were

routinely low, with the range between 10 - 608/mL and an average of 210/mL prior to disinfection.

An ANOVA and Scheffe's test were performed to determine if these measurements affected the log reduction of MS2 and *G. muris* cysts. All P-values indicated that there was no significant effect on the disinfection capabilities of chlorine and chlorine dioxide.

Table 1.0

Recoveries of Viable and Formalin Inactivated *G. lamblia* Cysts from Seeded Water Using Different Centrifugation Schemes

		Time of	Formalin inactivated *G. lamblia* cysts			Viable *G. lamblia* cysts		
RCF	Vol.	centrifugation	no. seeded	no. recovered	% recovery	no. seeded	no. recovered	% recovery
1300 x g	200 mLs	20 mins.	2.0×10^5/mL	5.0×10^3/mL	2.5 %	1.7×10^6/mL	2.1×10^6/mL	> 100%
1300 x g	200 mLs	30 mins.	2.0×10^5/mL	7.0×10^3/mL	3.5 %	1.7×10^6/mL	2.1×10^6 /mL	> 100%
1300 x g	50 mLs	12 mins.	2.0×10^5/mL	9.2×10^3/mL	4.6 %	1.7×10^6/mL	1.5×10^6 /mL	88.2 %
1300 x g	50 mLs	20 mins.	2.0×10^5/mL	9.0×10^3/mL	4.5 %	1.7×10^6/mL	1.5×10^6/mL	88.2 %
12,100 x g	30 mLs	10 mins.	2.0×10^5/mL	3.8×10^5/mL	> 100%	3.4×10^6/mL	3.1×10^6/mL	91.2 %

Table 1.1

Recoveries of Viable *G. muris* Cysts from Seeded Water Using Different Centrifugation Schemes.

RCF	Vol.	Time of centrifugation	Viable *G. muris* cysts no.seeded	no. recovered	% recovery
1300 x g	200 mLs	20 mins.	1.0 x 10^6/mL	0	0 %
1300 x g	200 mLs	30 mins.	1.0 x 10^6/mL	0	0 %
1300 x g	50 mLs	12 mins.	1.0 x 10^6/mL	0	0 %
1300 x g	50 mLs	20 mins.	1.0 x 10^6/mL	0	0 %
12,100 x g	30 mLs	10 mins.	1.0 x 10^6/mL	1.4 x 10^4/mL	1.4 %
12,100 x g	30 mLs	10 mins.	1.0 x 10^6/mL	4.8 x 10^5/mL	48.0 %

Table 2.0

Log Reduction of *Giardia muris* Cysts and MS2 by Cl_2 and ClO_2 at Bench and Pilot Scales

Log Reduction

Disinfectant	Organism	Scale	Mean	Std. Dev.	Std. Error	Count
Chlorine	*Giardia muris*	bench	2.67	1.64	.21	60
Chlorine	*Giardia muris*	pilot	1.20	1.50	.75	4
Chlorine	MS2	bench	3.21	2.56	.12	470
Chlorine	MS2	pilot	2.48	1.81	.30	36
Chlorine Dioxide	*Giardia muris*	bench	2.65	1.57	.16	93
Chlorine Dioxide	*Giardia muris*	pilot	.99	1.17	.37	10
Chlorine Dioxide	MS2	bench	3.90	2.93	.20	212
Chlorine Dioxide	MS2	pilot	1.97	1.78	.27	44

Table 2.1

CT Values of *G. muris* Cysts and MS2 for Cl_2 and ClO_2 at Bench and Pilot Scales

Disinfectant	Organism	Scale	Mean	Std. Dev.	Std. Error	Count
Chlorine	*G. muris*	Bench	14.37	11.76	1.52	60
Chlorine	*G. muris*	Pilot	24.29	28.10	14.05	4
Chlorine	MS2	Bench	7.03	12.85	.59	470
Chlorine	MS2	Pilot	4.62	6.51	1.09	36
Chlorine Dioxide	*G. muris*	Bench	5.77	7.25	.75	93
Chlorine Dioxide	*G. muris*	Pilot	29.17	33.48	10.59	10
Chlorine Dioxide	MS2	Bench	3.71	5.98	.41	212
Chlorine Dioxide	MS2	Pilot	4.04	5.00	.75	44

Table 3.0

Log Reduction of *G. muris* Cysts and MS2 by Cl_2 and ClO_2 as Function of pH at Bench and Pilot Scales

Log Reduction

Disinfectant	Scale	pH*	Mean	Std. Dev.	Std. Error	Count
Chlorine	bench	6.0	3.83	2.62	.20	181
Chlorine	pilot	6.0	2.26	1.85	.32	34
Chlorine	bench	7.0	2.97	2.24	.15	231
Chlorine	pilot	7.0	2.53	1.68	.59	8
Chlorine	bench	9.0	2.47	2.44	.23	118
Chlorine Dioxide	bench	6.0	3.11	2.51	.27	86
Chlorine Dioxide	pilot	6.0	1.78	1.73	.29	37
Chlorine Dioxide	bench	7.0	3.42	2.66	.24	119
Chlorine Dioxide	pilot	7.0	1.84	1.76	.46	15
Chlorine Dioxide	bench	9.0	3.98	2.72	.27	100
Chlorine Dioxide	pilot	9.0	1.49	2.11	1.5	2

*pH values at pilot scale
pH 6.0 = pH values ranging from 4.9 - 6.4
pH 7.0 = pH values ranging from 6.5 - 7.5
pH 9.0 = pH values ranging from 8.8 - 9.1

Table 4.0

Log Reduction of *G. muris* Cysts and MS2 by Cl_2 and ClO_2 as Function of pH at Bench and Pilot Scales

				Log Reduction			
Disinfectant	**Organism**	**Scale**	**pH**	**Mean**	**Std. Dev.**	**Std. Error**	**Count**
Chlorine	*G. muris*	Bench	6.0	2.38	1.41	.26	30
Chlorine	*G. muris*	Pilot	6.0	1.29	1.51	.61	6
Chlorine	*G. muris*	Bench	7.0	3.03	1.87	.48	15
Chlorine	*G. muris*	Bench	9.0	2.89	1.82	.47	15
Chlorine	MS2	Bench	6.0	4.12	2.72	.22	151
Chlorine	MS2	Pilot	6.0	2.47	1.87	.35	28
Chlorine	MS2	Bench	7.0	2.97	2.27	.15	216
Chlorine	MS2	Pilot	7.0	2.53	1.68	.59	8
Chlorine	MS2	Bench	9.0	2.41	2.52	.25	103
Chlorine Dioxide	*G. muris*	Bench	6.0	2.83	1.56	.29	30
Chlorine Dioxide	*G. muris*	Pilot	6.0	1.30	1.84	1.30	2
Chlorine Dioxide	*G. muris*	Bench	7.0	2.42	1.51	.24	40
Chlorine Dioxide	*G. muris*	Pilot	7.0	.72	.82	.33	6
Chlorine Dioxide	*G. muris*	Bench	9.0	2.81	1.70	.36	23
Chlorine Dioxide	*G. muris*	Pilot	9.0	1.49	2.11	1.49	2
Chlorine Dioxide	MS2	Bench	6.0	3.52	2.89	.39	56
Chlorine Dioxide	MS2	Pilot	6.0	1.81	1.75	.30	35
Chlorine Dioxide	MS2	Bench	7.0	3.93	2.97	.33	79
Chlorine Dioxide	MS2	Pilot	7.0	2.58	1.88	.63	9
Chlorine Dioxide	MS2	Bench	9.0	4.33	2.87	.33	77

Table 5.0

ANOVA Table for Log Reduction of MS2 by Cl_2 as Function of pH

	DF	Sum of Squares	Mean Square	F-Value	P-Value
pH	2	155.75	77.87	12.89	<.0001
Residual	503	3039.16	6.04		

Split By: Organism, Disinfectant type

Cell: **MS2, Cl_2**

Table 5.1

Scheffe Table for Log Reduction of MS2 by Cl_2 as Function of pH

pH Values	Mean Diff.	Crit. Diff.	P-Value	
6.0, 7.0	.91	.61	.0012	**S**
6.0, 9.0	1.45	.75	<.0001	**S**
7.0, 9.0	.54	.71	.1822	

Split By: Organism, Disinfectant type

Cell: **MS2, Cl_2**

Significance Level: 5%

Table 6.0

ANOVA Table for Log Reduction of MS2 by ClO_2 as Function of pH

	DF	Sum of Squares	Mean Square	F-Value	P-Value
pH	2	118.20	59.10	7.60	.0006
Residual	253	1967.06	7.78		

Split By: Organism, Disinfectant type

Cell: **MS2, ClO_2**

Table 6.1

Scheffe Table for Log Reduction of MS2 by ClO_2 as Function of pH

pH Values	Mean Diff.	Crit. Diff.	P-Value	
6.0, 7.0	-1.09	1.026	.0343	**S**
6.0, 9.0	-1.64	1.06	.0009	**S**
7.0, 9.0	-.55	1.07	.4573	

Split By: Organism, Disinfectant type

Cell: **MS2, ClO_2**

Significance Level: 5%

Table 7.0

Log Reduction of *G. muris* Cysts and MS2 by Cl_2 and ClO_2 as Function of Temperature at Bench and Pilot Scales

Log Reduction

Disinfectant	Scale	Temp. (°C) *	Mean	Std. Dev.	Std. Error	Count
Chlorine	Bench	4.0	3.83	2.85	.23	159
Chlorine	Pilot	4.0	1.84	1.41	.35	16
Chlorine	Bench	10.0	3.40	2.74	.27	102
Chlorine	Pilot	10.0	1.62	1.70	.60	8
Chlorine	Bench	24.0	2.66	1.98	.21	269
Chlorine	Pilot	24.0	3.03	1.97	.47	18
Chlorine Dioxide	Bench	4.0	3.40	2.59	.34	58
Chlorine Dioxide	Pilot	4.0	1.647	1.82	.38	23
Chlorine Dioxide	Bench	10.0	4.75	3.12	.48	43
Chlorine Dioxide	Pilot	10.0	2.14	1.83	.51	13
Chlorine Dioxide	Bench	24.0	3.29	2.50	.18	204
Chlorine Dioxide	Pilot	24.0	1.68	1.57	.37	18

* temperature values at pilot scale
4.0°C = 3.3°C - 6.5°C
10.0°C = 10.0°C - 15.0°C
24.0°C = 18.5°C - 28.5°C

Table 8.0

Log Reduction of *G. muris* Cysts and MS2 by Cl_2 and ClO_2 as Function of Temp. at Bench and Pilot Scales

Log Reduction

Disinfectant	Organism	Scale	Temp. (°C)	Mean	Std. Dev.	Std. Error	Count
Chlorine	*G. muris*	Bench	4.0	3.10	2.02	.52	931
Chlorine	*G. muris*	Pilot	4.0	.85	1.20	.85	2
Chlorine	*G. muris*	Bench	10.0	2.78	1.76	.43	17
Chlorine	*G. muris*	Pilot	10.0	1.48	2.09	1.48	2
Chlorine	*G. muris*	Bench	24.0	2.37	1.30	.25	28
Chlorine	*G. muris*	Pilot	24.0	1.55	2.19	1.55	2
Chlorine	MS2	Bench	4.0	3.90	2.91	.24	144
Chlorine	MS2	Pilot	4.0	1.94	1.42	.38	14
Chlorine	MS2	Bench	10.0	3.53	2.89	.31	85
Chlorine	MS2	Pilot	10.0	1.67	1.78	.73	6
Chlorine	MS2	Bench	24.0	2.69	2.04	.13	241
Chlorine	MS2	Pilot	24.0	3.22	1.94	.49	16
Chlorine Dioxide	*G. muris*	Bench	4.0	3.33	2.06	.57	13
Chlorine Dioxide	*G. muris*	Pilot	4.0	.88	1.90	.45	6
Chlorine Dioxide	*G. muris*	Bench	10.0	2.33	2.09	.74	8
Chlorine Dioxide	*G. muris*	Pilot	10.0	1.15	1.44	.72	4
Chlorine Dioxide	*G. muris*	Bench	24.0	2.56	1.40	.16	72
Chlorine Dioxide	MS2	Bench	4.0	3.42	2.74	.41	45
Chlorine Dioxide	MS2	Pilot	4.0	1.95	1.97	.48	17
Chlorine Dioxide	MS2	Bench	10.0	5.30	3.08	.52	35
Chlorine Dioxide	MS2	Pilot	10.0	2.58	1.88	.63	9
Chlorine Dioxide	MS2	Bench	24.0	3.69	2.87	.25	132
Chlorine Dioxide	MS2	Pilot	24.0	1.68	1.57	.37	18

Table 9.0

ANOVA Table for Log Reduction of MS2 by ClO2 as Function of Temperature

	DF	Sum of Squares	Mean Square	F-Value	P-Value
Temp. (°C)	2	85.19	42.60	5.15	.0065
Residual	209	1728.01	8.27		

Split By: Disinfectant type, Organism, Scale

Cell: **ClO_2, MS2, Bench Scale**

Table 9.1

Scheffe Table for Log Reduction of MS2 by ClO_2 as Function of Temperature

Temp. Values(°C)	Mean Diff.	Crit. Diff.	P-Value	
4.0, 10.0	-1.88	1.60	.0158	**S**
4.0, 24.0	-.27	1.22	.8619	
10.0, 24.0	1.61	1.35	.0140	**S**

Split By: Disinfectant type, Organism, Scale

Cell: **ClO_2, MS2, Bench Scale**

Significance Level: 5%

Table 10.0

ANOVA Table for Log Reduction of MS2 by Cl_2 as Function of Temperature

	DF	Sum of Squares	Mean Square	F-Value	P-Value
Temp. (°C)	2	144.28	72.14	11.55	<.0001
Residual	467	2918.20	6.249		

Split By: Disinfectant type, Organism, Scale

Cell: **Cl_2, MS2, Bench Scale**

Table 10.1

Scheffe Table for Log Reduction of MS2 by Cl_2 as Function of Temperature

Temp. Values (°C)	Mean Diff.	Crit. Diff.	P-Value	
4.0, 10.0	.38	.84	.5381	
4.0, 24.0	1.22	.65	<.0001	**S**
10.0, 24.0	.84	.77	.0299	**S**

Split By: Disinfectant type, Organism, Scale

Cell: **Cl_2, MS2, Bench Scale**

Significance Level: 5%

Table 11.0

ANOVA Table for Log Reduction of MS2 by ClO_2 as Function of Temperature

	DF	Sum of Squares	Mean Square	F-Value	P-Value
Temp. (°C)	2	82.20	41.10	5.19	.0062
Residual	253	2003.06	7.92		

Split By: Disinfectant type, Organism

Cell: **ClO_2, MS2**

Table 11.1

Scheffe Table for Log Reduction of MS2 by ClO_2 as Function of Temperature

Temp. Values (°C)	Mean Diff.	Crit. Diff.	P-Value	
4.0, 10.0	-1.73	1.37	.0084	S
4.0, 24.0	-.43	1.05	.5955	
10.0, 24.0	1.30	1.19	.0281	S

Split By: Disinfectant type, Organism

Cell: **ClO_2, MS2**

Significance Level: 5%

Table 12.0

ANOVA Table for Log Reduction of MS2 by Cl_2 as Function of Temperature

	DF	Sum of Squares	Mean Square	F-Value	P-Value
Temp. (°C)	2	107.60	53.80	8.77	.0002
Residual	503	3087.31	6.14		

Split By: Disinfectant type, Organism

Cell: **Cl_2, MS2**

Table 12.1

Scheffe Table for Log Reduction of MS2 by Cl_2 as Function of Temperature

Temp. Values (°C)	Mean Diff.	Crit. Diff.	P-Value	
4.0, 10.0	.33	.80	.5941	
4.0, 24.0	1.02	.62	.0003	S
10.0, 24.0	.68	.74	.0786	

Split By: Disinfectant type

Cell: **Cl_2, MS2**

Significance Level: 5%

Table 13.0

ANOVA Table for Log Reduction of *G. muris* Cysts by ClO_2 - Bench vs. Pilot Scale

	DF	Sum of Squares	Mean Square	F-Value	P-Value
Scale	1	24.91	24.91	10.52	.0016
Residual	101	239.17	2.37		

Split By: Disinfectant type, Organism

Cell: **ClO_2, *G. muris***

Table 13.1

Scheffe Table for Log Reduction of *G. muris* Cysts by ClO_2 - Bench vs. Pilot Scale

Scale	Mean Diff.	Crit. Diff.	P-Value	
bench, pilot	1.66	1.02	.0016	**S**

Split By: Disinfectant type, Organism

Cell: **ClO_2, *G. muris***

Significance Level: 5%

Table 14.0

ANOVA Table for Log Reduction of MS2 by ClO_2 - Bench vs. Pilot Scale

	DF	Sum of Squares	Mean Square	F-Value	P-Value
Scale	1	135.19	135.19	17.61	<.0001
Residual	254	1950.07	7.68		

Split By: Disinfectant type, Organism

Cell: **ClO_2, MS2**

Table 14.1

Scheffe Table for Log Reduction of MS2 by ClO_2 - Bench vs. Pilot Scale

Scale	Mean Diff.	Crit. Diff.	P-Value	
bench, pilot	1.93	.90	<.0001	**S**

Split By: Disinfectant type, Organism

Cell: **ClO_2, MS2**

Significance Level: 5%

Table 15.0

ANOVA Table for Log Reduction of *G. muris* Cysts by Cl_2 - Bench vs. Pilot Scale

	DF	Sum of Squares	Mean Square	F-Value	P-Value
Scale	1	10.35	10.35	3.91	.0523
Residual	64	169.33	2.65		

Split By: Disinfectant type, Organism

Cell: Cl_2, *G. muris*

Table 15.1

Scheffe Table for Log Reduction of *G. muris* Cysts by Cl_2 - Bench vs. Pilot Scale

Scale	Mean Diff.	Crit. Diff.	P-Value
bench, pilot	1.38	1.39	.0523

Split By: Disinfectant type, Organism

Cell: Cl_2, *G. muris*

Significance Level: 5%

Table 16.0

ANOVA Table for Log Reduction of MS2 by Cl_2 - Bench vs. Pilot Scale

	DF	Sum of Squares	Mean Square	F-Value	P-Value
Scale	1	17.96	17.96	2.85	.0921
Residual	504	3176.95	6.30		

Split By: Disinfectant type, Organism

Cell: **Cl_2, MS2**

Table 16.1

Scheffe Table for Log Reduction of MS2 by Cl_2 - Bench vs. Pilot Scale

Scale	Mean Diff.	Crit. Diff.	P-Value
bench, pilot	.73	.85	.0921

Split By: Disinfectant type, Organism

Cell: **Cl_2, MS2**

Significance Level: 5%

CHAPTER IV

Discussion

Most of America's water is clean enough for wildlife, agriculture, recreation, and is acceptable for drinking purposes if adequately treated. Most people take clean water for granted. Increasingly, however, America's water is threatened by pollution from industrial, domestic, and agricultural sources. The quality of the nation's drinking water is compromised by excessive amounts of carcinogens, pesticides, heavy metals and microbes that society contributes on a daily basis. Our water is responsible for innumerable cases of gastrointestinal illness, lead poisoning, cancer, and for damage to wildlife.

The Clean Water Act of 1972 and the Safe Drinking Water Act of 1974 were passed to ensure that America's water is safe for drinking, fishing, recreation, and wildlife. The implementation of a national program has undoubtedly improved the quality of the nation's water. Federal dollars dispersed to state and local agencies has significantly impacted the goal to protect and clean up the waterways. Discharges from point sources of pollution - especially from industrial facilities - have decreased considerably. But those concerned with clean water have become focused on other sources

of water pollution. Non-point sources such as runoff from streets and roadways, runoff of pesticides from our lawns as well as agricultural areas, are receiving more attention. This problem is further accentuated with the rapidly increasing population. These sources of pollution are more difficult to control because it means changing the attitude of people. Society needs to be better informed of the conditions of our source waters and understand that actions of individual members of society have tremendous impact on the welfare of this critical natural resource.

In addition to non-point source pollution, the national agenda should include the development and implementation of programs to protect and enhance our groundwater; prevention of toxic pollutants to our waterways; certainty of sufficient financial and professional resources to sustain and improve treatment facilities, and public awareness of the importance of personal efforts to protect our water sources.

The overall condition of our lakes, rivers, and coastal waters have improved since the passage of federal regulations that mandate water standards. These standards are continually being evaluated, reassessed, amended and enforced. Unfortunately, federal financial assistance has not also been made a priority. Federal grants are no longer available and the only financial aid attainable is from a deficient loan program. How then do drinking water facilities and sewage treatment plants upgrade their infrastructures to comply with the mandatory standards set by the

government's agencies? More than likely, these upgrades will be funded by homeowners and businesses in addition to state and local tax revenues. The cost of clean water in large urban areas is astronomical and compliance is exceedingly difficult. However, without financial assistance from federal or state programs, small communities will be the hardest hit. Some suppliers may not be able to continue functioning. Consequently, the only source of drinking water may come from unregulated private wells and/or bottled water.

The absence of financial aid threatens the renewal of regulations and standards that have protected the nation's water resources for decades. The idea of weakening standards has emerged from fiscal pressures, not from public health and environmental concerns. Relinquishing the investment of protecting and improving our water will not only undo the accomplishments of years of regulation, but will undoubtedly lead to potentially disastrous effects on the environment and public health. Unfortunately, a major public health problem is inevitable if we abandon the committment to clean water. The occurrence of a waterborne outbreak like the Milwaukee *Cryptosporidium* outbreak of 1993 in which over 400,000 became ill after exposure to *C. parvum* through the public water supply indicates that our current standards are not adequate to prevent transmission of infectious agents (Gradus et al., 1994). Intensive efforts and cooperation between federal, state, and local agencies, drinking water utilities, the medical community, and

consumers is required to ensure the safety of drinking water for all citizens.

The need for the evaluation of current treatment practices for the removal of pathogenic microorganisms has become increasingly important with the occurrence of *Giardia* and *Cryptosporidium* outbreaks. The increased incidence of other pathogenic microbes in source waters has also contributed to the concern about the effectiveness of drinking water treatment technologies. Despite existing regulations, waterborne outbreaks continue to occur. Outbreaks have occurred in systems that have been in compliance with current regulations.

There is a considerable degree of uncertainty and contradiction regarding the efficiency of chlorine and other disinfectants. This is due largely to the lack of standardization in disinfection studies and because of varying quality of source waters that influence microbial disinfection of water. Consequently, there is widespread concern about the public health status and the most commonly used drinking water treatment technologies. Understanding the main factors that influence the microbial disinfection of water is fundamental to the interpretation of results generated from disinfection studies.

Factors Influencing Microbial Disinfection of Water

Type of Microbe

A wide variety of human pathogens may be found in the excrement

from humans and animals. These microbes differ in their sensitivity to adverse environmental stresses and disinfection. In general, the order of sensitivity to both disinfection and environmental stresses is: vegetative bacteria > viruses > acid-fast bacteria, protozoan cysts, and bacterial spores (Sobsey, 1989). Even among isolates of the same species of bacteria, there can be differences in sensitivity to disinfection. For example, Ward et al. (1984) reported 6-fold differences in sensitivity to combined chlorine among 6 isolates of *E. coli*. Laboratory strains of viruses have been shown to be more sensitive to disinfection than isolates recovered from chlorinated drinking water (Shaffer, 1980). Prior exposure to a disinfectant may result in the selection of resistant survivors. Microorganisms that survive initial exposure of a disinfectant may be modified by the chemical (mutational change in one or several genes) so that further exposure fails to kill them. Differences in disinfection resistance of various microorganisms are important with regard to the use of our current bacterial indicator system of fecal pollution.

Physiological State of the Microorganism

The physiological state of a microbe influences its response to disinfection and its ability to be detected and quantified. Pathogens as well as indicator organisms can be injured from aquatic stress factors and prior exposure to disinfectants, resulting in sublethal cellular lesions and alterations in their physiology (Singh and McFeters, 1990). For example, Berg

et al. (1982) showed that *E. coli* was more sensitive to disinfection by chlorine dioxide when grown at lower temperatures and reduced growth rates. Studies with injured coliforms indicated an immediate increase in intracellular ATP concentration, aerobic respiration, glucose transport and utilization, as well as alteration of other activities related to the cytoplasmic membrane (Singh and McFeters, 1990).

The method or condition used for assaying the viability of microorganisms is an important factor in detection and quantification. Bacteria that have been exposed to a disinfectant (Sobsey, 1989) or to simulated natural aquatic environments (Singh and McFeters, 1990) may be unable to grow on standard media, but may still be viable and virulent. This may occur if a disinfectant damages a microorganism (a bacteriostatic situation), but not to the point where the organism is lysed or denatured. This condition, "viable but nonculturable", is a notable phenomenon to take into consideration when assessing the response of disinfection by using standard viability assays. The actual number of viable microbes may be underestimated and a true public health hazard may go undetected. This poses an even bigger danger with subpopulations that are at higher risk of infection such as the immunocompromised and the elderly.

Microbial Aggregation and Attachment to Surfaces

Aggregation or clumping is typical of waterborne microbes. Aggregation greatly reduces the efficiency of disinfection because the microbes

inside the aggregates are protected from exposure to the disinfectant. A study by Chen et al. (1985) reported that the inactivation of *Naegleria gruberi* cysts by chlorine dioxide was reduced by 65-88% because of cyst clumping. Similarly, attachment to or colonization of surfaces such as pipe walls protect bacteria from inactivation by disinfectants (Sobsey, 1989). This is attributed to stearic hindrance and increased disinfectant demand, both of which are protective properties of biofilm formation.

Water Quality

Water quality factors that significantly influence disinfection efficiency include particulates (suspended solids; turbidity), dissolved organic matter, pH, and temperature. Others factor such as inorganic compounds and reactor design also affect disinfection, but only those factors that were directly measured in this project will be discussed.

Suspended solids can have an adverse effect on disinfection in two ways. Particles, such as fecal material, can protect microbes from disinfection by the formation of aggregates surrounding the microbes. Secondly, particles can react with and consume the disinfectant, thereby decreasing the concentration of disinfectant available to inactivate the microorganism(s).

Dissolved organics can similarly react with and consume the disinfectant, reducing the concentration of disinfectant available to react with microbes. Alternatively, the association between the organics and disinfectant can result in the formation of reaction products having weak or

no microbiocidal activity. For example, it has been well documented that the reaction of chlorine with organic amines to form organic chloramines results in reduced microbial inactivation (Lawrence and Block, 1968).

Hydrogen ion concentration (pH) greatly influences microbial inactivation, especially for oxidizing agents such as chlorine, chlorine dioxide, and chloramines. Inactivation of bacteria, viruses, and protozoan cysts by chlorine is increased at lower pH levels (4.5-6.0) where HOCl predominates. Chlorine dioxide, however, appears to be more effective at higher pH levels (8.5-10.0). Microbial inactivation by chloramines also appears to be pH-dependent. Most studies indicate that chloramines at pH 6.0-7.0 are more microbicidal than at pH 8.0-10.0, but there are some that report that high pH levels are more effective. (Sobsey, 1989).

The influence of temperature on microbial disinfection kinetics can be more easily explained. In general, increased temperature produces increased rates of inactivation. More specifically, inactivation rates of microorganisms are dependent on the activation energy of a given disinfectant. The larger the value of the activation energy, the more sensitive is the rate of the reaction to change in temperature (Lawrence and Block, 1968). For example, if a disinfectant were to be used at temperatures considerably below room temperature, an agent having a relatively low inactivation energy (and therefore relatively independent of changes in temperature) would be more effective. The extent to which temperature affects inactivation rates is still

questionable.

Disinfection Studies

Optimization of *Giardia* Cyst Recoveries

Despite the increased concern in monitoring and regulating *Giardia*, very little research has been done to determine recovery efficiencies of the existing methods to detect *Giardia* cysts in water. A study conducted by the Water Quality Department of the Erie County Water Authority, Erie, NY., which compared the abilities of laboratories to recover known concentrations of cysts from cartridge filters, reported recovery rates between 4.5% to 14.2% (Clancy, 1994). The American Society of Testing Materials (ASTM) method, which is considered to be the method of choice, was used in the Erie County study. The low recoveries from the Erie County study suggest the need for improved methods for recovery and enumeration of these pathogens from our environment.

Experimental trials at the pilot plant required optimization of recovery techniques of *G. muris* cysts. Initial trials were performed with both formalin inactivated *G. lamblia* cysts (less expensive) and with viable *G. muris* cysts. These trials yielded good recovery of *G. lamblia* cysts (80.0-90.0%), but poor recovery of *G. muris* cysts (2.0 - 10.0%). Experiments to determine the cause of poor *G. muris* cyst recovery from samples at the pilot plant indicated that the cysts were not sedimenting completely during centrifugation. *Standard*

Methods for the Examination of Water and Wastewater and ASTM suggest centrifuging at 1050 x g (or less), but this relative centrifugal force (RCF) was not sufficient to pellet the samples completely. Consequently, experiments were performed to determine optimal cyst recovery from samples.

The recoveries of *G. lamblia* and *G. muris* cysts following different centrifugation schemes suggest that there is a difference in sedimentation rates between the two species. Results indicate consistently better recovery of *G. lamblia* cysts (both formalin inactivated and viable) than *G. muris* cysts for every scheme tested. Formalin treating cysts also seems to affect the sedimentation rate of *G. lamblia* cysts. The results of these experiments may provide a partial explanation of the low recovery rates and subsequent detection of *Giardia* cysts reported in the literature. There is an obvious need to modify the currently practiced techniques to increase the sensitivity of these assays.

Disinfection research which uses *G. muris* to model *G. lamblia* (due to cost and safety), must use caution when assuming that both species behave in a similar fashion. Without the use of careful controls, conclusions derived from *G. muris* data may not accurately reflect the fate of *G. lamblia.*

Chlorine vs. Chlorine Dioxide - Which is More Effective?

The most commonly used disinfectant throughout the United States is chlorine. It is the most cost-effective means of treatment to provide potable

water (Olivieri, 1986). Chlorine, however, has several major drawbacks. Viruses and protozoan cysts which are more resistant to disinfection than bacteria are not always susceptible to the typical levels of chlorine recommended for drinking water (Leahy et al., 1987). Secondly, the interaction of free chlorine with certain precursors found in drinking water results in the formation of carcinogenic by-products, such as trihalomethanes (THMs) (Craun, 1988). These shortcomings have spurred the need to evaluate alternative disinfectants. Chlorine dioxide appears to be a viable option that offers several advantages. It does not formTHMs, it has more than twice the oxidizing capability of chlorine, and it is easy to maintain a residual throughout the distribution system. As a result of these and other shortcomings of chlorination, the need to compare chlorine and chlorine dioxide as disinfectants for surface waters became apparent.

A comparison of average CT values and average log reductions indicate chlorine dioxide is a better disinfectant than chlorine at the bench scale. Average CT values of chlorine dioxide with *G. muris* cysts and MS2 are much lower than the average CT values of chlorine with *G. muris* cysts and MS2. However, average log reductions for both organisms are higher with chlorine dioxide than with chlorine. At the pilot scale, however, chlorine dioxide does not appear to be more effective than chlorine. For *G. muris* cysts, the average CT value for chlorine dioxide is higher than that of chlorine, yet the average log reduction is lower for chlorine dioxide than it is

for chlorine. This indicates that chlorine is more cysticidal than chlorine dioxide at the pilot scale. For MS2, however, there is not a difference between the two disinfectants at the pilot scale. The average CT values and log reductions of MS2 by chlorine and chlorine dioxide are notably similar.

Caution should be taken in interpreting the statistical analysis of pilot data generated from this study. It is important to be aware of the significant variation in the pilot results due to factors that are mentioned throughout the discussion. The statistical power of analysis is further limited by the number of data points obtained.

Despite the aforementioned limitations, chlorine dioxide does appear to exhibit strong disinfecting capabilities and should be considered as a viable alternative to chlorine. Further studies need to be performed to determine its effectiveness against other microorganisms and the potential of producing harmful byproducts following its application.

Cl_2 vs. ClO_2 as a Function of pH

Disinfection efficacies as a function of pH and temperature were also determined. Results from bench scale studies indicate that chlorine is more effective against MS2 at a lower pH value such as pH 6.0 than at pH values of 7.0 and 9.0. At pH 6.0, hypochlorous acid is the dominant species of chlorine. The superiority of hypochlorous acid over hypochlorite ion is attibuted to hypochlorous acid's enhanced ability to associate with microorganisms

(Harakeh, 1984). It is the most active species of chlorine.

Chlorine dioxide, however, appears to be more effective against MS2 at pH 9.0 rather than at pH values 6.0 and 7.0. These results are consistent with data generated from Noss and Olivieri (1985) who demonstrated that inactivation of f2 bacteriophage by chlorine dioxide increased as pH increased. The increased inactivation of MS2 at pH 9.0 is probably not the result of the disproportionation products such as chlorite or chlorate because the amount of chlorite and chlorate formed at high pH values is less than 5%. Increased inactivation of MS2 at pH 9.0 may be due to chemical and/or structural changes in MS2 that increase the reactivity of chlorine dioxide with the virus at elevated pH values. Another possibility is that hydroxyl ions are necessary for the reaction to occur, resulting in a lower rate of disinfection as the pH decreases (Katz, 1980).

For both disinfectants at the bench scale, *G. muris* cyst log reductions are not significantly different between any of the pH values tested. This is undoubtedly related to the physiological modes of inactivation of *G. muris* cysts by the two disinfectants and the remarkable resistance of the cysts. To date, the mechanisms of inactivation are unknown, however they may be similar to bacteria and viruses. Both chlorine and chlorine dioxide have been shown to inhibit certain enzyme systems and to damage the permeability of bacterial walls. For viruses, the disinfectants react with tyrosine residues on the capsid protein, resulting in the inhibition of viral adsorption to host

bacteria. They also target viral nucleic acid. The mechanism of *G. muris* cyst inactivation is probably also related to the reaction of the disinfectants with the cyst wall and their ability to gain access across the cyst wall.

The cyst wall functions as a selective barrier to the movement or diffusion of molecules found in the environment. The cyst wall consists of two components: an inner and outer cyst wall. The inner wall is approximately 0.25 um thick and contains 2 membranes. The outer cyst wall is a tightly packed network of thin filaments (7-20 nm in diameter) measuring 5-9 nm in thickness (Adam, 1991). The composition of these walls has not been well studied, but evidence of galactosamine and chitin have been presented. Studies have demonstrated that the tightly interwoven cyst wall filaments disassociate themselves from one another upon induction of excystation. The stimuli to induce excystation include low pH (2.0-3.0) which mimics the passage of ingested cysts through the stomach, and exposure of the cysts to proteases such as trypsin or chymotrysin which mimics the passage of cysts into the lower duodenum. Based on the fact that cysts are not adversely affected by the acidic pH of the stomach, there is little reason to believe that disinfection experiments with pH values between 6.0 and 9.0 would negatively affect the cysts. If however, chlorine or chlorine dioxide were to pass through the thick filamentous walls of the cysts, the disinfectants could then target the nucleic acid and metabolic processes, resulting in lysis and death of the cysts. These mechanisms would occur, regardless of the pH

of the disinfectants.

Results of this study indicate that *G. muris* cyst log reductions are not significantly affected by pH for both chlorine and chlorine dioxide. The mechanism by which these disinfectants react with the components of the cyst walls needs to be elucidated before the affect of pH can be fully explained. Perhaps more extreme pH values would indicate an affect of pH on the viability of *G. muris* cysts.

Results from pilot scale trials indicate that inactivation rates of *G. muris* and MS2 are not significantly affected by pH for both chlorine and chlorine dioxide. This is most likely related to the experimental conditions. There are several factors at the field scale that affect the efficacy of the disinfectant and the physiological state of the microorganisms that are not present in laboratory, bench scale experiments. Water quality factors such as dissoved organic matter and suspended solids decrease the effectiveness of disinfectants by consuming the disinfectant and by forming aggregates surrounding the microbes which protect them from the disinfectant. The physiological state of the microorganisms may also influence their response to disinfection. This response may be different at the field scale level, resulting in alterations of membranes or proteins necessary for inactivation. Therefore, the effect of pH at the field scale level may be diminished as a result of the numerous other factors that effect disinfection efficacy.

Cl_2 vs. ClO_2 as a Function of Temperature

Statistical analysis of the log reductions of *G. muris* and MS2 by chlorine and chlorine dioxide as a function of temperature at the bench scale indicate that higher inactivation rates occur at lower temperatures. More specifically, the mean log reductions of both organisms by chlorine are higher at 4.0°C and 10.0°C than at 24.0°C. Based on microbial disinfection kinetics, increased temperatures should produce increased rates of inactivation. These results, however, contradict this general rule. Upon examination of the raw data, the reason for the higher log reductions at the lower temperatures became evident. In performing the bench scale experiments with MS2 and chlorine at 24.0°C (room temperature), samples were taken at 0.5, 1.0, 1.5, and 2.0 minute time intervals. The experiments at 10.0°C and 4.0°C were usually run with more time intervals and with longer contact times. This was done to ensure a 4 log reduction of MS2 which was anticipated to occur at a slower rate due to the decrease in temperature. The longer contact times resulted in viral inactivation significantly higher than 4-logs (ie: 6 or 7 logs). As a result of more samples and longer contact times, the average of the log reductions at the lower temperatures are higher than at room temperature. The same trend of higher log reduction at lower temperatures is seen with *G. muris* cysts, but the differences between temperatures do not result in a significant P-value.

Mean log reductions of MS2 by chlorine dioxide also indicate

higher inactivation at 4.0°C and 10.0°C than at 24.0°C. However, the significant differences are seen between 4.0°C and 10°C and between 10.0°C and 24.0°C. The descriptive statistics shown in Table 7.0 indicate that the average log reductions of MS2 by chlorine dioxide for bench scale studies are highest at 10.0°C (5.30), followed by 24.0°C (3.69), and lowest at 4.0°C (3.42). Once again, a closer look at the raw data provides an explanation for the reasons behind the contadictory and significant P-values. The majority of the experimental runs at 4.0°C were done with sample time points at 1, 2, 5, and 10 minutes, although a few had longer contact times at 20 and 30 minutes. As a result, the log reductions that occurred in the first few minutes were usually less than 3.5 logs. This accounts for the relatively low average of log reduction at 4.0°C. The same time points were chosen for the experiments conducted at 24.0°C, but high log reductions ($\geq$ 4.0 logs) occurred in the first few minutes and total kill (7.0 to 8 logs) achieved after these first few minutes. This explains the reason why the average log reductions at 24.0°C are relatively high. The highest average log reductions of MS2 are calulated for experiments at 10.0°C. This, too, can be expained by the time points that were chosen to achieve a 4-log reduction. Considering the low log reductions achieved in the first few minutes at 4.0°C, these time points were omitted and longer contact times (5, 10, 20, 30) were selected. These longer contact times were sufficient to achieve a minimum reduction of 3 logs at the first time point, yielding overall higher log reductions. Had earlier time points been

included in the series of 10.0°C experiments with chlorine dioxide and MS2, the typical trend of higher inactivation rates at higher temperatures would be evident.

Experiments performed at the pilot plant indicate that inactivation rates are higher at higher temperatures throughout the year. At the pilot scale, average log reductions by chlorine for both organisms are highest at 24.0°C (summer runs) than at the lower temperatures, indicating that temperature affects inactivation rates. The log reduction of *G. muris* is higher at 10.0°C than at 4.0°C (no 24°C available) for trial runs at the pilot plant with chlorine dioxide. However, the summer run with MS2 and chlorine dioxide does not show the same effect. This result may be due to the effect of pH on the effectiveness of chlorine dioxide. Chlorine dioxide is more biocidal at high pH values. This particular experimental run was performed at pH 4.9, the natural pH of the source water on the day of the experiment. Additional pilot studies need to done to determine if temperature has a significant effect of inactivation rates of microorganisms in the environment.

Bench vs. Pilot Scale Studies

The majority of disinfection studies are conducted at the bench scale level. This design allows for the careful control of experimental factors and decreases the variability both among and between treatment groups. While useful, results generated from laboratory experiments do not necessarily correspond with those yielded from field scale conditions (discussed in

introduction). This study provides support that bench scale results can not be extrapolated to pilot scale studies.

Results indicate that higher log reductions are achieved at the bench scale level than at the pilot scale level for both organisms and both disinfectants (Table 1.0). Statistical analysis indicates that there is a significant difference between bench and pilot scale studies for the log reduction of both *Giardia muris* and MS2 by chlorine dioxide (Tables 12.0 - 13.1). The same statistical analysis using chlorine and both microorganisms does not illustrate a significant difference between bench and pilot scale studies, but the P-values strongly suggest that these experimental conditions are divergent (Tables 14.0 - 15.1).

Log reductions at the bench and pilot scale are significantly different for chlorine dioxide, but not for chlorine. Possible contributing causes need consideration. Experiments with chlorine dioxide at the pilot plant relied on the generation of chlorine dioxide on site. The chlorine dioxide was produced by combining hydrochloric acid and sodium chlorite in a reactor vessel. Immediate mixing was essential to obtain a good yield (less than 5% exces chlorine) of chlorine dioxide. Chlorine dioxide was generated at the bench scale level by adding acetic anhydride to a solution of sodium chlorite. While both processes to generate chlorine dioxide are favored, perhaps they are sufficiently different in terms of yield. Alternatively, perhaps the chlorine dioxide generated at the pilot plant was not as stable as the chlorine

dioxide produced at the bench scale. Stability pertains to the lack of reactivity with constituents other than microorganisms and is an estimate of persistence in the treated system. An additional contributing factors may have been the presence of interfering compounds that can alter the microbicidal activity of chlorine dioxide.

G. muris is More Resistant to Disinfection than MS2

Results from bench and pilot experiments done in this study indicate that the average log reductions of MS2 are higher than those of *G. muris* cysts for both chlorine and chlorine dioxide. This supports previously generated data that protozoan cysts are more resistant to disinfection than viruses (Jarroll, 1981). This greater resistance is attributed to the outer and inner cyst wall that protects the cyst from the environment.

It has been documented repeatedly that protozoan cysts are more resistant to environmental stresses and disinfection than viruses. Results from this research demonstrate that *G. muris* cysts are more resistant to chlorine and chlorine dioxide than MS2 bacteriophage. The average CT value of *G. muris* with chlorine at the bench and pilot scale is about 3 times higher than that of MS2, yet there is about a log difference between the two organisms. This indicates that *G. muris* is approximatesly 30 times more resistant than MS2 to chlorine disinfection (Table 1.1). Using the same rationale, experiments with chlorine dioxide indicate that *G. muris* cysts are

approximately 45 times more resistant than MS2 to chlorine dioxide disinfection (Table 1.1).

Does Turbidity, Total and Fecal Coliforms, and Heterotrophic Bacteria Affect Inactivation?

Source water quality is determined by several factors including turbidity, coliform levels, and heterotrophic bacteria levels. All of these factors can exert a chlorine demand and can decrease the effectiveness of the disinfectant (Sobsey, 1989). Treatment facilities are required to monitor these measurements periodically throughout the day or week. Turbidity, an indirect measurement of the particulate matter in water, is a crucial quality control practice. For instance, prior to the *Cryptosporidium* outbreak in Milwaukee, Wisconsin, there was a marked increase in the turbidity of treated water at one of the city's water treatment plants. It was later discovered that the difficulty in maintaining low turbidity was most likely due to difficulties in determining the appropriate amounts of polyaluminim chloride and alum during the coagulation process. As a result, the treatment plant has installed continuous turbidity monitors on each filter bed.

One objective of this project was to determine if these factors affect the log reductions of *G. muris* and MS2 at the pilot scale. The quality of the water at the Concord Water Treatment Facility is exceptionally good as indicated by low turbidity levels, low total and fecal coliform counts, and low

heterotrophic plate counts. Statistical analyses demonstrated that none of the factors affected the disinfectants' ability to inactivate *G. muris* cysts and MS2. It would be useful to repeat this study with source water of relatively poor quality to determine if these factors significantly effect the disinfection capabilities of chlorine and chlorine dioxide under those conditions.

Problems Encountered in Performing Pilot Plant Studies

Research from this study indicates that disinfection results obtained in laboratory studies are not suitable for predicting performance under field conditions. Factors that limit the usefulness of laboratory-derived studies have been previously mentioned. Difficulties and impracticalities encountered at the pilot treatment plant in Concord, NH have provided valuable information on problems such as batch-to-batch variation of *Giardia* suspensions, the lack of funcdamental data for *Giardia*, difficulties in achieving a full 3 log reduction, and the inadequacy of CT values. These problems warrant consideration in conducting field research.

Batch-to-Batch Variability of *Giardia* Suspensions

The relative resistance and susceptibility of cysts to adverse environments and disinfection processes have implications for drinking water regulations. Results from this work indicate that there was variation between experimental trials due to the change in the "health" of the *Giardia*

cysts. The cysts used for all experimental trials were obtained from the same supplier. However, there were several trials at the pilot plant that were cancelled because of unacceptably low viability of *G. muris* cyst samples. Any suspensions that were below 80% viable were not used in any experimentation as they may have been more susceptible to environmental stresses and injury than "healthy" cysts. This was one measure taken to decrease sample to sample variation. Several pilot runs generated no usable data because the viability of the cysts recovered from the control filter had decreased below the acceptable limit (80%) by the time of sample analysis (within 72 hours).

In one other instance, a pilot run with chlorine dioxide yielded very unusual results. During the enumeration of the disinfected sample and prior to excystation, instead of cysts making up the majority of the sample, most of the sample was trophozoites. Trophozoites are typically fragile in nature and are not known to thrive in the environment. These results were surprising due to the fact that the sample had not been exposed to any condition that would induce excystation and the chlorine dioxide dosage was relatively high (1.0 mg/L). Based on the fragile nature of trophozoites and the level of chlorine dioxide, it seems unlikely that the excystation occurred before or during the experimental run. This suggests that excystation occurred while on the filter or during sample analysis. However, there was no deviation in performing any steps of the procedure nor did this reoccur in any of the trials.

While this unusual experimental run was not successful in generating analyzable data, it highlights two important points that need consideration. Firstly, it emphasizes the lack of fundamental information that is available on the how *G. muris* cysts behave in the environment and to disinfection, and how laboratory manipulations can affect the viability of this organism. Much still remains to be learned about the nature of *G. muris* and related species. Secondly, it may help explain why waterborne disease outbreaks still occur despite the treatment methods that are currently in practice. The variability of *G. muris* cyst resistance lends support to the question of whether or not the current regulations are providing potable water that is safe enough to drink.

The integrity of the cysts relies on methods used to propagate relatively high concentrations of cysts in the laboratory, which leads to ask the question "how much variation in resistance/susceptibility of cysts exists naturally in the environment?" Several studies have demonstrated variability in surface proteins among *G. lamblia* isolates. Meng et al. (1993) attributed morphological and functional differences of cysts to antigenic shifts occurring after the completion of encystation and before subsequent regrowth after excystation *in vitro*. Udezulu et al. (1992) also confirmed antigenic heterogeneity among *G. lamblia* trophozoites. These clones indicated differences in membrane proteins, antigenic profiles, infectivity, and virulence. These differences in isolates appear to enhance the survival of the

trophozoites in the small intestine, therefore affecting their ability to successfully cause symptoms associated with giardiasis. The degree of diversity among isolates undoubtedly alters the basic host-parasite relationship, but further research is necessary to elucidate how this variability may provide resistance to currently used treatment processes. This information may be useful to regulatory agencies which are assessing the effectiveness of disinfection methods and also in determining the risk from public health hazards like giardiasis.

Despite the increased concern in monitoring and regulating this parasite, little research has been done to evaluate the hardiness or health of *Giardia* cyst populations. Several studies have generated data on the methods available for determining the viability of *Giardia* cysts including dye exclusion, excystation *in vitro*, and animal models for infectivity. However, these techniques do not indicate the resistance or susceptibility of the cysts to disinfection or environmental stresses. Precautions were taken to minimize batch-to-batch variation in this research project, yet there were still differences in viability between cyst suspensions. Some samples remained healthy and retained their viability for extended lengths of time while others did not remain viable for more than 48-72 hours. Variations of viability in cyst populations undoubtedly influence their resistance to adverse conditions such as disinfection.

To date, there is no method to measure the resiliency of *Giardia* cysts.

A technique that determines cyst viability after subjecting a subsample of a given population to a moderately unfavorable condition (ie: mild heat shock) would indicate the relative resistance of that population prior to disinfection. Such a technique could provide a system of standardizing cyst suspensions and consequently confer validity to results generated from disinfection studies. Without a method to standardize the resiliency of cyst suspensions, the data generated from disinfection studies will remain difficult to assess and the overall conclusions may be misleading. *Giardia* has received increasing attention over the past decade, however much of the basic research to define the nature of this microorganism is still lacking. Despite the shortage of information regarding *Giardia*, regulatory agencies have intensified efforts to set valid standards and regulations. Additional research will be necessary to confirm the efficacy of the regulations currently enforced.

Lack of Fundamental Data for *Giardia*

Another major obstacle encountered at the pilot plant that directly relates to the SWTR is the lack of fundamental disinfection data for *Giardia* species. Experimental trials at the pilot plant required optimization for the recovery of *G. muris* cysts from the system. Initial recovery trials were successful when working with suspensions of less expensive formalin activated *G. lamblia* cysts. Subsequent trials with *G. muris* cysts yielded poor recovery rates (2.0 - 10.0%). Experiments to determine the cause of the loss of

cysts determined that the cysts were not sedimenting completely during the centrifugation steps. Procedures in the literature and in *Standard Methods for the Examination of Water and Wastewater* suggest centrifugation at 1050 x g, but this relative centrfugal force was not sufficient to pellet the samples completely. Following a series of experiments in which different centrifugation schemes were examined, determination of optimal cyst recoveries indicated that *G. muris* and *G. lamblia* have different sedimentation rates and densities. These data suggest that more research is needed to determine the similarities and differences amongst these two related species. In addition, laboratory techniques for the detection and quantification of *Giardia* spp. need improvement. This is an important aspect to the development of standardized disinfection data, since non-pathogenic *G. muris* is used as a model for pathogenic *G. lamblia*. Information used to evaluate the adequacy of the currently used laboratory methods for manipulating and detecting *Giardia* spp. influences research priorities and water quality regulations.

Difficulties in Achieving 3 log Reduction

The majority of the trial runs at the pilot plant did not yield a full 3 log reduction of *G. muris* cysts. During the course of Phase II studies, the pilot plant was adjusted several times to provide a long enough contact time (based on bench scale studies) to reach a 3 log reduction, however even these

elevated CT values did not achieve the target inactivation required. Based on the CT values provided by the USEPA, CT values used at the pilot plant should have achieved at least a 3 log reduction of cysts.

The most widely accepted method for determining cyst viability, which in turn is used to determine log reduction, is *in vitro* excystation (Rice and Schaefer, 1981). This method requires the enumeration of both the dead and living organisms which yields the survival ratio. This method is a time-consuming and tedious procedure. Because the SWTR mandates a 3 log reduction for *Giardia* cysts, this method has a disadvantage in that a minimum of 1,000 organisms must be assessed. This factor proved problematic with the Phase II work at the pilot plant, as recovery efficiencies from the disinfection basin are low and the cost of preparing cyst suspensions of high concentration is expensive. Therefore, a need for an alternative procedure for cyst enumeration, determination of viability and log reduction became apparent.

Currently, a volumetric method for assessing *Giardia* inactivation is being employed (Haas, et al., 1994). This technique requires only the concentration of viable organisms be determined. In this method, the number of trophozoites in the control (nondisinfected) sample and the number of trophozoites in the disinfected sample are counted, and the volume of sample from which these trophozoites are enumerated is determined. A survival ratio is then calculated. Because only trophozoites

need to be counted, the labor of this method is decreased. More importantly, it does not requre the analysis of a large number of nonviable cysts to determine high levels of inactivation (> 2 logs).

Haas analyzed and compared the results of disinfection studies obtained from the volumetric and standard methods (Haas, 1994). He concluded that within the intrinsic variability of performing the disinfection (bench-scale) studies, the volumetric and standard methods produce results that are indistinguishable. Therefore, this method appeared to be the best option for determining high levels of inactivation at the pilot scale level due to its equivalence with the standard method and its practicality in counting and cost of cysts.

Based on the high CT values achieved and the exceptional recovery rates (> 1%) of *Giardia* cysts at the pilot plant, it was disconcerting to fall short of the expected 3 log reductions. One explanation for not achieving a full 3 log reduction is that the volumetric method is not applicable to larger-scale experiments that are performed with large volumes. Haas et al. (1994) used relatively small volumes of sample to obtain the necessary cyst counts. Also related to this explanantion is the difficulty in distinguishing between cysts and trophozoites recovered from field samples. Although the samples are purified using a percoll-sucrose gradient, there are still suspended solids and material impurities that remain which obstruct the view of the researcher. This results in a less accurate estimate of the actual number of cysts and

trophozoites in the samples. In addition, the methods used to concentrate and clean the samples are conditions that may be injurious to the cysts, resulting in unhealthy or atypical looking cysts. Both methods to determine the inactivation of cysts depend on the ability to accurately count the number of trophozoites and/or cysts. However, the volumetric method may be so sensitive that a difference between 2 or 3 trophozoites can have an enormous effect on the log reduction. Alternatively, the volumetric method is a reliable technique and the log reductions are accurate calculations of the strength of the disinfectants employed.

The mere fact that viable cysts were detected following relatively high CT values lends support to the occurrence of waterborne outbreaks in which *Giardia* is the etiologic agent. Research has yet to provide reliable and practical methods for evaluating inactivation of pathogenic protozoa at the field scale level.

CT Values May Be Inadequate

Data generated since the promulgation of the SWTR suggest that the required CT values to achieve the 3 log removal/inactivation of *Giardia* and the 4 log removal/inactivation of viruses may not be sufficient if a system uses source water of poor quality. Studies conducted by LeChevallier et al. (1991) revealed that *Giardia* and *Cryptosporidium* were detectable in 97% of raw water samples and 39% in drinking water samples. The densities of

Giardia and viruses in source waters may be too great for the treatment processes to adequately control for pathogens (Payment, 1991). Systems that are supplied by source water of poor quality that must reduce the levels of disinfection to meet the new Disinfectant/Disinfectant By-Product (D/DBP) regulations for *Giardia* may significantly increase the risk of microbial contamination (USEPA, 1994). The SWTR requirements were developed with an annual health risk goal of no more than one *Giardia* infection per 10,000 people per year (USEPA, 1989). This goal was developed prior to the knowledge of such high pathogen densities in our source waters of poor quality, which reflects an actual health risk of elevated probablility. To address this shortcoming, the EPA suggested revisions of the SWTR that may tailor ample treatment levels to known *Giardia* concentrations in source waters.

Another factor which suggests that the current CT values may not be adequate to provide potable water free of viruses and protozoan cysts is that the CT values provided by the EPA were generated from laboratory studies that used a free, non-aggregated strain of Hepatitis A Virus (HAV), and purified suspensions of *Giardia lamblia* cysts. Microbes in the environment are usually in aggregates or are associated with cell debris or particulates, making them more resistant to disinfection than free or monodispersed microorganisms. Furthermore, microbes in the environment tend to be hardier and less susceptible to adverse conditions. Another factor to consider

is that some pathogenic viruses and protozoa such as *C. parvum* are more resistant to treatment processes than HAV and *Giardia lamblia*. Most importantly, results from this project indicate that there is little correlation between bench and pilot scale studies. This fact alone places considerable doubt as to whether the current CT values are sufficient to inactivate the microorganisms it addresses.

Conclusions

Regulatory Aspects

The EPA has recently proposed a revision to the SWTR to provide additional protection from the contamination of pathogens from source waters of relatively poor quality into our drinking waters. At the time of the development of the SWTR (effective Dec. 31, 1990), there was a shortage of data on *Cryptosporidium* oocyst, *Giardia* cyst, and viral occurrence in source waters and their susceptibility to treatment. There was also a lack of information on pathogen variation over time and seasonal influences, and a need for field data on the effectiveness of different types of water treatment for controlling these pathogens. As a result of these shortcomings of the SWTR, the EPA is currently developing the Enhanced Surface Water Treatment Requirements (ESWTR).

To generate the ESWTR, the EPA has developed an extensive data collection program, the Information Collection Rule (ICR) (USEPA, 1994).

This program will provide the EPA with needed information on the concentrations of *Giardia, Cryptosporidium,* and enteric viruses in source waters of varying quality. The monitoring will also supplement the existing data on densities of microorganisms from season to season and determine whether additional treatment is necessary. The EPA also needs more information on the effectiveness of different types of treatment and the possible production of carcinogenic byproducts. All of the data will be used to develop the Enhanced Surface Water Treatment Rule (ESWTR).

In selecting suitable requirements to be included in the upcoming ESWTR, there is a crucial need to develop a quantitiative assessment of risk from pathogens in drinking water. Quantifying the level of protection from disease provided by treatment would allow the EPA to make appropriate decisions about what changes in water treatment are necessary. Modeling the risk from *Giardia, Cryptosporidium,* and virus contamination in drinking water has not been given adequate attention. This is due to limitations in accurately enumerating pathogen occurrence, uncertainties associated with infectivity and virulence, and variability and diversity of organism occurrence. In order to estimate the risk from microbes in water, the distribution of pathogens in water and the sensitivity of the human population to these microorganisms must be considered. These are two variables which the EPA has not given sufficient attention. The continued occurrence of outbreaks provides compelling evidence for the need of risk

assessments from pathogens in water.

Although outbreaks still occur, the SWTR has provided a framework within which water treatment facilities have been relatively successful in delivering safe, potable water. The SWTR's approach of emphasizing treatment as the condition for compliance rather than estabishing maximum contaminant levels (MCLs) for microorganisms has merit; however, the effectiveness of using CT values may be overestimated and oversimplified. While this approach provides a relatively easy system for water treatment facilities to determine compliance, the reliability of CT values remains debatable especially with source waters of poor or varying quality. For systems that have continual difficulties complying with the SWTR (thereby creating an increased health risk) and where the cost of upgrading cannot be met, perhaps the best solution is that boiled water serve as the permanent supply of potable water.

The revision to the SWTR places a heavy burden on state drinking water programs which have the responsibility of implementing and enforcing the standards. The new regulations also necessitate long and short-term planning by water utilities for compliance. Drinking water suppliers will be forced to stay informed about proposed or anticipated regulations and plan accordingly for compliance so that the public may benefit from drinking tap water of unquestionable quality.

Multiple Barrier Approach

The prevention of waterborne disease outbreaks depends on three water supply practices that are considered essential parts of the multiple barrier concept: adequate sewage collection and disposal, protection of water sources, and effective water treatment techniques (White, 1986). Adequate sewage treatment and natural barriers that protect source waters from discharges of human sewage are the first steps in preventing waterborne transmission of disease. Protection of raw water quality is becoming more apparent with increasing evidence of the importance of domestic and wild animals in the transmission of waterborne disease. Equally important are the practices of filtration and disinfection, which are the last lines of defense, for surface waters.

Inherent in the multiple barrier concept is that protection against waterborne transmission of disease does not rely solely on one type of treatment process. The preceding barriers must reduce the microbiological population and remove interfering substances for disinfection to be an effective final barrier. By providing effective filtration and pretreatment to reduce the chlorine demand, lower concentrations of chlorine can be used, resulting in decreased levels of chlorinated by-products. Also important is the maintenance of barriers in the distribution system (ie: adequate water pressure and disinfectant residuals) to protect against contamination after water treatment.

Outbreaks that have occurred in systems in compliance with current regulations illustrate the importance of maintaining the multiple barriers of treatment and multiple points of control between sewage discharges and water supply intakes. Relying on treatment of polluted water supplies to protect public health instead of preventing pollution in the first place is not universally effective in providing pathogen-free drinking water. It has been documented repeatedly that outbreaks result primarily from inadequate or interrupted disinfection, especially in systems that provide disinfection as the only treatment. More emphasis should be placed on the design and operation of treatment facilities if they are to be effective against pathogens.

The prevention of waterborne outbreaks is a responsibility that shoul be shared between federal, state, and local agencies and drinking water utilities who all play an important role in protecting the public's health. Government and private water utilities must continue to recognize and accept the challenge to improve the quality of water supplied to consumers. Systems that are modified to satisfy the EPA standards must strive to provide drinking water with sufficient disinfectant to prevent the risk of infectious

diseases and minimize exposure to chemical toxins while not greatly increasing the monthly water bill.

Local and state health departments must continue to play an active role in the control, prevention, and risk assessment of disease by investigating outbreaks and conducting surveillance programs. The identification of the etiologic agents of waterborne outbreaks is of paramount importance as new organisms may require different or supplemental methods of control (ie: *Cryptosporidium*,*E. coli* 0157:H7, and *Cyclospora*). Additionally, an emphasis on pollution prevention through the use of incentive programs to increase individual and collective responsibility for protecting water resources are needed. Addressing the social causes of water quality problems is essential to the development of long-term solutions.

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APPENDIX A

PREPARATION OF MEDIA

Brilliant Green Lactose Bile Broth (2%)

40.0 g brilliant green lactose bile media (Difco, Detroit, MI)

Suspend in 1L distilled or deionized water and warm slightly to dissolve medium completely. Dispense required amount in test tubes. Place an inverted fermentation vial in each tube. Place closure on tubes and sterilize in autoclave for 15 minutes at 15 lbs. pressure (121°C). Final pH 7.2 ± 0.2 at 25°C.

E.C. Broth

37.0 g E.C. media (Difco)

Same as above.

Lauryl Sulfate Broth

35.6 g lauryl sulfate broth media (Becton Dickinson, Cockeysville, MD)

Dissolve in 1 L purified water. Dispense in test tubes, containing inverted Durham tubes, in 10.0 mL amounts for testing 1.0 mL or less of samples. For testing 10.0 mL quantities of samples, dissolv 71.2 g of powder in 1 L pure water and distribute in 10.0 mL amounts. Sterilize by autoclaving at 121°C, preferably for 12 minutes, but not exceeding 15 minutes. After sterilization, cool broth as soon as possible.

Plate Count Agar

23.5 g plate count agar (Difco)

Dissolve in 1 L distilled or deionized water by heating until boiling. Sterilize in the autoclave for 15 minutes at 15 lbs. pressure (121°C). Final pH 7.0 ± 0.2 at 25°C.

Tryptic Soy Agar

40.0 g tryptic soy agar (Difco)

Suspend in 1 L distilled or deionized water and boil to dissolve completely. Sterilize at 121°C for 15 minutes. Pour into sterile petri dishes when cooled.

Tryptic Soy Broth

30.0 g tryptic soy broth media

Suspend in 1 L distilled or deionized water and boil to dissolve completely. Sterilize at 121°C for 15 minutes.

2X Soft Overlay

30.0 g tryptic soy broth (Difco)
5.0 g yeast extract (Difco)
7.5 g Bacto-agar (Difco)
0.8 g calcium chloride (Baker)

Dissolve in 500 mL distilled or deionized water. Sterilize at 121°C for 15 minutes. Dispense 5.0 mL into sterile test tubes . Keep at 50 - 52°C until use.

APPENDIX B

BUFFERS AND SOLUTIONS

Elution Solution

1X PBS	800.0 mL
1% SDS	100.0 mL
1% Tween 80	100.0 mL

Hank's Balance Salt Solution

Mix 25.0 mL 10X Hank's solution into (total volume) 250.0 mL dH_2O. Supplement with 0.067 M glutathione (MW 307.3) and 0.029 M L-cysteine hydrochloride (MW 175.6). Filter sterilize and refrigerate.

1M $MgCl_2$

Dissolve 20.3g into (total volume) 100.0 mL dH_2O.

Percoll Sucrose Solution

Percoll	45.0 mL
2.5 M sucrose	10.0 mL
dH_2O	45.0 mL

Mix with stir bar over low heat. Measure with hydrometer at room temperature or 4.0°C (1.09 - 1.10).

10 X PBS

NaCl	80.0g
KCl	2.0g
KH_2PO_4	4.0g
Na_2HPO_4	20.0g
Phenol red	0.05g
Deionized H_2O	1000.0 mL

Mix together. Adjust pH to 7.2-7.3. Filter sterilize.

KH_2PO_4

Dissolve 17.01g KH_2PO_4 in 250.0 mL dH_2O (pH = 4.4).

0.1M $NaHCO_3$

Dissolve 0.42g $NaHCO_3$ into (total volume) 50.0 mL dH_2O.

1% SDS

Dissolve 10.0g SDS into (total volume) 1000.0 mL dH_2O.

Na_2HPO_4

Dissolve 17.75g Na_2HPO_4 in 250.0 mL dH_2O (pH = 9.9).

1M $Na_2S_2O_3$

Dissolve 248.2g into (total volume) 1000.0 mL dH_2O.

1% Tween 80

Dissolve 10.0g Tween 80 into (total volume) 1000.0 mL dH_2O.

Tyrode's Salt Solution

NaCl	18.0g
$CaCl_2$	0.4g
KCl	0.4g
$NaHCO_3$	2.0g
glucose	2.0g
$MgCl_2$	0.2g
NaH_2PO_4	0.1g
dH_2O	2000.0 mL

Filter through a 0.45 um or 0.22 um filter.